Our group has been involved for more than two decades with questions relating to stem cell transplantation. In particular, two major fields of investigation are being pursued, namely, transplantation of hematopoietic stem cells for recipients without a matched donor and, more recently, the use of embryonic committed stem cells as a new source for organ transplantation.

Tolerance induction by veto CTLs

One major challenge in BMT is how to achieve durable engraftment of allogeneic stem cells with minimal toxicity so that it will be an acceptable practice, not only in the treatment of terminal leukemic patients, but also as a safe tool for tolerance induction as a prelude for organ transplantation or cell therapy. Clearly, while 'mega dose' stem cell transplants can overcome rejection in heavily conditioned leukemia patients, the cell composition of the transplant must comprise additional facilitating cells when attempting to overcome more difficult immune barriers in minimally reduced recipients. In this context, several veto cells have been described, referring to the activity of alloantigen-bearing cells capable of inactivating T cells that recognize them. Recently we have demonstrated that anti–third-party CTLs generated under IL-2 deprivation afford a suitable source for effective veto cells that can enhance BM allografting without GVHD in lethally irradiated mice. Our results suggest that allograft rejection mediated by naive or memory T cells can be overcome by veto CTLs. This conclusion is highly relevant to the prospect of clinical application of anti-third party veto cells in leukemic recipients of bone marrow transplantation, in whom rejection is often mediated by residual memory cross reactive T cells. A protocol for effective large scale production of human veto CTLs has been described recently and clinical trials in leukemia patients are in the final stages of preparation.
The mechanism by which the anti-3rd party CTLs exert the veto effect is a major subject of intense research in our laboratory (Fig 1). While early studies emphasized the role of CD8 mediated apoptosis, we showed that the veto activity of anti-3rd party CD8+ CTLs is dependent upon the simultaneous expression of both CD8 and FasL. Thus, the interaction of CD8 on the veto cells with MHC class I x3 domain on the effector cells is associated with an increased susceptibility of the effector cells to FasL killing. Further investigation of signal transduction pathways involved in the veto effect revealed that pretreatment of effector cells with the MEK1,2,5 inhibitor U0126 inhibits the apoptosis induced by the veto cells. No inhibition of veto activity could be found with specific inhibitors of other signaling molecules such as JNK, P38, PI3K or PKC. Considering that Fas expression on the effector cells is critical for the veto activity, it is interesting that the ERK inhibitor didn’t affect the level of Fas on the effectors and only partially affected their stimulation. Also, this inhibition is not likely mediated by affecting the veto CTLs, as pretreatment of the latter cells with ERK inhibitor didn’t diminish the veto effect. Furthermore preliminary studies suggest that phosphorylation of ERK leads to down regulation of the apoptosis inhibitors such as xIAP thereby allowing for Fas-FasL apoptosis to proceed.

**Tolerance induction by third party “Off-The-Shelf” CD4+CD25+ Treg Cells**

In the search for additional approaches for facilitating allografts, we investigated whether donor CD4+CD25+ Treg cells would be synergistic with veto CTLs or conversely would suppress tolerance induction by veto CTLs. When using a Rapamycin (RAPA) based conditioning, Treg cells alone led to moderate enhancement of engraftment and RAPA at different doses was synergistic with the Treg cells. However addition of veto CTLs to the Treg cells enabled reducing the effective RAPA dose by four-fold. We further tested in this model whether third party Treg cells could be used instead of donor or host Treg cells to overcome rejection of BM allografts and found that freshly isolated third party Treg cells were as effective as the donor type cells in preventing graft rejection. Thus, third party Treg cells could afford a new viable ‘off-the-shelf’ source for tolerance induction. The use of third party Treg cells in contrast to donor type cells could allow advanced preparation of a large bank of Treg cells, with all the appropriate quality controls required for cell therapy.

**Eradication of B Lymphoma cells in TCR independent mechanism by human anti-3rd party Veto CTLs.**

Very recently we demonstrated that human anti-3rd party CTLs, generated by stimulation of PBMC against EBV transformed B cell lines, are endowed with an effective capacity to eradicate tumor cells from patients with B-CLL, as well as different human B cell Burkitt’s lymphoma lines, by induction of apoptosis. This T cell receptor independent recognition between veto hCTLs directed against 3rd party HLA and the B-CLL cells was found to be mediated by ICAM-1 binding on target B cells through the LFA-1 expressed on effector T cells. Upon this initial binding, MHc class I signaling occurs via interaction with CD8 molecules on the CTL membrane. This capacity of the anti 3rd party hCTLs implies an important role in graft versus tumor effect in addition to engraftment facilitation in BMT for malignant disorders. The mechanism of this novel HLA class1 apoptosis signaling is currently being investigated.

**Embryonic pig tissue transplantation: Pancreas for the treatment of diabetes.**

Transplantation of embryonic pig pancreatic tissue as a source of insulin has been suggested for the cure of diabetes. However, previous limited clinical trials failed in their attempts to treat diabetic patients by transplantation of advanced gestational age porcine embryonic pancreas. We examined growth potential, functionality and immunogenicity of pig embryonic pancreatic tissue harvested at different gestational ages.

Implantation of embryonic pig pancreatic tissues of different gestational ages in SCID mice reveals that E42 (embryonic day) pig pancreas can enable a massive growth of pig islets for prolonged periods and restore normoglycemia in diabetic mice. Furthermore, both direct and indirect T cell rejection responses to the xenogeneic tissue demonstrated that E42, in comparison to E56 or later embryonic tissues, exhibits markedly reduced immunogenicity. Finally, fully immunocompetent diabetic mice grafted with the E42 pig pancreatic tissue and treated with immunosuppression protocol comprising CTLA4-Ig and anti-CD40 ligand, attained normal blood glucose levels, eliminating the need for insulin (Fig 2). Taken together, our data provide a proof of principle for the promising potential of E42 embryonic pig pancreatic tissue as a novel source for transplantation. Studies in a nonhuman primate model are warranted in order to investigate the scope of reduced immunogenicity, the issues of implanted dose and transplantation site, as well as functionality of the growing embryonic pancreas. If successful, the use of embryonic porcine tissues might offer an attractive source of unlimited pancreatic tissue.

**Fig. 2** Insulin (blue) and glucagons (green) expression within islets developing from E42 pig pancreatic graft, 8 months post transplantation.

Reduced immunogenicity and enhanced growth of fetal liver in non-injured host liver.

Disappointing results of isolated hepatocyte transplantation in clinical trials may be explained in part by negligible proliferation of the transplanted cells in the quiescent host liver. Fetal tissues, which might exhibit higher levels of proliferation, could potentially afford an advantageous source for transplantation. In our present study we demonstrate that porcine fetal liver tissue fragments in which potential cell-cell and cell-stroma
interactions are spared, display remarkable growth and differentiation upon implantation into SCID mice, compared to isolated fetal hepatocytes harvested at the same gestational age (E28). The proliferative advantage of fetal pig liver fragments was attained in the setting of a quiescent host liver without need for liver injury. A second important advantage of early fetal liver tissue as a source for transplantation was demonstrated upon co-transplantation with human lymphocytes. Implants of E28 tissue were less infiltrated and survived better compared to implants harvested at later gestational time points, suggesting reduced immunogenicity. Furthermore, E28 liver could engraft, grow and differentiate in fully immunocompetent mice under a mild immunosuppressive regimen. Taken together, these results suggest that transplantation of fetal liver fragments can potentially resolve the obstacles associated with isolated hepatocytes and could offer a novel curative approach in the treatment of acute liver failure or metabolic diseases.

Selected publications

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