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Islet xenograft rejection: Studies in the pig-to-rodents and pig-to-primate models

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The aims of this thesis was: 1) to compare islet allo- and xenograft rejection in rats, using immunosuppression with CsA in a mixed allogeneic-xenogeneic islet transplantation model, 2) to evaluate the efficacy of various immunosuppressive drugs, with special reference to Leflunomide, in preventing islet xenograft rejection in the pig-to-rat model, 3) to perform an immunohistochemical study of porcine islet xenograft rejection in primates including primates treated with CsA+DSG, and 4) to study the metabolism and excretion of porcine C-peptide in mice, with special reference to the use of donor-specific C-peptide for monitoring islet xenograft function. In summary, the results were as follows:

1). After transplantation of mixed allogeneic-xenogeneic islet grafts into rats treated with CsA, a cell-mediated rejection destroyed the xenogeneic islets but left the allogeneic islets intact. Thus, the ongoing islet xenograft rejection did not initiate rejection of the allogeneic islets as well. The xenogeneic islets were destroyed by a massive cellular infiltration, similar to that observed after transplantation of xenogeneic islets alone. We conclude that the xenogeneic islets were rejected by a cell-mediated process, most likely qualitatively different from islet allograft rejection.

2). 12 days after transplantation, fetal porcine ICC transplanted into untreated rats were destroyed by a massive cellular infiltration. Treatment with CsA+LEF+MMF prevented rejection of ICC's for up to 24 days after transplantation. Immunosuppression with CsA+DSG (10 mg/kg BW) prevented rejection for up to 12 days. It can be speculated that the preventive effect resulted from a suppressive effect on macrophage activation by xenoreactive T cells. However, a direct suppressive effect on macrophages cannot be ruled out. CsA+LEF+RPM, CsA+CYP+plasmapheresis, or CsA+plasmapheresis also prevented ICC xenograft rejection. However, these three protocols all caused significant toxicity. Treatment with CsA+LEF, CsA+RPM, and LEF+RPM also had an inhibitory effect but the rejection was not prevented. The protective effect of CsA+LEF was probably not caused by inhibition of purine nucleotide biosynthesis or from the suppression of xenoreactive antibody production.

3). Fetal porcine ICC transplanted under the kidney capsule of cynomolgus monkeys were rejected by a cell mediated process, during the first 6 days after transplantation. This process was not dependent on recipient xenoreactive antibodies or C3-binding to
the graft. There was no evidence of an ADCC since there were no deposits of IgM, IgG, C1q or C3 in the grafts. The rejection of ICC was more vigorous in the primates than in rodents. In contrast to the finding in rodents, in which macrophages were the main infiltrating cells, islet xenograft rejection in the primates was dominated by infiltration with CD8-positive T cells. The rejection was delayed in cynomolgus monkeys treated with CsA+DSG, but not as significant as in the pig-to-rat model. In these animals, there was a marked reduction in the infiltration of CD8-positive T cells. However, no, or only a marginal, reduction of the macrophage infiltration was observed. On the basis of these observations, we can speculate that rejection of a fetal porcine ICC xenograft in the cynomolgus monkey depends on at least two qualitatively different mechanisms. In non-immunosuppressed primates, a rapid and vigorous CTL-mediated rejection process dominated. When this mechanism was inhibited in the primates immunosuppressed with CsA+DSG, a second mechanism far less sensitive to treatment with CsA+DSG and dominated by a massive infiltration of macrophages was revealed.

4). Injections of porcine C-peptide into mice did not result in the excretion of the C-peptide in the urine. In contrast, when porcine C-peptide was injected into nude mice, small amounts of the C-peptide were excreted. Presumably, this is because of more pronounced immunological reactivity to, and subsequent accelerated degradation of, the xenogeneic peptide in immunocompetent mice than T cell deficient mice. After s.c. injection of radioactively labeled porcine C-peptide into mice, the radioactivity in urine was at background levels. The probable explanation of this finding is that the xenogeneic C-peptide had been degraded and that the radioactive tracer had been separated from the peptide during the degradation process. Furthermore, following injection of radioactively labeled porcine C-peptide, radioactive uptake in tissues belonging to the mononuclear phagocytic system, including Kupffer cells, was significantly increased in mice which had previously been injected with porcine C-peptide for several weeks. This may reflect an increased immunological reactivity to, and a subsequent increased degradation of xenogeneic C-peptide. Consequently, in islet xenotransplant recipients, determinations of donor-specific C-peptide may not properly reflect islet xenograft function. In fact, negative findings concerning xenogeneic C-peptide in the urine may occur in spite of islet xenograft function.

Key words: Xenotransplantation, islets, rejection, porcine, immunosuppression, C-peptide.