Pig Production for Xenotransplantation

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SUMMARY

Clinical transplantation has become one of the major treatments for end stage organ failure since the introduction of chronic immunosuppressive drugs in the mid 1980s. One of the major approaches to dealing with the limited supply of human organs is the utilization of alternative species as a source of organs (xenotransplantation). The pig is considered the primary alternative species due to ethical considerations, breeding characteristics, infectious disease concerns and its compatible size and physiology .

A major barrier to progress in pig-to-primate organ transplantation is the presence of terminal a(1,3) galactosyl (gal) epitopes on the surface of pig cells. Humans and Old World monkeys have lost the corresponding galactosyltransferase activity in the course of evolution and therefore produce preformed natural antibodies against the epitope that are responsible for hyperacute rejection of porcine organs. The temporary removal of recipient anti-gal antibodies through affinity adsorption and expression of complement regulators in transgenic pigs has allowed survival of pig organs beyond the hyperacute stage. However, returning antibody and residual complement activity are believed to be responsible for the acute and delayed damage that severely limit organ survival even in the presence of high levels of immunosuppressive drugs and other clinical intervention. Competitive inhibition of galtransferase in H-transferase transgenic pigs has resulted in only partial reduction in epitope numbers.

Similarly, attempts to block expression of gal epitopes in N-acetylglucosaminyltransferase III transgenic pigs also resulted in partial reduction of gal epitopes number, but failed to significantly extend graft survival in primate recipients. Given the large number of gal epitopes present on pig cells, it seems unlikely that any dominant transgenic approach of this nature can provide sufficient protection from anti-gal mediated damage. In contrast, a genetic knockout of the a(1,3) galactosyltransferase (GGTA1) locus in pigs would provide permanent and complete protection.

Viable a(1,3) galactosyltransferase knockout mice have been produced by ES cell technology. Development of nuclear transfer technology has provided a means for locus-specific modification of large animals, as demonstrated by the production of viable sheep using *in vitro* targeted somatic cells. Successful cloning and production of transgenic pigs by nuclear transfer of genetically modified somatic cells have been reported. Attempts at targeting the GGTA1 locus in pigs and sheep have been reported also, but failed to result in live birth of animals with the desired modification. In both cases, difficulties in obtaining viable targeted donor cell clones were encountered.

We chose to knockout the GGTA1 locus in a highly inbred, MHC-defined miniature pig line. Descendent from lines long used for xenotransplantation studies, this line is an ideal size match for eventual use in clinical transplantation and has animals that consistently test negative for transmission of porcine endogenous retrovirus (PERV) to human cells *in vitro*. Cells were isolated from 1 male and 3 female fetuses at day 37 of gestation for production of donor cell lines. A gene trap targeting vector, pGalGT, was used for homologous replacement of an endogenous GGTA1 allele.The vector contains approximately 21 kilobases of homology to the GGTA1 locus, with the coding region upstream of the catalytic domain disrupted by insertion of a selection cassette consisting of a Bip internal

ribosome entry site followed by sequences encoding G418 resistance. Following transfection and 14 days of G418 selection, viable cell clones were passaged in triplicate for further analysis and cryopreservation. Nuclear transfer was performed by using in vitro matured oocytes and cryopreserved donor cells without further culture. Embryos were transfer to a surrogates and four live pigs in which one allele of the a(1,3) galactosyltransferase locus has been knocked out were produced.

The next step will be to create a(1,3) galactosyltransferase null (homozygous knockouts) either by l breeding to a heterozygous male produced by nuclear transfer or by sequential nuclear transfer modification of cell lines produced from the four female pigs reported here. Since a(1,3) galactosyltransferase null mice have already been produced it is not anticipated that this genetic modification will be lethal in the null animals. We hope that a(1,3) galactosyltransferase null pigs, will not only eliminate hyperacute rejection but also ameliorate later rejection processes, and in conjunction with clinically relevant immunosuppressive therapy, permit long term-survival of transplanted porcine organs. At a minimum, availability of galactosyltransferase null pigs will allow a clearer evaluation of approaches currently in development aimed at overcoming potential delayed and chronic rejection mechanisms in porcine xenotransplantation.