Mircea Iancu
Dr. Med.

Use of tryptophan metabolites for the control of heart allograft rejection in rats

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

The alloimmune response, initiated following organ transplantation, leads to graft rejection and loss of the transplanted organ. To prevent graft rejection, a large palette of immunosuppressive drugs has been developed. These drugs lead to a "general shutdown" of the immune reaction, leaving transplant recipients vulnerable to ubiquitary pathogens and cancer development. Therefore, a specific inhibition of the immune response which reacts against the graft, while preserving the patient's protective immunity to other antigens is the ultimate goal in organ transplantation. This state is referred to, as immunological tolerance.
Pregnancy is a natural model of immunological tolerance. The semi-allogeneic fetus is accepted by the immune system of the mother. Munn and Mellor's reports showing that a modified tryptophan (trp) catabolism induced by an enzyme called IDO is a possible mechanism mediating the immune acceptance of the fetus, were followed by endeavors to transfer these findings to organ transplantation.
In the last 4 decades, recipient preconditioning with donor-specific or -unspecific blood transfusions prior to transplantation (Tx) has been proven to prolong graft survival. Among others, based on the results of experimental studies, generation of leukocyte subpopulations with tolerogenic capacities have been proposed as a strategy for induction of tolerance in clinical transplantation.

In the present work we tried to combine the suppressive effect of tryptophan metabolites with that of donor-derived blood transfusion. To this end, recipients were transfused prior to Tx with blood leukocytes treated with natural (kynurenine) or synthetic (Tranilast) tryptophan metabolites. Allogeneic heterotopic heart Tx in rats served as experimental model.

Initial findings showed that rat peripheral blood mononuclear cells (PBMCs) treated *in vitro* with kynurenine (kyn) or Tranilast suppress allogeneic T-cell proliferation in a popliteal lymph node assay. Moreover, recipient preconditioning with Tranilast- and especially kyn-treated donor PBMCs prolonged the survival of a subsequently transplanted heart. Recipient

preconditioning 7 days before Tx showed a stronger suppressive effect in comparison to that on the day of transplantation. IFN-γ stimulation of kyn-treated PBMCs which enhances trp and kyn degradation, did not further enhance their graft protective effect.

As an alternative to *in vitro* treatment of PBMCs with kyn, donors were treated with kynurenine. In this case too, the donor PBMCs enhanced graft survival.

Recipient preconditioning with kyn-treated whole blood induced the longest prolongation of graft survival.

The next question was which component of kyn-treated donor blood exerted the suppressive effect. In our experimental set-up neither monocytes nor granulocytes or plasma alone were sufficient to significantly prolong graft survival.

The suppressive effect of pre-Tx blood transfusion was donor-specific since no significant effect could be noted on third party heart allograft survival.

Following previous reports regarding induction of apoptosis by tryptophan metabolites, we observed that upon treatment with Tranilast, increased apoptosis of transfused cells correlates with longer graft survival. This observation supports previous findings describing prolongation of solid allograft survival by pre-Tx transfusion of apoptotic leukocytes.

In vitro treatment of leukocytes with trp metabolites induced downregulation of the expression of costimulatory molecules (CD80 and CD86). Stronger downregulation in whole blood in comparison with isolated PBMCs, as well as the more potent effect of kyn in comparison to Tranilast correlated with longer graft survival. These results are in line with previous findings which state that insufficient costimulation by antigen presenting cells inactivate the allospecific T-cell response and suppress graft rejection.

Further investigations regarding the mechanism of suppression showed that seven days post-Tx, lower cytotoxic Ab formation, hyporesponsive spleen T-cells as well as increased $CD4^+CD25^+FoxP3^+$ T-cells can be detected in recipients preconditioned with kyn-treated blood. It remains to be seen to which extent these components of the immune system contribute to prolonged graft survival.

In conclusion, this study shows that treatment of donor blood leukocytes *in vitro* or *in vivo* with kynurenine as well as, to a lesser extent, with Tranilast generates suppressive cells which are able to induce donor-specific prolongation of rat heart allograft survival. The downregulation of costimulatory molecules upon *in vitro* treatment of blood with kynurenine as well as the reduced cytotoxic Ab titers, hyporesponsiveness of spleen T-cells and induction of $CD4^+CD25^+FoxP3^+$ T-cells after recipient preconditioning with kyn-treated donor blood

recommends these results for further basic investigations and development of a clinical application.