Homeostasis and function of T cells in healthy individuals and renal transplant recipients
Havenith, S.H.C.

Citation for published version (APA):
Havenith, S. H. C. (2012). Homeostasis and function of T cells in healthy individuals and renal transplant recipients

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
SUMMARY AND CONCLUSIONS
Cytomegalovirus (CMV) infection has a tremendous effect on the human PB T cell compartment, in that highly differentiated CD27^-CD45RA+ effector-type CD8^+ T and CD28^-CD27^- cytolytic CD4^+ T cells appear in the circulation\(^1\). With aging the effector-type CD8^+ T cells further increase in numbers\(^2\). The increase of these cells is assumed to lead to competition for “immune space” with other T cells, which will eventually cause increased susceptibility to infections and impaired responses to vaccination\(^3\). This process is also referred to as immunosenescence.

In the second chapter of this thesis, we studied a large cohort of patients awaiting kidney transplantation. In this cohort we found nearly twice as many circulating CD8^+ T cells in CMV-seropositive as compared to CMV-seronegative individuals. This indicates that CMV-specific CD8^+ T cells do not displace other memory CD8^+ T cells, but rather enlarge the total CD8^+ T cell pool. Next, we demonstrated that CMV-specific and CD27^- effector-type CD8^+ T cells are much less dominant in LNs than in the PB, while percentages of other virus-specific cells in LN and PB are similar. Furthermore, we demonstrate that cytolytic GranzymeB^+ CD4^+ T cells, which have previously been shown to appear as a consequence of CMV infection\(^4,5\), are also hardly present in the LNs (chapter 3). From this we conclude that in the LNs, a site where adaptive immune reactions are initiated, CMV-infection does not limit immunological space.

In humans, T helper lineages in the PB compartment have been thoroughly studied. Other compartments, such as the secondary lymphoid compartment, are seldom studied because of their scarce accessibility for human research. Tonsils, which are surgically removed because of recurrent or chronic inflammation, are sometimes used in studying the human secondary lymphoid compartment. However, because of the chronic inflammatory conditions in these tonsils, the data may represent a skewed image.

In chapter three, we studied the distribution of the different CD4^+ T cell lineages in PB and resting LN. Naïve, central memory and effector memory cells as well as the different CD4^+ T cell lineages consisting of Th1, Th2, Th17 and Tregs were equally represented in both compartments. On the other hand, cytotoxic CD4^+ T cells were strikingly absent in the lymph nodes. Follicular T helper cells (Tfh), which are CD4^+ T cells specialized in supporting expansion, activation and differentiation of B cells, are essential in the development of adequate antibody responses\(^6,7\). Expression of CXCR5 and downregulation of CCR7 allows the Tfh cells to migrate from the T cell zone to follicular areas and germinal centers, where they encounter antigen primed B cells\(^8,9\). In the human peripheral blood (PB) compartment CXCR5^-CD4^+ T cells, which have a CCR7^- central memory phenotype and which lack the expression of other Tfh cell markers, have been identified\(^10\). We here demonstrate that the resting LN compartment comprises significantly more CXCR5^-CD4^+ T cells than the PB compartment and that these CXCR5^-CD4^+ T cells express significantly more Tfh-markers than their PB counterparts. Furthermore, LN CXCR5^-CD4^+ T cells are better in providing B-cell help required for Ig production. When comparing our data to the data from previous studies on tonsil derived CXCR5^-CD4^+ T cells, we noticed that in
the LNs the percentage of CXCR5^+CD4^+ T cells is much lower and we can’t distinguish a clear population of CXCR5^+ICOS^+CD4^+ T cells, germinal center (GC) Tfh cells, in the LNs. These differences can be explained by the fact that the analysed tonsils represent highly inflamed lymphoid tissue, whereas the LNs in our present study represent lymphoid tissue during homeostatic conditions.

In conclusion, functionally and phenotypically CXCR5^+CD4^+ T cells derived from resting LN and PB differ substantially. Therefore, one should be cautious with the interpretation of studies which only analyse circulating CXCR5^+CD4^+ T cells.

Next, this thesis addresses T cell homeostasis and repopulation in lymphocytopenic conditions. In chapter four, we describe T cell repopulation following rabbit antithymocyte globulin (rATG) treatment. We longitudinally analysed peripheral blood samples of renal transplant recipients who received rATG as a treatment for acute humoral or steroid resistant cellular rejection. As previously described^{11-14} we found that rapidly repopulating CD8^+ T cells consist mainly of highly differentiated CD27^-effector-type cells. We here demonstrate that this rapid repopulation of effector-type CD8^+ T cells was only present in the CMV-seropositive patients and was most pronounced in the patients developing CMV-reactivation. From this we concluded that CMV is the driving force for the rapid effector-type CD8^+ T cell repopulation. Although more ‘immune space’ is available in the PB compartment of the CMV-seronegative as compared to the CMV-seropositive patients, naïve CD4^+ and CD8^+ T cells apparently do not have an advantage in repopulation. Thus, also during T cell repopulation in lymphocytopenic conditions, “immune space” does not play a significant role.

Additionally, we show that naïve CD4^+ and CD8^+ T cells were depleted almost completely from the circulation, and that the repopulation upon this depletion succeeded at a very slow pace. The naïve CD4^+ T cells that repopulated were largely CD31^+. CD31 expression has been shown to correlate with T cell receptor excision circle (TREC) content in naïve CD4^+ T cells^{15}. TREC’s are small DNA loops, which are a by-product of VDJ recombination of the T cell receptor genes^{16}. Thus, high expression of CD31 on repopulating naïve CD4^+ T cells might indicate that the thymus contributes to naïve T cell repopulation following rATG treatment. However, TREC content and thus likely also CD31-expression cannot be used as a direct measure for thymic output, because TREC content declines with peripheral division of naïve T cells and increases with thymic output but it can also increase with cell loss^{17}. Through long term in vivo deuterium labelling and label-decay studies, it is possible to make a reliable estimate of production and cell loss rates of naïve T cells^{18}. In addition, a combination of a deuterium labelling study and longitudinal analysis of TREC contents of naïve T cells has made it possible to make a very adequate estimation of direct thymic output. With this very elegant method, it has recently 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^{19}. A correct estimate of direct thymic output during lymphocytopenia remains unexplored. We are currently
executing a project in which we are studying T cell proliferation in renal transplant recipients following rATG treatment by in vivo labelling with deuterium\textsuperscript{20}. Because the effects of basic immunosuppressive drugs on the thymic output and peripheral homeostatic proliferation are unknown up to now, we are also studying T cell kinetics in renal transplant recipients who where not treated with rATG.

Solid organ transplantation is only possible when accompanied by controlled immunosuppressive therapy. Transplant recipients are usually treated with a combination of various immunosuppressive drugs and all these drugs have distinct effects on our immune system. Immunosuppressive drugs adequately inhibit rejection in organ transplantation, but their most common side-effect is immunodeficiency, leading to an increased risk of infection and cancer. The immunosuppressive potential of mTOR inhibitors has long been ascribed to interference in the cell cycle, leading to inhibition of T-cell proliferation\textsuperscript{21}. However, in several animal models, mTOR inhibition has been demonstrated to play an important role in increasing both the quantity and quality of memory T-cell responses\textsuperscript{22,23}.

In chapter five, we demonstrate that in renal transplant recipients, treated with prednisolone and the mTOR inhibitor everolimus (P/EVL), circulating CMV-specific and total CD27\textsuperscript{-} effector-type CD8\textsuperscript{+} T-cells as well as CD28\textsuperscript{-}CD27\textsuperscript{+} effector-type T-cells significantly increase in time after transplantation. In contrast, in patients who were treated with a combination of prednisolone and cyclosporine (P/CsA) or mycophenolate (P/MPS), this increase in effector-type CD8\textsuperscript{+} and CD4\textsuperscript{+} T cells was not observed. As mentioned earlier, CD27\textsuperscript{-} effector-type CD8\textsuperscript{+} T-cells and CD28\textsuperscript{-}CD27\textsuperscript{-} cytolytic CD4\textsuperscript{+} T-cells appear as a consequence of CMV infection\textsuperscript{1,5}. Thus, we concluded that patients treated with P/EVL mount a quantitatively larger T-cell response against CMV as compared to P/CsA and P/MPS treated patients. This offers at least in part an explanation for the low incidence of CMV viremia and infection in mTOR treated patients, as has previously been reported\textsuperscript{24,25}. Furthermore, our group has previously shown that immunosuppressive therapy, consisting of prednisolone and everolimus, preserves humoral immune responses and leaves secondary T-cell dependent immune responses after vaccination intact\textsuperscript{26}. The increase in pathogen-specific T-cell responses by mTOR inhibitors contrasts with the effectiveness of these drugs in clinical transplantation. A solid explanation to this paradox is as yet absent. Previous experiments have revealed that by increasing the strength of both the T-cell receptor (TCR) signal and co-stimulatory signals in vitro, anti-proliferative effects of mTOR inhibitors could be overcome\textsuperscript{27}. We hypothesize that the difference in the effect of mTOR inhibitors on allo- versus CMV-specific responses may be partly explained by a difference in TCR-signal strength.

Currently, mTOR inhibitors are not so popular in the immunosuppressive regimens for solid organ transplantation, because of their relatively high incidence of adverse effects. However, conversion from cyclosporine or mycophenolate to mTOR inhibitors may be worth considering in case of prolonged CMV disease.
Finally, in chapter six, we extensively studied CD161++IL-18Rα+ CD8+ T cells. On the one hand this subset has been described as mucosal associated invariant T (MAIT) cells. On the other hand these cells are proposed to be memory “stem” cells, which express the ABC-B1 multidrug transporter and c-KIT. Via the expression of the ABC-B1 transporter the CD161++IL-18Rα+ memory CD8+ T cells are able to efflux potentially harmful xenobiotics. Thus, in a for other memory T cells toxic environment the CD161++IL-18Rα+ memory CD8+ T cells have a survival benefit.

The concept of memory “stem” cells aroused our interest. In order to maintain life-long protection against pathogens, the concept of memory T cell with stem cell characteristics is very appealing. Memory “stem” cells can be of great importance in our defence against viruses, but may also be a significant player in allograft rejection. Ideally, a “real” memory “stem” cell will have to fulfils a couple of additional criteria. In chapter six we demonstrate that CD161++IL-18Rα+ memory CD8+ T cells are not capable of preserving their telomeres by high expression of telomerase, nor are they easily activated or able to detoxify aldehydes. From this we concluded that the CD161++IL-18Rα+ CD8+ T cells do not have any other characteristics marking them as stem cells. Moreover, they have several additional characteristics, such as KLRG1 expression and high BLIMP-1 expression, which coincide with highly differentiated effector-type CD8+ T cells. More recently a long-lived memory T cell subset, within the naïve-like T cell compartment, has been identified. The cells contained within this subset possess various stem cell-like properties and may represent true memory “stem” cells.

Although we have shown that CD161++IL-18Rα+ memory CD8+ T cells are not memory “stem” cells they remain an interesting subset of T cells. The ABC-B1 transporters can efflux a wide variety of frequently administered drugs. Immunosuppressive drugs often used in solid organ transplantation, namely glucocorticoids, cyclosporine, tacrolimus and sirolimus are among the drugs which are effluxed by the ABC-B1 transporter. Therefore, we hypothesised that CD161++IL-18Rα+ memory CD8+ T cells might play an important role in allograft rejection. We demonstrate in chapter 5 that a small percentage of CD161++IL-18Rα+ memory CD8+ T cells is indeed able to respond in mixed lymphocyte culture. Additionally, we retrospectively analysed the percentage of CD161++IL-18Rα+ memory CD8+ T cells in two groups of renal transplant recipients. One group developed an acute rejection shortly after transplantation and the other group had an uncomplicated post-transplantation course. Before transplantation and during acute rejection we did not find any differences in the percentage of circulating CD161++IL-18Rα+ memory CD8+ T cells between the two groups (unpublished data). However, this does not mean that these cells are not present in the kidney during acute rejection. Therefore, it would be interesting, in future experiments, to study the presence of CD161++IL-18Rα+ memory CD8+ T cells in renal biopsies of patients with and without acute rejection.
REFERENCE LIST


34. Choudhuri S, Klaassen CD. Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. Int.J.Toxicol. 2006;25:231-259.