Human T cell responses in xenotransplantation
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Summary

Over the last four decades, improvements in surgical techniques and immunosuppressive regimens have led to an increased allograft and patient survival. However, donor organ shortage and an increasing patient population eligible for a transplant led to an extensive waiting list. In the USA, an average 35 percent of the patients on this list will actually receive a transplant. The time spent on the waiting list and the chances of actually receiving a transplant vary widely depending on the organ required. The organ shortage has driven the search for alternatives. Besides the use biomechanical solutions like dialysis, artificial livers and heart assist devices, the use of animal derived or xenogeneic organs is being explored.

Xenotransplantation is a major concern of the general public, especially in Europe. The recent outbreak of bovine spongiform encephalopathy (BSE), also known as "mad cow disease", made the public aware of the risks of zoonosis. Chimpanzees and baboons xenogeneic organs probably have a higher risk of transmitting infectious diseases to closely related human beings than the current choice of porcine organs. Nevertheless, there are serious examples of pig borne diseases transmitted to man. The Spanish flu that killed millions of people at the beginning of the 20th century was thought to originate from pigs as was the more recent outbreak of encephalitis caused by the Nipah virus in Malaysia. In addition, the recipient of a xenogeneic organ is most likely severely immunosuppressed creating an ideal environment for the proliferation and possible mutation of microorganisms. Therefore, xenogeneic transplantation requires serious consideration and the benefits of the individual have to be weighed against the risks of the human population as a whole.

Despite ethical concerns and yet unpredictable risks of possible transmission of zoonotic infections, basic research continues to solve these and other problems. Pigs are currently considered the most likely source for xenogeneic kidneys, hearts, livers and possibly lungs to be implanted in human recipients. However, kidneys are the most feasible and bear the least risk for the patients. The patient can survive on hemodialysis if the pig kidney were to fail, whereas the other suggested organs are vital and have no alternative, and failure will lead to the death of the patient. Besides the mortality risk factor, the pig heart may be too weak to sustain blood circulation and the pig liver may produce too many incompatible proteins to sustain the life of the recipient.
To make xenotransplantation a clinical reality, the vigorous immunological response induced by the xenograft has to be controlled. The first hurdle of hyperacute rejection caused by preformed antibodies against α galactoside, a molecule present on cells of most organisms except on man, apes and Old World monkeys, seems to be manageable by the use of genetically engineered pig expressing human complement regulatory factors. Therefore, we have to anticipate the next hurdle of cell-mediated responses induced by porcine solid organs. This thesis investigated the human T cell response to porcine xenoantigens in vitro to evaluate and design possible strategies to eliminate or contain harmful T cell mediated anti-graft responses.

Under normal physiological circumstances antigens are processed by antigen presenting cells and these present the processed peptides to T cells in an MHC restricted fashion. The nature of the presented antigen determines if an immune response is mounted. Initially, it was thought that antigens originating from a xenograft would be presented through this indirect pathway and only cause a modest immune response. As demonstrated in allogeneic transplantation, T cells and APCs from individuals with different haplotypes can interact despite MHC restriction. This direct presentation pathway causes a potent immune response, which can be reduced by matching the donor organs to the recipient prior to transplantation. Surprisingly, human T cells and porcine APCs can interact across species through this direct pathway also and this response is at least equal or more potent than an allogeneic direct response. We showed that the xenogeneic direct response is twice as potent as the allogeneic response and that the allogeneic direct response is more potent than the xenogeneic indirect response. This demonstrates that porcine APCs are potent stimulators that induce human T cell proliferation and therefore may have an important role in triggering an immune response against the xenograft.

T cell proliferation requires, besides the interactions between the T cell receptors and the MHC molecules, a second or costimulatory signal. Without this costimulatory signal, a T cell cannot proliferate. We demonstrated that xenogeneic interactions between human T cells and porcine APCs are similar to allogeneic interactions in that they both require costimulatory signals provided through CD28/B7 and the CD40/CD40L receptor/ligand interactions. This was demonstrated by the lack of T cell proliferation when both costimulatory signals were
inhibited simultaneously. At the same time, we demonstrated that the allogeneic direct and the xenogeneic indirect responses were not as efficiently inhibited by the same protocol. This suggests that alternative costimulatory signals may be present when both the T cells and APCs are of human origin.

Current immunosuppressive drug treatment used in allotransplantation and in preclinical xenograft models are not without risk. Most of these drugs, which have to be taken as long as the graft is present, have eventually toxic effects or sensitize the patients, causing serious consequences. The drugs suppress the immune system as a whole and therefore render the patients susceptible to life-threatening infections and cancers. Even possible treatment with costimulation blockers will affect the overall immune status of the patient. The development of a more specific immunosuppressive therapy, that does not negatively affect the overall immune status of the patient, would be instrumental in reducing the associated risks.

We investigated the possibility of eliminating T cells specifically proliferating in response to a xenogeneic porcine stimulus. To do so we needed to identify an epitope that is uniquely expressed on these proliferating T cells to enable elimination by a monoclonal antibody. One such unique marker is the Vβ chain of the T Cell Receptor. The human Vβ repertoire consist out of 25 known families (Vβ1-25) and the literature suggest that the selection of the Vβ family, expressed on the responding T cell, is driven by antigens. Our hypothesis was that the strong porcine antigens would induce proliferating T cells expressing the same Vβ family, which would allow removal of these T cells prior to xenogeneic transplantation.

The experiments revealed that the Vβ repertoire in the individuals tested had common and unique Vβ families responding to porcine antigen presented through the direct pathway. Furthermore, these experiments demonstrated for the first time that the individual Vβ repertoire could be induced repeatedly when stimulated with the same pig. Stimulation with other pigs led to similar Vβ repertoires within the individuals but not between individuals. Since there is no obvious Vβ family responding to pigs, the importance of each proliferating Vβ family regarding graft rejection has to be assessed before a general antibody therapy seems feasible. A customized monoclonal antibody therapy is still a possibility since the
patient's Vβ repertoire induced by the potential donor can be established with the described method (chapter 5) prior to xenogeneic transplantation.

In the final study the *in vitro* anti-pig responses of patients, possibly eligible for a xenogeneic kidney transplant if the procedure were available, was assessed. Patients awaiting a kidney transplant, who are sensitized for allogeneic MHC class I or MHC class II antibodies, are considered for the first clinical experiments since they have a slim chance to receive a matching donor organ. However, it has been reported that allogeneic anti MHC antibodies can cross react with porcine cells and MHC class II sensitization is correlated with troublesome, difficult to control, rejection episodes in clinical allogeneic transplantation. We showed that patients already sensitized for allogeneic Class II MHC have a more vigorous *in vitro* response to xenogeneic antigens. This *in vitro* data does not necessarily predict the *in vivo* graft survival but it warrants further investigation prior to clinical trials that involve these patients.

It is likely that pig organs sensitize the human recipient through similar mechanisms as found in allogeneic transplantation. The consequences of this sensitization are unpredictable but it may be possible that pig organs sensitize patients in such a way that subsequent allogeneic graft survival is negatively affected. This would exclude the temporary use of xenografts to bridge the period until an allogeneic graft becomes available. History suggests, that this precaution is warranted since two patients that temporarily received baboon kidneys both died 5-6 weeks after implantation of allografts with unknown pathology, as shown by Starzl in 1964. However, this important question is likely to remains unsolved until further clinical trials can be done.

In summary, since hyperacute rejection seems to be controllable, studies involving human T cells responses against porcine antigens become increasingly important. This thesis suggests that simultaneous blockade of the costimulatory signal as well as customized specific targeting of the responding T cells may be pursued as alternative methods to prevent xenograft rejection. Furthermore, preclinical trials with MHC class II allosensitized recipients should be conducted with caution, as heightened cellular anti-porcine responses were measured *in vitro*.