Human Endothelial Protein C Receptor Overexpression Protects Intraportal Islet Grafts in Mice

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ABSTRACT

Islet transplantation can potentially cure type 1 diabetes mellitus, but it is limited by a shortage of human donors as well as by islet graft destruction by inflammatory and thrombotic mechanisms. A possible solution to these problems is to use genetically modified pig islets. Endothelial protein C receptor (EPCR) enhances protein C activation and regulates coagulation, inflammation, and apoptosis. We hypothesized that human EPCR (hEPCR) expression on donor islets would improve graft survival and function. Islets from an hEPCR transgenic mouse line strongly expressed the transgene, and hEPCR expression was maintained after islet isolation. Islets were transplanted from hEPCR mice and wild-type (WT) littermates into diabetic mice in a marginal-dose syngeneic intraportal islet transplantation model. The blood glucose level normalized within 5 days in 5 of 7 recipients of hEPCR islets, compared with only 2 of 7 recipients of WT islets ($P < .05$). Transplanted hEPCR islets had better preserved morphology and more intense insulin staining than WT grafts, and they retained transgene expression. The improved engraftment compared with WT islets suggests that inflammation and coagulation associated with the transplant process can be reduced by hEPCR expression on donor tissue.

INTRAPORTAL pancreatic islet transplantation has been shown to be an effective treatment for type I diabetes mellitus. Successful engraftment can result in freedom from exogenous insulin and a reduction in diabetic complications compared with current standard treatment. However, there are three major factors preventing widespread use of this modality in the clinic. The first is a critical shortage of human donors, of which only 2.1% are suitable islet donors [1]. Second, the technically difficult isolation procedure, coupled with tissue that is particularly susceptible to ischemic and traumatic injury, results in an inefficient process [2–4]. Multiple transplants for a single patient are often needed to achieve insulin independence [5,6]. Finally, the embolization process induces severe coagulation around the islets, resulting in thrombosis within the hepatic microvasculature [7]. The net effect is a significant reduction in islet viability [8]. An alternate source of islets and/or techniques to improve engraftment may be necessary to fulfill both current and future demands.

There are two strategies currently being pursued to increase islet supply. The first is manipulating precursor cells to differentiate into insulin-producing cells in response to hyperglycemia [9]. Progress on this front has been limited, largely because of incomplete understanding of the factors required to promote specific differentiation [10,11]. The second strategy is porcine islet xenotransplantation, which has achieved proof-of-principle success in preclinical pig-to-primate trials [12,13]. However, long-term graft survival of ≥6 months with clinically acceptable immunosuppression in recipient non-human primates, the accepted minimal criteria for human trials, has not been achieved [14,15]. One

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significant factor that may be preventing this goal is the potent thrombogenic and proinflammatory properties of pig islet xenografts [16]. Pig islets lose αGal xenoantigen expression with age, so adult pig islets have negligible αGal expression [17]. However, other factors, such as tissue factors, collagen, and monocyte chemotactic protein 1, trigger the "instant blood-mediated inflammatory reaction" (IBMIR) to islet grafts, causing rapid destruction [8]. IBMIR is largely a consequence of removal of islets from their natural environment rather than a result of immune-mediated processes [18].

Endothelial protein C receptor (EPCR) enhances generation of activated protein C (APC) by the thrombin/thrombomodulin complex. APC has potent anticoagulant, anti-inflammatory, and cytoprotective properties [19,20]. APC administration improved the function of syngeneic intraportal islet grafts in a mouse model [21], suggesting that enhancing the protein C pathway may be effective. However, long-term APC administration is not clinically feasible for several reasons, including side-effects related to systemic anticoagulation [22]. Moreover, there is increasing evidence to suggest that anti-inflammatory and anti-apoptotic cell signaling via the protein C pathway originates from protein C occupancy of the EPCR receptor [22,23]. Therefore, the aim of EPCR overexpression is to protect islets from thrombosis while enhancing the cytoprotective effects of the protein C pathway. Although the benefits of human EPCR (hEPCR) overexpression have been demonstrated in a pig-to-primate lung model [24,25], this has not been examined for islets. In the present study, we examined whether transgenically expressed hEPCR improved engraftment in a mouse model of syngeneic intraportal islet transplantation.

METHODS

Mice

Male C57Bl/6 mice transgenic for hEPCR [26] and wild-type (WT) littermates were used as islet donors at 10–14 weeks of age. Aged matched diabetic male Immunology Research Centre (IRC) mice [27] on the C57BL/6 background were used as recipients.

The recipient mouse line was generated at IRC by coinjection of C57BL/6 oocytes with a rat insulin promoter–driven expression construct for the rtTA2-M2-Tet-ON transactivator and a TRE-Tight VEGF-A construct. This line, named IRC, did not express vascular endothelial growth factor A (unpublished data) but developed spontaneous diabetes (see Results), likely as a result of beta-cell–specific Tet-ON expression interfering with islet development and/or function.

All mice were housed in ventilated cages in a specific pathogen–free environment. Experiments were performed blinded. All experiments involving animals were performed according to Australian National Health and Medical Research Council guidelines on the use of laboratory animals and were approved by the Animal Ethics Committee of St Vincent's Hospital, Melbourne.

Islet Isolation

Mice were killed by means of cervical dislocation and the abdominal cavity exposed by means of midline incision. The pancreas was perfused with 0.3 mg/mL collagenase P (Roche, Basel, Switzerland) reconstituted in Hank buffered salt solution containing 20 mmol/L HEPES and 2 mmol/L $CaCl_2$, excised, and digested at 37°C for 16 minutes. Digested pancreata were disrupted, filtered through a 500-μm nylon sieve, and washed with the use of serum-free RPMI medium (Invitrogen, Carlsbad,

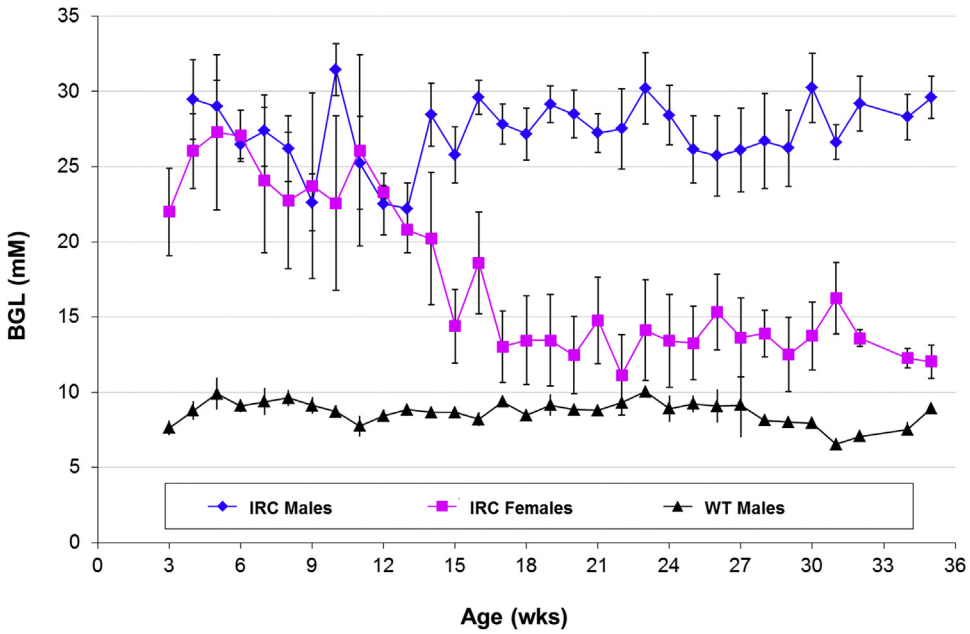

Fig 1. Blood glucose levels (BGLs) in spontaneously diabetic IRC transgenic mice. The mean ± SEM of ≥5 mice for each group per age is shown.