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Role of NK cell and Treg subsets in renal transplantation

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In our previous study, increased NK cells in late post-transplant recipients did not damage the graft. There was further evidence that NK cell reactivity might affect graft outcome in transplant recipients and pregnancy in women. Thus, this study focuses on NK cell subsets that might contribute to good long-term allograft acceptance and might have immunoregulatory potential.

Blood samples were obtained from 39 healthy controls, 38 renal transplant recipients, 32 end-stage renal disease patients, and 32 recurrent miscarriage patients. Plasma from those samples was used to investigate cytokines levels. Lymphocytes, NK subsets and Tregs were determined in the blood using 4- and 8-color fluorescence flow cytometry. To analysis NK cells function in-vitro, isolated PBMCs were co-cultured with the K562 tumor cell line. Surface and intracellular markers as CD107a, Perforin and GranzymeB and INFγ of NK cells were investigated before and after stimulation.

First, we compared the absolute counts of lymphocyte subsets in the peripheral blood of HC and different groups of patients. Late post-transplant patients showed significant decreases of CD56dim+CD16+ but not of CD56bri+NK subsets.

Second, 13 tubes analyzed using 8 multicolor fluorescence flow cytometry were used to detect different NK subsets in HC and different groups of patients. More than 150 NK subsets were analyzed and proportions and absolute counts were calculated and compared. Mann-Whitney U test was performed to compare parameters between different groups. We found that cytotoxic NK subsets such as Perforin+GranzymeB+ CD56dim+CD16+ NK cells and CD8+ NK cells were lower in late post-transplant recipients and strikingly higher in RM patients. In contrast, IL10+CD56bri+ and CD25+CD56bri+ NK subsets were higher in late post-Tx than RM patients.

Third, in order to evaluate the function of NK cells, isolated PBMCs from HC, ESRD, transplant and RM patients were co-cultured and stimulated with K562 cells using an E:T ratio of 5:1. Changes of CD107a was measured after 2 hours stimulation while other markers like Perforin, GranzymeB, IL-10, INFγ, IL-4, TGF-β1, IL-17 and FoxP3 were analyzed after 6 hours stimulation. Perforin expression was obviously decreased while CD107a and INFγ were upregulated during stimulation. Notably, late post-transplant recipients showed less release of perforin than healthy individuals and less upregulation of INFγ than RM patients, indicating impaired NK cytotoxic function in patients late post-transplant.

Fourth, we investigated associations between different NK subsets and serum creatinine as well as GFR value. Impaired graft function appears to be associated with higher cytotoxic NK cell levels (Perforin+GranzymeB+CD56dim+CD16+ NK cells and CD8+ NK cells) in the blood. In-vitro, low cytotoxic function of NK cells (release of Perforin during stimulation) was associated with high GFR in late post-transplant recipients. In contrast, high IL10+CD56bri+ NK and CD25+CD56bri+ NK subsets in the peripheral blood were strongly associated with low serum creatinine and high glomerular filtration in late post-transplant recipients, indicating the immunoregulatory role of those subsets in renal transplantation.

Fifth, we tested the correlation between NK subsets and immunosuppressive drugs doses as well as blood levels. Our data showed a potential induction of immunoregulatory IL10+CD56bri+NK cells by the administration of cyclosporine and an inhibition of cytotoxic NK cells by treatment with higher doses of steroids.

Sixth, we analyzed the levels of Tregs and the associations between Tregs and cytotoxic as well as potential regulatory NK subsets among different groups of patients and HC. Results showed that Treg cells were positively associated with IL10+CD56bri+NK subsets while negatively associated with cytotoxic NK cells in late post-transplant recipients. Interestingly, INFγ+ Tregs showed a negative association with IL10+CD56bri+NK subsets in RM patients.

Seventh, we measured the plasma levels of 26 different cytokines. Higher levels of IL-10 and TGF-β were found in late post-transplant recipients compared to HC and RM patients, and higher levels of IL-10 were negatively associated with NK cell counts in patients late post-transplant. We speculate that high levels of IL-10 might inhibit the cytotoxic NK cells and induce regulatory NK subsets which contribute to good graft acceptance in late post-transplant recipients.