Xenotransplanting Pancreatic Islets

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Today there are approximately 3.5 million patients afflicted with insulin-dependent diabetes mellitus (IDDM) worldwide. It is estimated that the number will have increased to 5 million by the year 2010, and to 30 million by 2025.[1] Even with stringent insulin therapy based on frequent glucose monitoring, the disease is progressive. Diabetes remains a leading cause of end-stage renal disease and blindness.

Transplantation of human islets of Langerhans would offer a logical means to treat IDDM, and sporadic attempts at such transplantations have been made over the last 20 years. However, poor outcome has hampered the evolution of this treatment modality. Among approximately 200 patients treated with human islet transplantation during the last decade, only a handful (8%), were insulin-independent at one year after transplantation. (International Islet Transplant Registry Report, 1999).

Breakthrough with Human Islet Transplantation Will Lead to Shortage of Human Islet Tissue

This year, the islet transplant group at the University of Alberta in Edmonton reported a series of seven consecutive diabetic patients, all of whom had attained insulin independence after islet transplantation (median follow-up 12 months).[2] Islets from two donor pancreatic glands were transplanted in six patients, and from three in one patient. If these highly encouraging results can be confirmed in a larger series, a large number of candidates for islet transplantation will come forward, and soon the supply of human islets will become a limiting factor. Alternative sources for insulin-producing cells would then have to be found. One option would be the use of islets prepared from animal tissue, xenoislets. Our research efforts are currently directed towards the use of pig islets for treatment of human diabetes. Other future possibilities include human stem cells or engineered human cells.

The pig as the source of xenoislets. When it comes to the source of xenoislets, several factors speak in favor of the pig. Porcine insulin was used for decades for the treatment of diabetic patients, pigs are easy to breed and have large litters. Since large numbers of pigs are consumed anyhow, using pigs as a source of tissue for transplantation should be ethically acceptable.

In a pilot study in the early 1990s, ten patients were transplanted with fetal porcine islets in Stockholm, Sweden.[3] Four of eight patients in whom the islets were given by intraportal injection had evidence of islet survival reflected in temporary excretion of small amounts of porcine C-peptide. Two patients had the islets placed under the kidney capsule. In one of these patients, a biopsy revealed surviving porcine islets 3 weeks after transplantation. Although no clinical benefit was observed, neither were there any untowards effects.

Mechanisms Orchestrating Islet Xenograft Rejection

The cell-mediated rejection of an islet xenograft has been assumed, by some investigators, to be similar to that of an islet allograft. However, data from recent studies support a role for additional mechanisms involved in xenoislet rejection. In support of this notion is the observation that immunosuppressive drugs effective in islet allotransplantation are essentially ineffective in islet xenotransplantation. However, these results have been generated in

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rodent models, and there is an urgent need to study xenoislet rejection in clinically-relevant animal models.

Recently we found that when fetal islet-like cell clusters were transplanted to cynomolgus monkeys, the rejection process was dominated by a massive infiltration of $CD8^+$ T cells, a finding which was in marked contrast to the findings in rodents, where macrophages dominate the cellular infiltrate.[4] These findings are, however, in accord with the findings of in vitro studies showing that the human anti-pig cell-mediated cytotoxic response is similar to the human anti-human response.[5,6]

The rejection of pig islets was significantly delayed when the monkeys were treated with cyclosporine and 15-deoxyspergualin.[4] Notably, the infiltration of $CD8^+$ T cells was markedly reduced, whereas the level of infiltration of macrophages, which correlated with destruction of the islet graft, was less affected. Based on these observations, it seems that rejection of a pig islet xenograft in the monkey model is dependent on two different cellular mechanisms. Presumably, there is first a recognition of the pig MHC by CTL via direct antigen presentation. Second, there is an immune response that is less sensitive to standard immunosuppressive drugs, and is characterized by infiltration of the graft by highly activated macrophages, this infiltration being elicited by T cells. This latter response shares some histopathological characteristics with a DTH-like response.

Such a two-stage model would provide an explanation for the apparent discrepancy between in vitro studies, which suggest that the cell-mediated response is similar towards xeno- and allografts, and in vivo experiments, which show stronger rejection against xenografts than against allografts. Furthermore, xenorejection is unresponsive to immunosuppressive drugs that are effective against allorejection.

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Blood-Islet Incompatibility

Most investigators have assumed that islet xenografts do not undergo hyperacute rejection as do organ xenografts. However, with intraportal injection of the islets (the established method for clinical islet transplantation), the islets will be directly exposed to blood. Recent studies in our laboratory, both in vitro and in a pig-to-cynomolgus monkey islet transplantation model, have shown that such exposure will result in a strong inflammatory reaction with subsequent islet injury.[7] This reaction, which we have named the instant blood-mediated inflammatory reaction (IBMIR) shares some characteristics with hyperacute rejection. However, IBMIR is not antibody-mediated, but rather the result of an activation of platelets and of the coagulation and complement systems. Complement inhibition with soluble complement receptor 1 abrogated the complement activation and reduced islet injury. Platelet inhibitors and anticoagulants might also be helpful in protecting islets against this early injury.

Islets from Transgenic Pigs

Current interest in xenotransplantation has been greatly stimulated by the advent of pigs transgenic for human complement inhibitors. When kidneys and hearts from such pigs were transplanted to non-human primates, hyperacute rejection did not occur and the xenografts could function for several weeks in immunosuppressed recipients. In our laboratory, we have recently studied whether islets prepared from hDAF pigs would be protected from early injury in vitro.[8] The results were disappointing in that we found no, or only marginal protection. Subsequent studies of the phenotypic characteristics of the islets revealed, however, that the transgene was very poorly expressed on the islets. Presently, studies are ongoing with islets from pigs transgenic for hMCP. These islets do express the transgene.

The use of islets expressing human complement inhibitors might, however, be potentially dangerous. It is known that porcine endogeneous retroviruses (PERV) released from pig cells are rapidly lysed by human complement. It could then be that PERV originating from transgenic cells expressing human complement inhibitors would escape this defense mechanism.

Clinical Prospects

When allo- and xenoislets were transplanted as a mixed graft to rats treated with cyclosporine, the xenoislets were promptly rejected while the alloislets remained intact; thus, the co-existence of xenoislets did not jeopardize the alloislets.[9] Based on this finding, we propose that clinical trials with pig islets should initially consist of mixed grafts, with a limited number of porcine islets transplanted in conjunction with human islets. The procedure would be potentially beneficial to the patient, by virtue of the transplanted human islets, and the

porcine islets would serve as a means to increase the islet mass. The functional survival of the two kinds of islets could be assessed by measuring the levels of the respective specific C-peptides. With increased experience, grafts containing more and more pig islets could be transplanted. The availability of a successful human islet transplantation program would thus be a prerequisite for the initiation of a clinical trial with pig islets.

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