Flavius Ionuţ Şandra-Petrescu
Dr. med.

Generation of suppressive blood cells for treatment of allograft rejection in organ transplantation: studies in a rat heart transplant model

Promotionsfach: Immunologie
Doktorvater: Prof. Dr. med. Peter Terness

Solid organ transplantation is the most effective therapy for end-stage organ failure. The surgical technique of transplantation has been successfully optimized. On the other hand, the introduction of immunosuppressive therapies led to a substantial improvement of short-term graft outcomes, whereas the long-term survival remained a challenge for the medical community. Immunosuppressants succeeded in reducing the rate of acute rejection but the life-long non-specific suppression of immune responses inhibited the defence against foreign antigens.

The ultimate goal in transplantation is the induction of operational tolerance. Cellular therapies have become a promising candidate for specific immunosuppression. The discovery of new cell markers, the isolation and even expansion of defined cell subpopulations allowed researchers to develop animal models of donor-specific tolerance induction and to initiate clinical trials. Among cell types, Tregs, DCs, mesenchymal stem cells and donor-derived blood cells have most frequently been used in order to modulate the host immune response. Donor-derived blood or blood cells merit a special attention since their isolation and use in the clinic is technically easy.

Previous studies of our laboratory showed that the chemotherapeutic drug mitomycin C (MMC) converts immunomodulatory into suppressive cells. Based on this finding we tried to develop a clinically relevant model for donor-specific immunosuppression in organ transplantation.

We treated donor derived PBMCs in vitro with MMC and injected them into recipients seven days before transplantation in a rat heart allograft model. This pretreatment strongly prolonged the allograft survival in a cell dose-dependent manner without any additional immunosuppressive treatment. Most importantly, tolerance could be induced when 10^8 MMC-PBMCs were injected. At the first glance, the blood subpopulation responsible for the suppressive effect was that of monocytes and/or lymphocytes. Depleting the monocyte

population from MMC-treated PBMCs abrogated the graft prolonging action. Surprisingly, isolation of donor-derived monocytes using magnetic microbeads showed that a monocyte-enriched population only weakly prolongs the allograft survival as compared to the 10^8 MMC-PBMCs. One can conclude that the combination monocytes–lymphocytes is mainly responsible for suppression, whereas other synergistically acting mechanisms might also be involved. The induction of tolerance was donor specific since third party hearts were rejected.

In order to determine local factors that protect the graft against rejection cardiac transplants were microscopically analyzed. Hearts of tolerant animals displayed infiltrations with mononuclear cells beginning early after HTx. When looking closer to the infiltrating cells an increased number of Tregs in the tolerant hearts could be observed. They reached a peak 30 days after transplantation declining thereafter. This suggests the presence of a regulatory protective mechanism in the graft. Further analysis of tolerated grafts surprisingly showed complement activation with a peak seven days after HTx similar to rejected grafts, suggesting an acute antibody-mediated attack. However, the humoral immune response against the graft was thereafter significantly reduced and the transplants were not rejected. In the periphery we found an increased number of $CD4^+CD25^+Foxp3^+$ T cells in the spleen and blood of tolerant in comparison to rejecting animals. Their regulatory capacity could be indirectly proven, in both SPCs and PBMCs, by successfully inducing tolerance when adoptively transferring these cells into syngeneic recipients. Moreover, the induced tolerance was so strong that the adoptively transferred animals showed a suppressed immune response against an additional allogeneic skin transplant from the same donor performed on day 100 after cell transfer.

Despite the donor-specific tolerance achieved *in vivo*, *in vitro* recipient T-lymphocytes preserved their capacity to respond to donor antigens, as expressed on monocyte-derived DCs. This phenomenon is called “split tolerance” and excludes the possibility of depletion of donor-reactive lymphocytes, suggesting the implication of regulatory mechanisms mediated by Tregs.

The cytokine milieu found in the serum of tolerant animals suggests a change of the Th1/Th2 balance toward Th2 cytokine early after HTx, possibly protecting the graft against rejection. IL-10 level increased on day 7 and 30 after HTx in comparison to naïve animals but remained lower than in rejecting ones; on day 30 its level was similar to the rejecting group and afterwards decreases on day 70 under the level of naïve animals. Th1 cytokines such as IFN-γ and IL-2 displayed a lower level on day 7 after Tx in comparison to rejecting animals.

In the long run, grafts of tolerant animals revealed a narrowing of the vascular lumen, a modification which is characteristic for chronic rejection. However, compared to the syngeneic group the difference was not significant. This shows that heart transplantation *per*

se in the absence of an immune reaction already leads to a certain degree of chronic vasculopathy.

Altogether, our results suggest that a single infusion of 10^8 MMC-PBMCs is able to induce donor-specific tolerance in a rat heart allotransplantation model without the concomitant use of immunosuppressive drugs. This model is easy to perform, has no side effects and therefore owns clinical relevance.