Clinical islet of Langerhans xenotransplantation: How close are we?

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Introduction

The encouraging results recently reported by the Edmonton group in patients receiving allogeneic islet grafts, all of whom achieved at least temporary insulin-independence, has rekindled interest in transplantation of islets of Langerhans as a cure for diabetes [1, 2]. As successful clinical islet transplantation currently requires 2–4 human donors per recipient [1], the shortage of organ donors might prevent most eligible diabetic patients from receiving a graft. Currently, less than 100 cadaveric organ donors become available for clinical transplantation each year in Switzerland. In contrast, the population of type 1 diabetic patients is estimated at approximately 0.2% of the total Swiss population, i.e. around 14,000 persons. Unlimited islet transplants could be performed if a suitable tissue source was identified. Xenotransplantation of porcine islets is a potential solution to this shortage.

Although in comparison to pigs, nonhuman primates are genetically closer to humans, but the pig remains the most suitable source of organs for humans due to a number of practical, safety, and ethical reasons [3]. With respect to the treatment of diabetes it is noteworthy that pig islets are known to be metabolically suitable, since pig insulin has for many years been used to treat diabetic patients; its structure differs from human insulin by only one amino acid residue. The implantation of xenogeneic tissue has provoked ethical and epidemiological controversies [4]. Balanced against the benefits of successful xenotransplantation is the possibility of transmission of porcine endogenous retroviruses (PERV) from porcine cells to the xenograft recipient, as infection of human cells has been demonstrated in vitro [5, 6]. However, neither PERV transmission nor clinical infection or disease have been observed in patients who have been exposed to living porcine tissues [7, 8].

The first clinical experience of porcine islet xenotransplantation into human patients was reported by the Swedish group headed by C. Groth in 1992 [9], several years before the identification of PERV. This surgical team transplanted a total of 10 diabetic patients with porcine islets. No reduction in insulin requirement was observed in any of these patients, but porcine C-peptide could be detected [10]. The results of this trial indicated that some xenogeneic islet cells were not acutely rejected in these patients receiving standard pharmacologic immunosuppressive therapy.

More recently, a new clinical trial of islet xenotransplantation has been initiated in Mexico by Valdes et al. [11]. Several patients have been transplanted with a combination of porcine islets and Sertoli cells. This trial has provoked skepticism in the scientific community, as it is performed in a country that has no legal regulatory conditions on xenotransplantation and that no pre-clinical animal experiments have been performed [12].

It should therefore be mandatory to perform experiments in relevant pre-clinical models before initiating new clinical trials. As the risk of pig-to-human viral transmission is not yet established or excluded, experiments in large animal models will allow to estimate if this risk is acceptable for the initiation of new clinical trials.

Present State of Knowledge in the Field of Islet Xenotransplantation

The major obstacle to successful xenotransplantation is the immunological incompatibility between pigs and humans. The predominant xenoantigen responsible for rejection of porcine grafts in humans has been identified as the carbohydrate epitope, galactoseα1-3galactose (Gal) [13]. All mammals, except humans, apes and Old World monkeys, express this oligosaccharide on the surface of their vascular endothelial cells. Primates that do not synthesize this carbohydrate produce antibodies directed against this antigen as a result of the stimulation of the immune system by Gal sugars on the surface of bacteria present primarily in their intestinal tract. These "natural" antibodies initiate the hyperacute rejection of xenotransplanted organs by activating complement on the surface of endothelial cells resulting in coagulation, thrombosis and haemorrhage in the graft [3].

Recently, the company PPL Therapeutics announced at the XIXth International Congress of

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the Transplantation Society in Miami in August 2002, that Gal-knockout pigs were born and were fully healthy and viable [14]. These pigs do not express Gal on their tissues and represent a major advance in the progress towards clinical trials of xenotransplantation. Organs of these pigs will be transplanted shortly into primates.

Xenotransplantation of islets presents some possible advantages over that of a whole organ. With non-vascularised grafts, such as islets, the absence of immediate vascularisation prevents contact between the recipient's circulating natural pre-existing antibodies and the endothelial cells of the islets. Until recently, cellular immunity was believed to be predominant in the rejection of tissue xenografts [15, 16], but the exact mechanism remains incompletely understood. Long-term survival of pig [17] and human [18] pancreatic islets in athymic nude

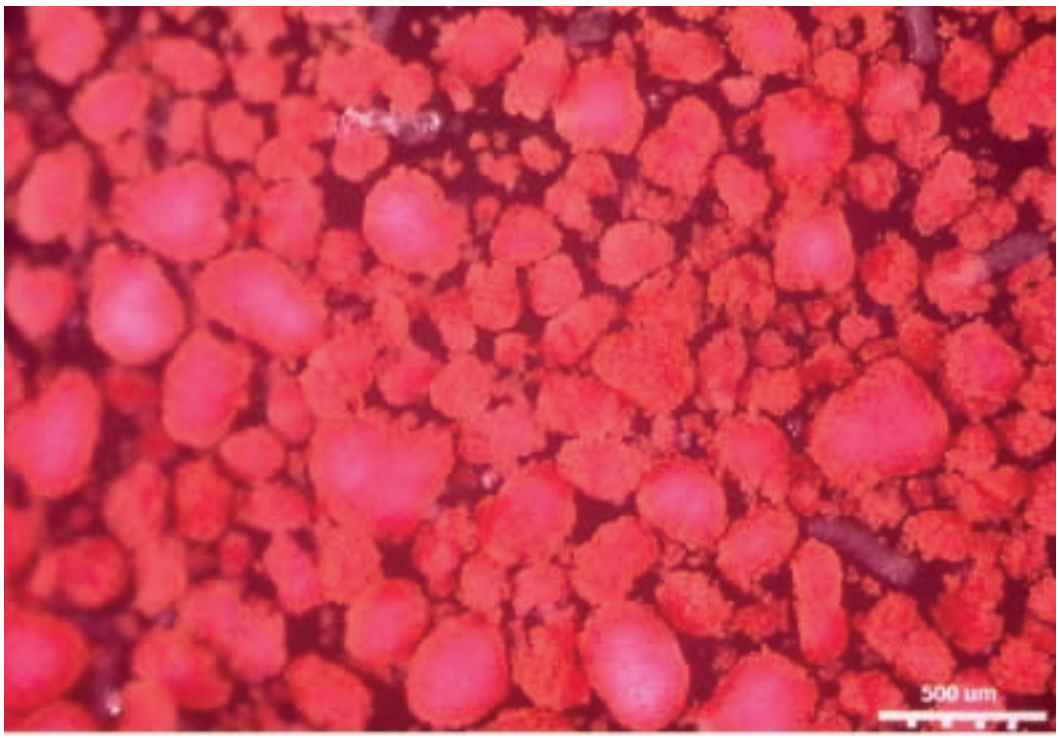

Figure 1.
Purified porcine islets stained with dithizone.

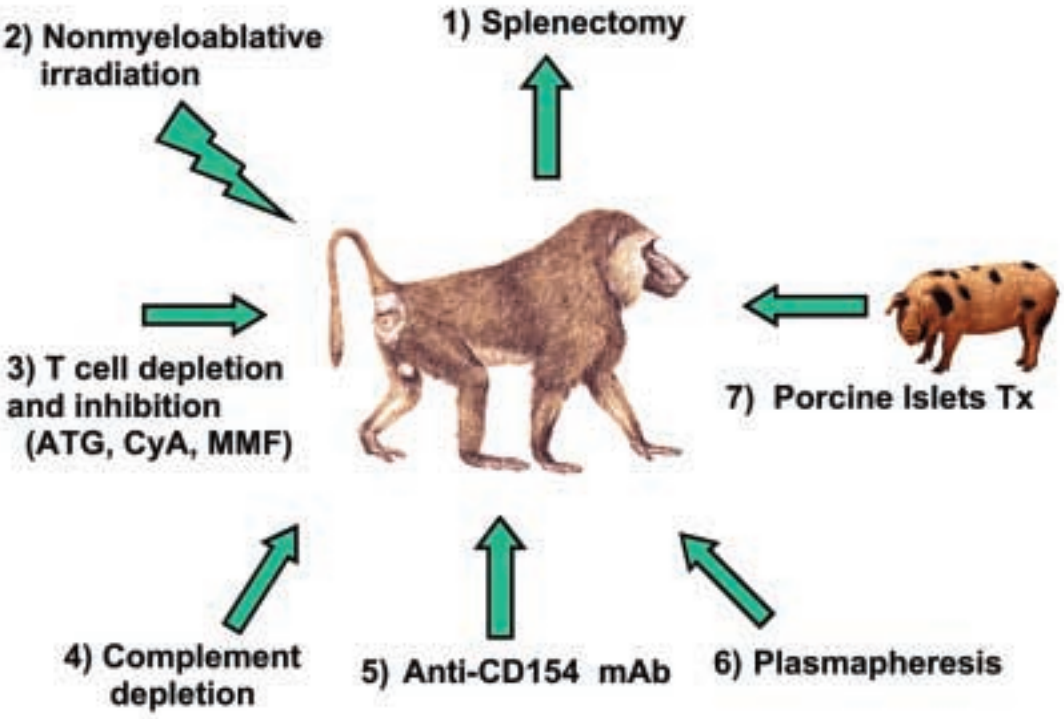

Figure 2.
Immunosuppressive regimen in baboons receiving porcine islets included splenectomy, nonmyeloablative whole body irradiation, T cell depletion (with ATG or anti-thymocyte globulin), cyclosporine (CyA), mycophenolate mofetil (MMF), complement depletion with cobra venom factor, a course of an anti-CD154 monoclonal antibody and anti-pig antibody adsorption using a specific plasmapherisis technique prior to porcine islets transplantation (Tx).

mice suggests a T cell-mediated process, in which CD4+ T cells have been shown to play a major role [19]. However, the use of conventional immunosuppressive agents that block the T cell response in immunocompetent recipients allows only a modest prolongation of survival of xenografted islets [20].

Regarding experiments using pig-to-nonhuman primate models, we have transplanted porcine islets [figure 1] by intraportal injection to baboons receiving either conventional triple drug immunosuppressive therapy, or a more intensive regimen [figure 2], including depletion of T cells and complement, removal of anti-Gal antibodies by a specific plasmapheresis technique and the use of a new monoclonal antibody blocking T cell signaling (anti-CD154 monoclonal antibody) [21]. In the group receiving conventional immunosuppression, porcine C-peptide was detected only transiently after porcine islet injection, and histological examination of liver biopsies taken between days 2 and 19 did not reveal viable islets. In the group receiving more intense immunosuppression, porcine C-peptide was detected up to 5 days after transplantation. Biopsies showed viable islets up to day 14, but not thereafter, with a progressive mononuclear cell and macrophage infiltration. These results suggest that powerful immune responses are involved in rejection of discordant xenogeneic islets and that adequate immunosuppressive regimens still need to be developed.

Outlook on the future

The transplantation of animal cells and tissues into humans could play an important role in the treatment of a great variety of disorders that result from tissue loss or dysfunction, diabetes being the most common. The serious shortage of available human organs for transplantation will certainly favor new clinical xenotransplantation trials.

New immunosuppressive therapies are continuously developed and tested. Our preliminary results have shown that innate immune responses are involved in the rejection of pig islets in baboons. The use of macrophage-depleting agents such as gadolinium chloride, has allowed significant prolongation of xenoislet survival in rodent models [22]. Experiments investigating its efficacy will be performed in large animal models.

New monoclonal antibodies inducing T cell signaling-blockade, such as anti-CD154, have been shown to efficiently block allograft rejection in small and large animal models of organ and islet transplantation [23, 24]. Only a few reports have investigated the ability of costimulatory blockade to prevent xenograft rejection. Anti-CD154 monoclonal antibody therapy is able to delay T cell-mediated rejection of porcine skin grafts in mice [25] and also to block T cell-dependent antibody production in the pig-to-baboon model [21, 26].

FTY720 is a new molecule that interferes with the circulation of T cells and thereby prevents interaction of T cells with the graft [27]. FTY720 in combination with cyclosporine has been shown to prolong survival of xenotransplanted islets [28].

Xenotransplantation also offers the first real opportunity for modifying the donor as opposed to the recipient. This opens up great possibilities, particularly in this era of rapidly developing techniques such as genetic engineering, gene transfer and cloning. The pig tissue may be transgenic for one or more human complement-regulatory proteins and, ideally, would express no or little Gal. The breeding of a pig with a vascular endothelial structure against which humans have no preformed antibodies should be a major advance.

To achieve successful xenotransplantation it will probably be necessary to combine several therapeutic techniques and/or agents, as is the case with allotransplantation today. There will almost certainly be several steps of development, but clinical application could start within the near future.

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