Immune responsiveness in immunosuppressed patients

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SUMMARY

The impact of the currently applied immunosuppressive medication regimens on CD4<sup>pos</sup> T cell functions in vivo is largely unknown. We therefore studied quantitative and qualitative aspects of CD4<sup>pos</sup> T cell immunity in renal transplant recipients. The first part of this thesis focuses on cellular immune reactivity against CMV in patients treated with the immunosuppressive drugs prednisolone and cyclosporin. Next, the influence of immunosuppressive drug therapy on immune responsiveness in vivo with an emphasis on a possible effect on Th1- versus Th2 balance is described. Finally, the possible application of recently developed new immunological assays to quantify antigen-specific CD8<sup>pos</sup> T cells was studied on alloreactivity.

T-lymphocytes that co-express CD4 and CD8 antigens may be found in small percentages in the peripheral blood of healthy individuals, and have a CD4<sup>bright</sup>CD8<sup>dull</sup> phenotype. CD4<sup>dull</sup>CD8<sup>bright</sup> T-lymphocytes have been found only in temporal association with some viral infections. In chapter 1, we describe a renal transplant recipient with cytomegalovirus infection in whom a small but clearly distinguishable subpopulation of CD4<sup>dull</sup>CD8<sup>bright</sup> double positive T lymphocytes was detected, that exhibited phenotypic characteristics of cytotoxic T-lymphocytes and were granzyme B positive. Furthermore, no naive cells appeared to be present within this subpopulation.

In chapter 2, we describe the kinetics and characteristics of cytomegalovirus (CMV) specific CD4<sup>pos</sup> T cells in the course of primary CMV infection in kidney transplant recipients. Our data revealed that, as the first sign of specific immunity, circulating CMV specific CD4<sup>pos</sup> T cells become detectable with a median of 7 days after first appearance of CMV-DNA in peripheral blood. These primary CMV specific T cells produce the T helper 1 type (Th1) cytokines IFNγ and TNFα, but not the T helper 2 type (Th2) cytokine IL4. In primary CMV infection the vast majority of these circulating virus-specific T cells have features of recently activated naive T cells, as they co-express CD45RA and CD45R0 and appear to be in cell cycle. In contrast, in persons who have recovered from CMV infection earlier in life, virus-specific T cells are non-cycling and are memory, i.e. CD45RA<sup>neg</sup>CD45R0<sup>pos</sup> T cells. After the initial rise, circulating virus-specific CD4<sup>pos</sup> T cells rapidly decline. During this phase, a strong rise in IgM and IgG anti-CMV antibody titers as well as a concomitant reduction of CMV-DNA occurs.

In chapter 3, we studied CD4<sup>pos</sup> T cell dynamics in peripheral blood in patients who were CMV-seronegative before transplantation and received an organ from a CMV-seropositive donor. We found that around the time of first detection of CMV–DNA, lymphocyte counts declined which was mainly due to a dip in the peripheral blood CD4<sup>pos</sup> lymphocyte counts To
our knowledge, the decline in CD4\textsuperscript{pos} lymphocyte counts in the absence of a serious decline of CD8\textsuperscript{pos} lymphocyte numbers just before detectable primary CMV infection was not previously documented. We speculate that this decrease of the CD4\textsuperscript{pos} lymphocyte number from the peripheral blood early during primary CMV infection might be due to redistribution of these cells to e.g. secondary lymphoid organs.

In chapter 4, we addressed the issue of CD8 dynamics in primary CMV infection by frequent sampling of peripheral blood and detailed subset analysis in patients, highly at risk of developing primary CMV infection. CD8 lymphocytosis rapidly followed the CMV-DNAemia. We found that NK-cells significantly add to the CD8-lymphocytosis during the first 50 days after the infection, but not thereafter. CD3\textsuperscript{neg} NK cell- and CD8bright T cell dynamics then segregate into an expanding CD8\textsuperscript{pos} T cell compartment and a shrinking NK cell compartment. However, it remains to be established how these lymphocyte dynamics contribute to reversal of the CMV infection into latency.

In chapter 5, the interaction between CD8\textsuperscript{pos} T cells and cytomegalovirus was studied. To this end, frequencies and phenotypes of CMV-specific CD8\textsuperscript{pos} T cells were determined in healthy individuals and compared to those in renal transplant recipients. In healthy donors, the function of circulating virus-specific CD8\textsuperscript{pos} T cells, as measured by peptide-induced IFN\gamma production, but not the number of virus-specific T cells enumerated by binding of specific tetrameric peptide/HLA-complexes, correlated with the number of CMV-specific IFN\gamma secreting CD4\textsuperscript{pos} helper T cells. Circulating CMV-specific CD8\textsuperscript{pos} T cells did not express CCR7 and may therefore not be able to recirculate through peripheral lymphnodes. Based on co-expression of CD27 and CD45R0 most CMV-specific T cells in healthy donors appeared to be memory-type cells. Remarkably, frequencies of CMV-specific CD8\textsuperscript{pos} T cells were significantly higher in immunosuppressed individuals than in healthy donors. In these patients CMV-specific cells predominantly had an effector phenotype, i.e. CD45R0\textsuperscript{pos}CD27\textsuperscript{neg}CCR7\textsuperscript{neg} or CD45RA\textsuperscript{pos}CD27\textsuperscript{neg}CCR7\textsuperscript{neg} and contained both granzyme B and perforin. Our data show that in response to immunosuppressive medication quantitative and qualitative changes occur in the CD8\textsuperscript{pos} T-cell compartment. These adaptations may be instrumental to maintain CMV latency.

In chapter 6, the effect of IFN\gamma on human antigen specific CD4\textsuperscript{pos} T cell reactivity after surgically induced immunosuppression was studied in a comparative trial of recombinant human (rh) IFN\gamma versus placebo in patients after abdominal surgery. Antigen-specific helper T cell immune reactivity was assessed by antigen induced cytokine production, intracellular cytokine staining and flow cytometry. A single dose of rhIFN\gamma was found to rescue downmodulation of antigen specific CD4\textsuperscript{pos} T cell reactivity, concomitant with an upregulation of TCR-ligands on antigen presenting cells.

In chapter 7 we defined the influence of distinct immunosuppressive treatment protocols on
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Primary and secondary cellular and humoral immune responses in groups of renal transplant recipients: patients treated with prednisolone and cyclosporin A (P/CsA); with IgA CD3 monoclonal antibody as a rejection treatment superimposed on prednisolone and cyclosporin A, (IgA CD3 mAb+P/CsA); and with prednisolone, cyclosporin A and mycophenolate mofetil (P/CsA/MMF). Primary in vitro proliferative responses to the protein antigen keyhole limpet haemocyanin (KLH) were not significantly disturbed in P/CsA treated patients, nor in IgA CD3 mAb+P/CsA and P/CsA/MMF treated patients. In vitro proliferative responses to the recall antigen tetanus toxoid (TT) were similarly unaffected. Antigen specific antibody responses to immunization with KLH and TT were not affected by treatment with P/CsA, nor by IgA CD3 mAb+P/CsA, but severely disturbed in patients treated with P/CsA/MMF. All patients displayed a profound inhibition of the delayed type hypersensitivity skin reactivity to KLH and recall antigens. Nevertheless, in most patients with P/CsA treatment, T cell infiltrates were observed in skin biopsies from the site of KLH challenge, while expression of intercellular cell adhesion molecule-1 (ICAM-1) expression in challenged skin was significantly decreased in these patients. The balance between T helper 1 and T helper 2 cells was unaffected by immunosuppressive treatments during 1 year of follow up. We conclude that immunosuppressive drug treatment with P/CsA inhibits delayed type hypersensitivity skin reactions to both primary and frequently encountered antigens. Histological studies indicated an effect on ICAM-1 expression, leaving the influx of CD3pos T cells unaffected.

Administration of a 10 day course of IgA CD3 mAb does not add profound immunosuppressive effects on the measured parameters. In contrast, addition of treatment with MMF profoundly decreases both primary and secondary humoral immune responsiveness in vivo. Finally, no effect of the currently studied immunosuppressive drugs on Th1/Th2 balance in vivo was measured.

In chapter 8, the influence of cyclosporin A or methotrexate on T helper cell cytokine secretion patterns or T cell migration patterns was investigated. Flow cytometric determination of interferon-gamma (IFNy) and interleukin 4 (IL4) producing T helper cell frequencies, as well as of cutaneous lymphocyte associated antigen (CLA) expressing T cell frequencies was performed in patients suffering from severe psoriasis, before, during and after a scheduled immunosuppressive regimen with either cyclosporin A or methotrexate. Both, cyclosporin A and methotrexate treatment reduced the psoriasis area severity index score after 12 weeks of treatment. Cyclosporin A treatment reduced the frequencies of IL4 producing CD4pos T cells, without significantly affecting the T helper 1 to T helper 2 (Th1/Th2) balance but in conjunction with decreasing the number of peripheral blood eosinophil counts. In methotrexate treated patients, the Th1/Th2 balance was unaffected. Cessation of both therapies resulted in increased numbers of IFNy- as well as IL4 producing CD4pos T cells as compared to before initiation of oral therapy. Methotrexate-, but not cyclosporin A treatment
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Reduced the frequencies of circulating skin homing $\text{CLA}^{\text{pos}}$ T cells. This effect was reversed by four weeks after withdrawal of methotrexate therapy. We conclude that 1) neither cyclosporin A nor methotrexate affect the balance between Th1 and Th2 cells, 2) exaggerated cytokine production by T helper cells after cessation of oral cyclosporin A or methotrexate drug treatment may contribute to reappearance of psoriatic skin lesions and 3) decrease of circulating skin-homing T cells may be responsible for part of the therapeutic effect of methotrexate in severe psoriasis.

Finally, in chapter 9, expansion and differentiation of allo-antigen reactive $\text{CD8}^{\text{pos}}$ T cells in mixed lymphocyte cultures was followed by measuring diminishment of carboxyfluorescein diacetate succinimidyl ester (CFSE) fluorescence of responder cells. Proliferation of $\text{CD8}^{\text{pos}}$ T cells became detectable at day 4 of culture and 2 days later over 60% of the $\text{CD8}^{\text{pos}}$ T cells in culture were dividing alloreactive lymphocytes. In parallel with expansion, $\text{CD8}^{\text{pos}}$ T-cell differentiation was initiated as evidenced by an increase in the number of $\text{CD45RA}^{\text{neg}}$ and $\text{CD27}^{\text{neg}}$ T cells and acquisition of the ability to produce interferon-γ after re-stimulation with the specific allo-antigen. Finally, although short term stimulation and measurement of intracellular cytokine production allows visualization of alloreactive $\text{CD8}^{\text{pos}}$ T cells expanded in vitro, this procedure did not detect circulating alloreactive $\text{CD8}^{\text{pos}}$ T cells activated in vivo in recipients of allogeneic kidney grafts.