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320.4

TEMRA CD8 T Cells from Human Kidney Transplant Recipients Exhibit Potent Anti-Donor Reactivity and Induce GVHD in Humanized Mouse Model

Lola Jacquemont[1,2], Gaelle Tilly[1,2], Virginie Huchet[1,2], Pierrick Guerif[1,2], Magali Giral[1,2], Sophie Brouard[1,2], Fabienne Haspot[1,2], Nicolas Degauque[1,2]
[1]Centre de Recherche en Transplantation et Immunologie UMR1064, INSERM, Nantes, France; [2]Institut de Transplantation Urologie Néphrologie (ITUN), CHU Nantes, Nantes, France.

Introduction: We have shown that an increase of TEMRA CD8 ($CD45RA^{+}CCR7^{-}$) evidenced 1 year post-transplantation is associated with a higher risk of kidney graft failure. The functionality of TEMRA CD8 and especially their reactivity upon donor-specific stimulation have never been investigated. In this study, we characterize before and one year after transplantation the functional response of TEMRA CD8 from Kidney Transplantation (KT) recipients after donor-specific stimulation and we setup a model of humanized NSG mice to investigate in vivo their pro-inflammatory response.
Methods: PBMC from 24 living-donor kidney-transplant recipients have been prospectively collected before and 12 month post-transplantation. Extensive phenotype of CD8 T cell subsets have been performed using multi-parameter flow cytometry. Naïve, TEMRA and EM CD8 from KT recipients have been cultured with irradiated donor-specific CD3-depleted PBMC. Expression of activation marker (CD25, CD69), cytotoxic marker (CD107a) and secretion of pro-inflammatory cytokines (Luminex Assay) have been analyzed after 24h and 48h of stimulation. Proliferation (CPD dilution) has been investigated after 5 days of culture. One to 5.10^{6} TEMRA CD8 purified from KT recipients were co-injected *i.v.* with 10^{7} T- and NK-depleted autologous PBMC in NSG HLA-A*0201 transgeneic mice. Xenogeneic GVHD was monitored by daily evaluation of the body weight and CD8 T cell infiltration was investigated using fluorescent microscopy in different organs.
Results: Kidney transplantation results in an increase of TEMRA CD8 (25 vs 39%), characterized by a high expression of GZMb and effector-associated transcription factor T-bet, and a concomitant decrease of NAÏVE CD8 whereas frequency of EM CD8 remains unchanged. Donor-specific stimulation results in the early expression of $CD25^{+}CD69^{+}CD107a^{+}$ by TEMRA CD8 whereas NAÏVE CD8 failed to upregulate CD25 or CD107a. Expression of cytotoxic molecule CD107a by TEMRA was even enhanced after kidney transplantation. Donor specific stimulation results in the proliferation of TEMRA CD8. Finally, the infusion of TEMRA CD8 from KT recipients induces GVHD in 7 to 10 days with an infiltration of TEMRA in colon.
Conclusion: We demonstrate that TEMRA CD8 exhibit potent pathogenic function both *in vitro* when challenged with donor cells and *in vivo* resulting in an acute GVHD in immunodeficient mice. Our study highlights the need to design TEMRA specific therapy to improve kidney graft outcome.

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320.5

Characterization of Acute Cellular Rejection in the Different Layers of Rat Transplanted Intestines

Pablo Stringa[1,2,3], David Romanin[1], Natalia Lausada[3], Rodrigo Papa Gobi[1], Leandro Vecchio[3], Gabriel Gondolesi[2], Martin Rumbo[1]
[1]Instituto de Estudios Inmunológicos y Fisiopatológicos IIFP-CONICET, Universidad Nacional de La Plata, La Plata, Argentina; [2]Instituto de Medicina Traslacional, Trasplante y Bioingeniería IMMeTyB-CONICET, Universidad Favaloro, Buenos Aires, Argentina; [3]Laboratorio de Trasplante de Organos y Tejidos - Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina.

Introduction: Intestinal transplantation (IT) faces many challenges, among them, the necessity to understand and detect rejection processes. Rodent models of IT are used to provide evidence for intervention strategies as well as improve knowledge of IT biology. Our aim was to determine the kinetics of small bowel rejection with emphasis in the characterization of acute cellular rejection (ACR) in the different layers of the graft since most of the information coming from the clinics is on mucosal layer, the only one that is accessible by endoscopic biopsies.
Methods: Allogeneic (ALLO) heterotopic IT in rats was performed following standard procedure. ACR was diagnosed by H-E staining analysis. Also, real-time PCR from microdissected samples (epithelial, muscular and serosa layer) and whole graft to determine gene expression was performed at 5 and 10-12 postoperative days (POD). An Isogenic IT group was performed as a control.
Results: ACR was observed since 5 POD in ALLO group with mild rejection as the most characteristic grade. Severe ACR was diagnosed in all ALLO samples at 10-12 POD. Descriptive analysis showed a well-preserved architecture at 5 POD; confluent and loose apoptotic cells in the intestinal epithelium and perivascular infiltrate in all layers were evident. At 10-12 POD, significant cellular infiltrate, epithelial damage, ulcers and an increase of apoptotic cells were observed. Muscular and serosa layers showed inflammatory cell infiltrate and intercellular edema.

At 5 POD, some markers were consistently increased in ALLO groups such as CXCL10 that showed a 120 ± 80-fold increase compared with nontransplanted tissue. Furthermore, IFNg and IDO showed a trend to be increased at these time points. Remarkably, other inflammation-related genes, such as CXCL1 and IL6 showed consistent increase in ALLO groups at 10-12 POD, when severe ACR was established. When a principal component analysis of the overall gene expression markers was performed, ALLO group at 10-12 POD clearly separated from the other conditions. The analysis of gene expression at different layers of the graft was coincident with whole tissue biopsies: higher levels of IL6 and CXCL1 were observed in ALLO groups at 10-12 POD with important activation of this response in serosa and muscular layer. Interestingly, IL22 expression was only measurable in epithelial layer in ALLO groups at 10-12 POD, indicating ACR-induced expression in this compartment. Serosa layer showed some of the highest relative increases in pro-inflammatory gene expression also in ALLO groups at 10-12 POD.
Conclusion: Although in the clinic mucosal rejection has been extensively characterized, in our animal model we could document that all graft layers are affected by ACR since the initial stages. Serosa and muscular layer show high expression of proinflammatory markers, with differential expression of IL22 in the epithelial compartment. This information could be useful in the search for early biomarkers of ACR.

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