

Mostafa Gaafar Osman Aly

Dr.med.

Über die Problematik (Effects of Vitamin D on T Helper 17 and IFN γ -positive Regulatory T cells in Renal Allograft Recipients)

Fach/Einrichtung: Immunologie

Doktorvater: Prof Dr.med. Volker Daniel

Currently, the immune-modulatory role of vitamin D is being extensively investigated. Growing evidence supports that vitamin D has the ability to bind to the promoter regions of different genes and, hence, the ability to modify the transcription of more than 900 genes. The role of vitamin D on Tregs and Th17 cells was infrequently tested in ESRD patients and renal transplant recipients. In addition, studies on the nature of association between Th17 and Tregs on one side and between Tregs or Th17 and each of the other lymphocyte subsets in ESRD patients and renal allograft recipients are limited. The intermediate Treg/Th17 cells were recently described in literature with anticipated immunosuppressive potential. These cells are not widely described in ESRD patients as well as renal allograft recipients.

Blood samples were obtained from one hundred and thirty-four renal transplant recipients, 46 ESRD patients, and from 23 healthy controls. In parallel, blood samples were obtained from an independent group of 11 patients before and 3 months post-transplant. The mean duration post-transplant was 3.8 ± 4.2 in the long-term transplant group, whereas the mean duration of dialysis in ESRD group was 3 ± 3.4 . Plasma 25 (OH) D₃ and 1,25 (OH)₂ D₃ in addition to IL-6, IL-17, IL-23, and TGF- β 1 were assessed. Lymphocytes, Treg subsets, intermediate Treg/Th17, and Th17 cells were determined in the blood using 4- and 8-color fluorescence flow cytometry.

First, prevalence of vitamin D sufficiency, insufficiency, and deficiency was assessed in ESRD patients and renal transplant recipients. Vitamin D insufficiency was prevalent in both ESRD patients and renal allograft recipients (37 % and 44% respectively). 25 percent of ESRD patients had normal vitamin D compared to approximately 30 percent of renal transplant recipients. Only 10 percent of ESRD patients showed vitamin D intoxication. In contrast, vitamin D intoxication was not detected in renal transplant population. In addition, we showed that plasma 25 (OH) D₃ drops significantly in the first 3 months post-transplant. In addition, we estimated the levels of 1, 25 (OH)₂ D₃ in 8 of the 11 patients at the same time points. In contrast to 25 (OH) D₃, the active 1, 25 (OH)₂ D₃ was found to significantly increase post-transplant. No significant correlation between 25 (OH) D₃ and 1, 25 (OH)₂ D₃ was found either pre- or 3 months post-renal transplantation in this cohort of patients.

Second, we tested the association between inactive or active vitamin D plasma levels and the absolute count of each lymphocyte subset. We found that vitamin D was poorly associated with

the absolute counts of Tregs, Th17, intermediate Treg/Th17, and NK cells. In contrast, we showed that 25 (OH) D was negatively associated with B cell counts in both ESRD patients and renal allograft recipients, and negatively associated with CD4⁺ T cells in renal allograft recipients only. In contrast to 25 (OH) D₃, 1, 25 (OH)₂ D₃ was positively associated with CD3⁺ and CD8⁺ T cell counts in ESRD patients and renal allograft recipients, respectively.

Third, we compared lymphocyte subsets in ESRD patients and short- and long-term renal transplant recipients. We found that the absolute count of Tregs was higher in ESRD patients compared to renal allograft recipients. No significant difference in Th17 or Treg-like Th17 cell (CD4⁺ Foxp3⁺ IL-17⁺) counts between ESRD patients and long-term allograft recipients was shown. However, a decrease in the absolute count of Th17 cells with a relative increase in T-bet⁺ Th17 cells 3 months post-transplant was observed. Furthermore, we showed that the Treg/Th17 ratio in ESRD patients was higher than in renal transplant recipients. CD19⁺ B cells were significantly lower in ESRD patients as well as renal transplant recipients compared to healthy controls. A significant decrease in CD16⁺ CD56⁺ NK cells was found in early as well as long-term transplant recipients compared to ESRD patients. These differences reveal the effects of immunosuppressive drugs in transplant recipients as well as the effects of uremic milieu in ESRD patients.

Fourth, we tested the association between Tregs or Th17 cells and CD8⁺ T, NK, and B cells. In addition we tested the associations between Treg and Th17 subsets. A positive correlation was shown between Tregs and CD8⁺ T, CD16⁺ CD56⁺ NK, and CD19⁺ B cells in both ESRD patients and renal allograft recipients although the correlation was statistically insignificant with CD8⁺ T cells in ESRD patients. Likewise, Th17 cells were positively correlated with CD8⁺ T and CD16⁺ CD56⁺ NK cells, however not with CD19⁺ B cells. As expected, the same correlations were insignificant 3 months post transplantation, a finding that might be attributed to intense immunosuppression post-transplant. Tregs and Th17 cells were positively correlated with a trend to significance in renal transplant recipients but not in ESRD patients. A positive correlation between Helios⁺ Tregs and Th17 cells in renal transplant recipients and ESRD patients was also shown, with a trend to significance in the latter group. The highest significant correlation was shown between Th17 cells and Helios⁺ CD38⁺ Tregs. These results highlight the non-antagonistic relationship between real or apparently effector lymphocyte subsets and Tregs especially the active tTregs and anticipate either a regulatory function of NK, B, Th17, and CD8⁺ T cells in stable renal function, or that Tregs are fueled when these effector cells are activated in order to suppress the progression of inflammatory response.

Fifth, we showed that the majority of Th17 cells in both ESRD patients as well as renal allograft recipients expressed Foxp3 and Helios. Since this cell population was only weakly correlated with Helios⁺ Tregs (tTregs), it is intuitive that these cells are originating to a small extent from tTregs that express IL-17 in addition to Th17 cells that coexpress Helios and Foxp3. The trend to positive correlation of Foxp3⁺ Th17 cells with eGFR suggests an immune-regulatory rather than an effector function of these cells.

Sixth, we tested the correlation between Th1-like Th17 that express T-bet or IFN γ and Tregs. We found that there was a significant positive correlation between T-bet⁺ Th17 cells and Helios⁺ as well as total Tregs only in ESRD patients. In addition, we found a positive correlation between T-bet or IFN γ and Foxp3 in Th17 cells, a finding that suggest that the intermediate Treg/Th17 cells are also Th1-like, and are likely to be immunosuppressive.

Seventh, we showed that plasma levels of IL-17, IL-23, and TGF- β 1, but not IL-6, were likely to change after renal transplantation. IL-17 and IL-23 were found to increase whereas TGF- β 1 was found to decrease post-transplant. The cytokine levels yielded infrequent significant correlations with Tregs and Th17 cells. This might be attributed to the fact that each of these cytokines can be secreted by different types of cells.

Finally, we tested the predictors of long-term graft function. We found that type of graft, cold ischemia time, proteinuria, number of HLA-mismatches, log-transformed CD16⁺ CD56⁺ NK cells were significant predictors of change in eGFR at 3.8 years post-transplant in the univariate analysis. Only log-transformed CD16⁺ CD56⁺ NK cell count was a significant positive predictor of outcome in the multivariate regression analysis. In contrast, active and inactive vitamin D levels, total Treg and Th17 cell counts were poor predictors of long-term graft function.