The individuality of (virus-specific) CD8 T cells
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GENERAL INTRODUCTION FOR THE LAYMAN
This thesis is divided in two parts: The first part, comprising chapters 1 to 4, concerns research into the traits and formation of human CD8+ T cells targeting different viruses, such as the human cytomegalovirus (hCMV), the Epstein-Barr virus (EBV) and the influenza (A) virus (influenza); the second part, comprising chapters 5 to 7, specifically concerns our research data on the differentiation and functional properties of human CD8+ T cells targeting polyomavirus BK. This is a virus that nearly all of us are infected with without being aware of it. However, it may cause significant disease in renal transplant recipients, who have a poorly functioning immune system due to the immunosuppressive medication that is necessary to prevent rejection of the transplanted kidney.

Each part is prefaced by an introduction chapter, concerning the traits and functional properties of antiviral αβ CD8+ T cells in the human circulation and tissues for part I, and the predominantly clinical aspects of BKV-induced pathology in transplant recipients for part II. Both parts concern manuscripts that were mainly written for specialists in these fields and may therefore be difficult to understand for outsiders. In order to better comprehend and interpret the topics described in this thesis, I wrote a general, and a bit simplified introduction on these subjects here. For a more detailed description of the topics discussed in this thesis please see chapters 1 and 5.

PART I

To better understand the relevance of the research described in part I, one must understand eight things:

1. CD8+ T cells (or T lymphocytes) are part of the specialized immune system, also known as the leukocytes (or ‘white blood cells’).
2. CD8+ T cells have the unique capacity to scan for disease in the inside of all cells that make up the human body. In contrast, the majority of the other leukocytes are mainly focused on controlling threats located on the outside of the cells (e.g. the circulation, the lymphatics etc.).
3. CD8+ T cells are typically known for their ability to ‘kill’ diseased cells, and are also known as cytotoxic (meaning toxic to cells) leukocytes.
4. Viruses are extremely small entities that consist of little more than a capsid that is holding genetic material.
5. Viruses are unable to replicate themselves without ‘hijacking’ the replicative machinery of the cells of a host to make offspring.
6. To hijack the replicative machinery of the host that a virus infects, viruses use various strategies to enter the interior milieu of their host’s cells, making them difficult to detect by the host’s immune system.
7. Finally, uncontrolled proliferation of viruses causes severe disease in humans, going beyond fever and malaise, sometimes even involving the emergence of cancer.
8. CD8+ T cells can form ‘memory’ cells that are able to ‘remember’ the virus they encountered cells and provide a highly effective and specialized defence when the body is attacked by the same virus in the future.

White blood cells are part of the specialized immune system, meaning that they are primarily dedicated to the defence of the body against pathology, like bacteria, fungi, parasites, viruses and cancer. All other cells in the body have also developed defensive mechanisms to protect themselves against threats. However, in contrast to the white blood cells, this is generally not their primary function. CD8+ T cells are uniquely capable of detecting and acting against threats found in the inside of cells through the use of the ‘T cell receptor’ (TCR). The CD8+ T cell TCR can be compared to a sophisticated scanning device that is able to interact with a special group of molecules. These are called the major histocompatibility complex class I (MHC I) molecules, and they can be found on the outside of nearly every cell in the body. The MHC I molecule can be seen as a chalice holding a small random fragment deriving from the inside of the respective cell. Such fragments originate from a process where cells continuously recycle nearly every component found in their insides, including components that should not be there. During this process, the small fragments are incorporated in these chalices, which are then transported to the exterior of the cells for the purpose of displaying these possibly pathologic components to the CD8+ T cells. As such, the CD8+ T cell does not probe directly into the inside of cells, it rather interacts with everything that is displayed on the outside of the cell in the MHC I molecules to see what is happening inside the cell. Because a single cell carries thousands of these MHC I molecules on its surface, each loaded with a fragment derived from the inside of the cell, the CD8+ T cell can get a fairly accurate image of what is going on in the interior of the cell after having carefully scanned the MHC I molecules (Figure 1). A small proportion of these fragments may also derive from the outside of the cells through a process called ‘cross-presentation’, which is a complicated topic that is beyond the scope of this general introduction.

Once a CD8+ T cell detect a potentially dangerous fragment from an MHC I molecule, it may be triggered to rather rigorously eliminate the entire cell (and not just the threat, e.g. the viruses, found in the inside of the cell). They do this by injecting the cell with substances (called serine proteases or granzymes) that initiate a self-destruct mechanism inside the cell, ultimately leading to an orderly process in which the cell carefully proceeds to dying, but without releasing its contents into the environment (e.g. viruses, which might then infect neighbouring cells). However, despite their moniker ‘cytotoxic T cell’, CD8+ T cells do more than killing to control infection. Amongst others, they are also capable of releasing substances (called cytokines and chemokines) into the environment that alarm neighbouring cells of the danger they found.

Viruses are a strange type of entity. First of all, it is not clear whether they are actually alive or whether they just ‘are’. They are incredibly small, some nearing only two-hundred times the size of a hydrogen atom, whereas being twenty times smaller than a bacterium, or around five-hundred times smaller than a human cell. To give one a better idea: if a human cell was a blue whale, then a virus would be a small goldfish (Figure 2).
Second, viruses are often nothing more than a simple container holding the virus’ genetic material. They do not have a cell wall or the normal organs (organelles) found in regular cells. Regardless, similar to all things ‘alive’ in this world they mysteriously strive to procreate, creating more copies of themselves. However, because they lack the means to copy their own genetic material, they have evolved methods to ‘borrow’ the replicative
machinery of cells, for example those found in the human body. The viruses, however, do not ask nicely whether they can borrow a cell's replicative machinery. They stealthily invade the cell, skillfully evading the cell's defence mechanisms and just take what they want. Because viruses replicate themselves faster as more of the cell's replicative machinery is active, they also utilize all kinds of devious tactics to artificially stimulate cellular replication. This in turn may be dangerous to the host organism (e.g. the human to which the cells belong) as uncontrolled stimulation of a cell’s replicative machinery and in turn uncontrolled cellular division may progress into the formation of cancer. As such, viral infection can be extremely dangerous if not controlled properly by the immune system.

CD8⁺ T cells are capable of detecting a viral presence in the inside of cells because the MHC I molecules also present fragments of the virus on the outside of the infected cell. Upon detection of danger, they will not only eliminate the danger, they also do something else called ‘memory cell formation’. During the acute phase of infection, where an individual also may have a fever and feels ill, large numbers of viruses are present in the body, infecting multiple cells, often located in a specific organ system. For example, the well-known influenza virus specifically targets cells in the airways and the lungs. Instead, the norovirus mainly infects intestinal cells, and either virus causes a different array of symptoms, depending on the organ system affected.

This is where CD8⁺ T cells are activated to fight the respective virus, which starts of by the expansion of single activated CD8⁺ T cells into large numbers of daughter CD8⁺ T cells that are specifically armed to effectively combat the virus. Indeed, each virus triggers a specialized population (or subset) of CD8⁺ T cells that carries a distinct set of weapons (i.e. the afore-mentioned serine proteases or granzymes and cytokines and chemokines). This is not entirely unexpected given the different, and often also highly specialized strategies by which different viruses enter and hijack the body's cells. The war between the CD8⁺ T cells (and the rest of the immune system) and the virus often takes several days in which the host may experience fever, pain, coughing, diarrhoea etc. Once the virus has been eliminated, the large expansion of CD8⁺ T cells are no longer necessary and may even be harmful to the host given their toxic weaponry and their metabolic demands. This is also where the CD8⁺ T cell number declines, only to leave a small population behind that may stay in the body for several years. These are special cells, experienced war veterans if you would, that have remembered how to best handle future infections with the same virus. Therefore they are called memory cells. In the case of a new infection with the same virus, these memory cells again expand to large numbers to fight the next round of combat, but they do this much more rapidly and effectively when compared to their parent's actions during the first encounter. This, amongst others is one of the reasons why adults are less often sick than infants, and when they do, the illness usually takes up a much shorter period of time. One of the holy grails of science would be to understand this process of memory formation so that it can be used to enhance the current vaccination strategies.
PART II

To better understand part II of this thesis, one needs to know eight things:

1. Polyomavirus BK (BKV) is a virus that infects nearly all of us without causing any symptoms in the presence of a well-functioning immune system.

2. Polyomavirus BK is never eliminated from the body entirely as it uses tactics to hide itself inside cells found in the kidneys and the bladder.

3. Individuals with a severely impaired or non-existent kidney function need a replacement kidney to clear the body from harmful substances. Replacement kidneys can be provided in the form of renal dialysis or in the form of a kidney transplantation where someone receives a functional kidney from a living or deceased organ donor.

4. Once an individual receives a kidney transplantation, the immune system recognizes the new kidney as ‘foreign’ and as potentially dangerous, and it will start to attack the donor kidney. Immunosuppressive medication must be given to these individuals to keep the immune system docile, thereby preventing immune-mediated damage to (or rejection of) the donor kidney.

5. These immunosuppressive agents unfortunately not only prevent immune-mediated damage to the donor kidney, they also suppress the immune system that is responsible for the defence against viruses, like BKV.

6. Owing to the immune-suppressed state of kidney transplant recipients, BKV frequently emerges from its hiding place, after which it is able to proliferate in a largely uncontrolled fashion.

7. This reactivation of BKV, mainly affecting the donor kidney can cause severe damage to the transplanted organ, sometimes even leading to loss of function of the affected kidney. This is of course very unwanted, since kidney transplant recipients generally must wait a couple of years for a suitable kidney-transplant.

8. There is currently no proven effective medication available that directly targets BKV and its actions. The only remedy is to reduce the amount of immunosuppressive medication so that the individual’s immune system can recuperate to subsequently regain control over BKV. This, however, also enhances the capacity of the immune system to attack to donor kidney.

BKV is thought to be transmitted by certain bodily fluids, amongst which urine, but possibly also saliva and is perhaps already transmitted in the womb. After entry, the human immune system quickly gains control over the virus, upon which BKV hides itself from the immune system inside certain cells of the body (mainly those found in the kidneys and urinary tract). This indeed means that it is never completely eliminated from the body after primary infection.

Kidney transplant recipients, i.e. individuals who have received a ‘new’ kidney from a living or a deceased donor, need to be treated with medication that suppresses the immune system to prevent the recipient’s immune cells from attacking
the ‘foreign’ donor kidney. This leads to the so called rejection of the transplanted organ. Unfortunately, these agents also severe hinder the immune system in its ability to control viral infection, including those viruses that were already ‘hiding’ in the cells of the body prior to transplantation, like BKV. As such, this creates a favourable environment for BKV to proliferate and to emerge from its hiding place. The large numbers of BKV virus particles, the so-called ‘virions’, then start infecting other neighbouring cells in the kidney. Because the viral growth is largely uncontrolled, the cells eventually burst open due to the large numbers of viruses that have accumulated inside the cell, after which these new virus particles indeed infect new neighbouring cells. This establishes a situation that threatens the entire kidney, and may cause a condition called BKV-associated (or induced) interstitial nephritis (BKVN) in up to 10% of the kidney transplant recipients. The foremost sign of this condition is a progressively deteriorating kidney function, accompanied by high levels of BKV virions found in the circulation. Ultimately, BKVN is diagnosed with a needle biopsy, during which tissue from the donor kidney is obtained for further microscopic examination by a pathologist.

Unfortunately, reactivation of BKV cannot be countered by specific antiviral agents. The only approach of proven value is to reduce the amounts of immunosuppressive medication so that the individual's own immune system can recuperate after which it can regain control over the viral proliferation. This is, however, a double-edged sword as it not only allows the immune system to better fight the virus, but also the foreign donor kidney, indeed leading to an increased chance on rejection. Therefore, there is a dire need for better treatment options. One interesting possibility involves the ‘use’ of an individual’s own T cells. As stated above, CD8+ T cells interact with BKV-infected cells through their T cell receptor and a MHC I molecule presenting a BKV fragment that is deriving from the inside of the cell. If we would be able to extract populations of CD8+ T cells that are activated only by BKV fragments, and not by any other fragments presented in the MHC I molecules prior to transplantation, then we could give such populations back to the individual after transplantation if BKV reactivates. These BKV-specific CD8+ T cells are unable to harm the uninfected part of the transplanted kidney (because they cannot interact with cells not presenting BKV peptides on their surfaces in the MHC I molecules) and they will specifically target only those cells infected with BKV. Moreover, these cells would thereafter, theoretically, form memory populations that could provide protection against BKV for a longer period of time. Such a mode of therapy would indeed be highly specific, and would not increase the chance of rejection of the donor kidney.

Nevertheless, various questions can be raised on this theory, all of them deserve intensive research before this can be tested in patients. First of all, when we started our studies, it was not known what type of CD8+ T cell (or subset) normally confers protection against BKV, and therewith automatically also the armamentarium that they employ. Second, we did not know what is happening to the (pre-existent) CD8+ T cell populations in patients that develop severe BKV reactivation and/or BKVN, and why they lose control over the virus (covered in this thesis). By the way, it is besides the truth to state that BKV is controlled by CD8+ T cells only, as other cells of the immune system are like to also
play an important role. However, CD8$^+$ T cells are very proficient at detecting and fighting intracellular pathology and have therefore been the focus of this thesis. Third, which is not covered in this thesis, it is unknown whether such infused (or adoptively transferred) BKV-specific CD8$^+$ T cell populations will also be affected by the immunosuppressive medication, and whether they will able to help regain control over BKV. Finally, if they will appear effective, then it would seem logical that the effectiveness of such BKV-specific CD8$^+$ T cell infusions increases with the size of the T cell population given to the patient. Would we be able to isolate large amounts of BKV-specific CD8$^+$ T cells prior to transplantation (without impairing the BKV-specific immune response already prior to transplantation), or could we artificially expand smaller populations of BKV-specific CD8$^+$ T cells (in a petri dish) without influencing the differentiation state and the ‘weaponry’ of the T cells prior to giving them back to an individual?

To conclude, this should largely cover the basics of the topics touched upon in the two parts that make up this thesis. As stated previously, each part will be headed by an Introduction chapter written on the level of experts in the respective field of research, which should now be better accessible to readers who are less experienced in these fields.

SCOPE OF THIS THESIS

For obvious reasons, the current understanding of CD8$^+$ T cell responses targeting viral infections largely derive from experimental mouse models. However, these models may not translate directly to the human situation, for one because mice do not normally get to become eighty years of age. The aim of this thesis is to investigate human CD8$^+$ T cell populations during health and disease in response to various viral infections. Of specific interest is the CD8$^+$ T cell response targeting polyomavirus BK. Whereas this is a virus that establishes a mode of latent infection in nearly all of us, we know very little about how this virus is normally controlled by the immune system, or how immunological control is lost in some immunocompromised individuals.