Homeostasis and function of T cells in healthy individuals and renal transplant recipients
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The human immune system

Throughout life, human beings encounter a wide variety of different pathogens such as bacteria, viruses and parasites. In the course of evolution we have developed a very adequate immune system, which can protect us against these pathogens. It is divided into two branches, the innate and the adaptive immune system.

The innate immune system primarily depends upon the recognition of pathogen components and is immediately available to combat. It is composed of natural killer (NK) cells, granulocytes, monocytes, macrophages, dendritic cells and the complement system. NK cells are cytotoxic cells that kill target cells which have down-regulated their major histocompatibility complex (MHC)-molecules. Most of the other innate immune cells express a wide variety of pattern recognition receptors (PRRs). Via these receptors, they can rapidly respond to pathogen-associated molecular patterns (PAMPs), which are broadly expressed by microorganisms.

The adaptive immune system has a lag phase of a few days and unlike the innate immune system, it can specifically recognize pathogens. Furthermore, it is capable of generating life-long immunological memory. Several lineages of lymphoid cells, like B lymphocytes, CD4+ and CD8+ αβ T lymphocytes belong to this system.

B lymphocytes are characterized by the presence of a unique B cell receptor (BCR), which is a membrane-bound immunoglobulin and can specifically bind to antigen. Upon recognition of its antigen, naïve B cells differentiate into memory cells or specialized immunoglobulin producing plasma cells. These immunoglobulins are important for the neutralization and elimination of extracellular pathogens.

The unique T cell receptor (TCR) of CD4+ T cells can interact with MHC class II molecules which present peptides. MHC class II molecules are mainly expressed by professional antigen presenting cells, such as dendritic cells, macrophages and B cells. The CD4+ T-cell pool consists of a variety of different T helper (Th) cell lineages, which will be discussed later.

CD8+ cytotoxic T cells are crucial in the defence against intracellular pathogens such as viruses. Virtually all nucleated cells in our body express MHC class I molecules, which continuously present peptides derived from cytoplasmic proteins. Virus-infected cells present peptides derived from viral proteins in their MHC class I molecules and can consequently be recognized and killed by virus-specific CD8+ T cells.

Apart from αβ T cells also natural killer T (NKT) cells, mucosal associated invariant T (MAIT) cells and γδ T cells are part of the human immune system. NKT cells express the invariant Va14-Jα18 T cell receptor and recognize the non-classical class I molecule CD1d which bind lipids and glycolipids. They can become activated by CD1d-dependent TCR stimulation, but also by pro-inflammatory cytokines, such as IL-12. Upon stimulation, they produce large amounts of mostly Th1-type cytokines, such as IFNγ. Recently MAIT cells have been described, expressing a semi-invariant TCR Vα7.2-Jα33, which are restricted by the MHC-related protein 1 (MR1). After stimulation with APCs that are fed with bacteria or fungi, they can become activated to produce
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high amounts of IFNγ. γδ T cells represent only a small subset of circulating T cells, but in epithelial-rich tissues, such as the skin and the gut, they can comprise up to 50% of T cells. They likely perform different functions according to their tissue distribution, the local microenvironment and structure of their antigen receptor. However, their exact function remains to be elucidated.

T cell development

αβ T cells are formed and ‘educated’ in the thymus. In the development from pluripotent hematopoietic stem cell to T cell, the formation of an antigen specific T cell receptor (TCR) is crucial. The TCR is formed by stepwise rearrangement of the antigen receptor genes, also known as VDJ recombination. This random recombination of genes gives each T cell a unique TCR. If the TCR can recognize either self MHC class I or II, the cell will be positively selected to respectively become a CD8+ or CD4+ T cell. Too strong interaction of the TCR with ‘self-peptides’ presented in the MHC molecules, will lead to negative selection. Following this selection process, the naïve T cell - equipped with a unique TCR - will migrate out of the thymus, now capable of recognizing either MHC class I or II in combination with specific ‘non-self peptides’. Although the TCR of γδ T cells is also rearranged in the thymus and in theory its diversity can be even greater than that of the αβ T cells, their repertoire of these cells is much more limited. The mechanisms responsible for this restriction remain unknown.

T cell differentiation

Professional antigen presenting cells (APC), in particular dendritic cells, are extremely important in initiating primary T cell responses. Upon encounter with an antigen, APCs will become activated and migrate to the lymph nodes. Once activated, they start presenting antigen derived peptides in their MHC molecules. Furthermore, they will start expressing co-stimulatory molecules and producing cytokines. Naïve T cells continuously patrol the lymph nodes in search of their antigen. Their activation requires TCR-initiated recognition of peptides presented by the APC in MHC molecules (signal 1), co-stimulation through a.o. CD28 ligation with CD80 or CD86 (signal 2) and additional stimulation via cytokines (signal 3). Activated naïve T cells will undergo clonal expansion and form a pool of highly specialized effector T cells, which can produce high amounts of pro-inflammatory cytokines and effector molecules.

During an immune response, CD4+ T cells can differentiate into a variety of Th lineages. The type of Th response that is mounted is determined by the character of the pathogen and is orchestrated by antigen presenting cells. In response to intracellular pathogens, the innate immune system produces IL-12, which promotes naïve CD4+ T cells to differentiate into IFN-γ producing Th1 cells. On the other hand, helminths and other extracellular pathogens induce Th2 responses. Through the secretion of IL-17, Th17 cells promote protection against extracellular bacteria and fungi and they can also promote autoimmunity. In addition, follicular T helper (Tfh) cells have been described, which are specialized in providing B cell help in the germinal centers (GCs).
MHC class I molecules continuously present peptides derived from endogenous proteins, which enables CD8+ T cells to detect transformed or infected cells. APC's which are not infected can present exogenous antigens via a mechanism called cross-presentation. Naïve CD8+ T cells can be primed in the lymph nodes by professional APC's via either direct- or cross-presentation of antigens. Once activated, the CD8+ T cells will clonally expand and differentiate into cytotoxic effector T cells, producing high amounts of IFNγ and cytotoxic molecules, such as perforin and granzyme B. These newly generated effector cells will migrate to infected tissue, where they subsequently kill virus infected cells. When the task of the effector T cells has been carried out and the infection is cleared, most of them will undergo apoptosis and only a small population of memory T cells will remain. The decisive factors, that determine which cells will become long-lived memory cells, are still unclear. T cell memory provides enhanced protection upon reinfection, mounting a faster and more efficient response.

Chemokine receptors and T cell trafficking

Lymphoid organs provide a specialized environment where antigen specific T cells recognize their antigen presented in MHC molecules of professional antigen presenting cells (APC). Chemokine-receptor expression patterns ensure that the right cell is positioned at the right place at the right time. Upon T cell activation and during T cell differentiation, the expression of chemokine-receptors is regulated. Naïve and central memory T cells continuously traffic from the blood to the secondary lymphoid organs in search of their cognate antigen. The chemokine receptor CCR7 and its ligands CCL19 and CCL21 have been identified as central conductors in the trafficking of T cells from the bloodstream to the lymph nodes. However, during an inflammatory response, T cells are also capable of homing to lymphoid tissue in a CXCR3-dependent fashion. In addition, chemokine receptors are critical in directing the homing of lymphocytes to nonlymphoid tissues. The expression of CCR9 on lymphocytes enhances localization to the small intestine, whereas CCR10 directs skin-tropic T cell trafficking. Chemokine receptors such as CXCR3, CCR5 and CXCR6 are increasingly expressed upon effector cell differentiation. These chemokine receptors enable T cells to migrate to inflamed sites, where they can directly execute their effector functions.

T cell homeostasis in steady-state conditions and during lymphocytopenia

Homeostasis is the tendency towards a relatively constant state. A variety of homeostatic mechanisms operate to keep the properties of the internal environment of organisms within fairly well-defined limits. The French physiologist Claude Bernard was the first to introduce the concept of the internal environment of an organism (1860s), upon which the term homeostasis as a unifying concept in human physiology was established by the American Walter Bradford Cannon in 1926. The maintenance of a functional and diverse T-cell pool is necessary to preserve immunity. There are several factors that contribute to the maintenance of the human T-cell pool. For their survival, naïve T-cells require short contact with self-MHC/peptide
complexes and contact with the homeostatic cytokine IL-7. Although memory T cells maintain their numbers independently of contact with self-MHC/peptide complexes, they are strongly dependent on contact with homeostatic cytokines. For survival and homeostatic proliferation memory CD4+ T cells mainly rely on IL-7, whereas for CD8+ memory cells both IL-7 and IL-15 are important.

Both naïve CD4+ and CD8+ T-cell numbers gradually decline in aging humans and the peripheral T-cell pool becomes dominated by antigen experienced cells. A proportion of the naïve T cells has differentiated into effector and memory T cells following consecutive antigen encounters. Furthermore, the maintenance of the naïve T cells through thymic output has declined, because of age-related atrophy of the thymus. Recently, it has been demonstrated that the majority of naïve T cells in healthy human adults is produced by peripheral proliferation of the established pool, rather than by thymic output.

T cell repopulation in lymphocytopenic conditions can be effectuated by thymopoeisis, homeostatic proliferation and antigen-driven expansion. Although each of these processes may play a role, it is unknown to which extent each separate process contributes. Naïve T cell repopulation in lymphocytopenic conditions has been shown to occur at a very slow pace and the contribution of the thymus to naïve T cell repopulation is still under debate. Naïve T cell recovery in lymphocytopenic conditions can be influenced by age related thymic atrophy. Total CD8+ T cells repopulate rapidly in lymphocytopenic conditions and consist mainly of highly differentiated CD62L-CD27-CD45RA-/-CD57+ effector-type cells. Since the presence of these highly differentiated effector-type CD8+ T cells in the circulation of healthy individuals has previously been associated with cytomegalovirus (CMV) infection, we hypothesize that repopulation of CD8+ T cells might be partly driven by CMV.

The impact of Cytomegalovirus on our immune system

Cytomegalovirus (CMV) is a β herpes virus, which induces a persistent infection in about 60-80% of healthy individuals. Although usually asymptomatic in healthy individuals, CMV can cause severe disease in immunocompromised hosts and primary infection during pregnancy can cause severe clinical symptoms in children. Furthermore, CMV has been suggested to be involved in the pathogenesis of atherosclerosis.

During latency, CMV resides in vascular endothelial cells and myeloid cells. Most likely, CMV has co-existed with man for a long time and it has evolved to avoid its elimination by tricking our immune system in various ways. Among others, it down-regulates human MHC class I molecules and - to prevent subsequent lysis by NK cells - infected cells are triggered to start expressing an MHC class I homologue. Although our immune system does not succeed in totally eliminating CMV, it is capable of controlling the infection. Especially CD8+ and CD4+ T cells but also NK cells, play an important role in the immune response mounted against CMV.

By monitoring CMV-seronegative renal transplant recipients who receive a donor organ from a CMV-seropositive donor, we have previously mapped CD4+ and CD8+ T cell responses upon primary CMV-infection. CMV-specific CD4+ and CD8+ T cells peak...
shortly after CMV-DNA becomes detectable in the peripheral blood. When CMV-DNA is no longer detectable the numbers of CMV-specific T cells decline. However, CMV-infection continues to exert a strong effect on the CD8\(^+\) T cell pool during latency. In healthy individuals, circulating CD27 CD45RA\(^+\) effector-type CD8\(^+\) T cells and cytolytic CD28\(^-\) CD4\(^+\) T cells mainly appear as a consequence of CMV-infection.

**The effects of immunosuppressive drugs on T cells**

To prevent rejection in solid organ transplantation, patients are often treated with a combination of immunosuppressive drugs. These immunosuppressive drugs can be classified into several groups, exerting different effects on the human immune system, where T cells are their prime target. Various drugs are known to target different steps in T cell activation. Glucocorticosteroids are the oldest immunosuppressive drug and have a very diverse mechanism of action. Among others, they can trigger apoptosis of T cells, inhibit Th1 polarization and suppress the production of pro-inflammatory cytokines. Tacrolimus and cyclosporin block the NFAT pathway by inhibiting calcineurin activity, which eventually leads to inhibition of the transcription of IL-2 and other cytokines. Mycophenolate and azathioprine prevent T cell proliferation via nucleotide synthesis inhibition. The immunosuppressive effects of mammalian target of rapamycin (mTOR) inhibitors have long been considered to be mediated by interference in the cell cycle, leading to inhibition of T cell proliferation. However, more recently it was demonstrated that rapamycin (sirolimus) can also selectively promote the expansion of regulatory T-cells (Tregs). Furthermore, mTOR inhibitors have been shown to possess immunomodulatory properties, playing an important role in memory T cell differentiation. To further reduce the risk of rejection the majority of renal transplant recipients are treated with induction antibody therapy. Polyclonal antithymocyte globulin (ATG) and monoclonal αCD52 (alemtuzumab), both lead to a prompt and durable T cell depletion. In contrast, the monoclonal antibody αCD25 (basiliximab) does not induce T cell depletion, but leads to T cell inactivation via blockade and down regulation of the CD25 molecule, being the IL-2 receptor. In the Netherlands αCD25 is preferably used as induction therapy, whereas ATG is mostly used as treatment for acute humoral rejection or steroid resistant cellular rejection.

**SCOPE OF THIS THESIS**

In this thesis we will mainly focus on homeostasis and function of T cells in healthy individuals and renal transplant recipients. In particular, we will focus on the effect of CMV infection on these processes. We had the unique opportunity to study human CD8\(^+\) and CD4\(^+\) T cells in paired lymph node (LN) and peripheral blood (PB) samples of renal transplant recipients. In the second chapter we compare the percentage, phenotype and functionality of PB and LN derived virus-specific CD8\(^+\) T cells. Additionally, we questioned whether the fingerprint that CMV infection leaves on the PB compartment is also present in the LNs. In the third chapter we studied the different CD4\(^+\) T helper cells lineages in LN as compared to PB. We especially focused
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on the Tfh / CXCR5+CD4+ T cells as these cells are specialized in providing B cell help in the LNs. In the fourth chapter we analyzed T cell repopulation and homeostasis in lymphocytopenic conditions in renal transplant recipients treated with ATG. We mainly aimed to determine the role of antigen-driven repopulation by studying the effects of CMV infection on T cell repopulation. Next, in chapter five, we studied the effect of different immunosuppressive drugs, administered to renal transplant recipients, on CMV-specific T cell responses. In chapter six, we studied CD161++/IL-18Ra+ CD8+ T cells. This subset contains virus-specific cells and has been assigned "stem cell" properties because of their expression of the ABC-B1 transporter. We aimed to further unravel the stem cell-like properties of these cells. At last, in chapter seven we will summarise this thesis and its most important conclusions.

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