Transplant International

Transplant International ISSN 0934-0874

COMMENTARY

Islet culture and counter-culture

Commentary on: Effect of short-term culture on functional and stress-related parameters in isolated human islets by Ihm *et al.*

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doi:10.1111/j.1432-2277.2008.00794.x

The publication of the Edmonton protocol caused a lot of excitement by reporting an almost overnight improvement in the rate of success of clinical islet of Langerhans transplantation [1]. Conventional wisdom holds that steroid-free immunosuppression and an increased transplanted islet mass, thanks to the use of multiple donors for each recipient, were the key determinants for the success of the Edmonton protocol. However, the protocol incorporated several additional strategies in the effort to improve the results of the method. One of these was the transplantation of freshly isolated islets within 2 h of the end of the isolation procedure [1,2]. This approach was novel, but based on intuition rather than on hard data. Indeed, in the previous years, islet preparations were commonly cultured prior to transplantation [3]. In the wake of the Edmonton report, many islet transplant centers attempted to reproduce its results by implementing the same protocol, including immediate transplantation of fresh islets, as was the case in the first ever multicenter trial of islet transplantation, funded by the Immune Tolerance Network [2]. There are, however, obvious logistical advantages in culturing islets prior to transplantation: it provides flexibility in the organization and planning of the procedure, which involves interventional radiology access and anesthesiology standby, and it allows the pooling of islet preparations sequentially isolated from different donor pancreata, to increase the islet graft functional mass. Indeed, it could be shown that transplantation of cultured islets could lead to seemingly similar success rates as those described by the Edmonton group [4,5].

The question thus arises whether culturing islets for a period of time could have a beneficial or detrimental impact on islet viability and function, and on what mechanistic basis. This question is addressed by Dr. Ihm *et al.* in a recent issue of Transplant International [6] [Correction added after publication 31 March 2009: in the preceding sentence, this issue was corrected to a recent issue]. This is an important question and it is noteworthy that this is one of very few publications that have addressed this topic studying human islets. Results obtained in animal models may not necessarily be extrapolated to the human, because of the species-specific impact of culture on islet function [7].

The authors show that short-term culture has a beneficial effect on islet vitality and viability. Two-day culture increased islet cell ATP contents and decreased the activity of protein kinases involved in pro-apoptotic signalling. These positive findings are balanced by the increased expression of certain pro-inflammatory genes induced by short-term culture.

What matters to the islet transplant community, however, is the overall impact of short-term culture on *in vivo* islet function. It is well known and a matter of concern that culturing islets leads to a loss of function and mass

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during culture [8]. This study, using a nude mouse transplant bioassay shows a rather positive impact of short-term culture on post-transplant function of human islets. The finding that the diabetes reversal rate achieved with a set number of islets counted before culture is higher than that of the same number of freshly isolated islets demonstrates the superior function of cultured islets, even after considering islet loss during culture.

There is of course a stronger rationale for culturing islets prior to transplantation than the mere logistical advantage it offers. The long-term results of the Edmonton protocol have been rather sobering, as a progressive loss of graft function has been observed in a majority of patients [2]. A variety of mechanisms, including rejection and recurrence of autoimmunity, are likely to be involved in islet graft loss, but it is generally considered that the major cause may be cellular exhaustion as a result of a marginal engrafted islet mass caused by immediate cell loss [9]. It has long been known that islet cells suffer from cytokine- and oxidative stress-mediated injury during the brain death and ischemia periods, as well as during the digestion and purification phases of the isolation procedure. As a result, a significant proportion of islets are necrotic or undergo apoptosis at the end of the islet isolation process [10]. Additionally, islets have to face attack from elements of the innate immune system at the site of implantation, i.e. macrophages in the liver microenvironment and the newly described IBMIR (instant blood-mediated inflammatory reaction) elicited upon contact of islets with the portal blood and mediated by the expression of tissue factor by islet cells [9]. Therefore, designing strategies to offset these phenomena appears to be a prerequisite for the long-term success of clinical islet transplantation.

This is where pretransplant islet culture can be of substantial value. Besides improving islet vitality, the culture period can be exploited to treat or modify the islets so that they recover from previously sustained inflammatory insults or better withstand those to come. To take a few examples, antiapoptotic/cytoprotective effects have been obtained by culturing islets in the presence of heme-oxygenase-1 inducers, the newly discovered peptide obestatin, glucagon-like peptide 1 (GLP-1) or, more plainly, glucocorticoids [11–14]. Obestatin and GLP-1 have shown additional proliferative effects on beta cells [11,12]. On another standpoint, counteracting IBMIR was achieved by coating the surface of islets with heparin complexes during the culture period [15]. Finally, there are prospects of implementing cytoprotective or immunomodulatory strategies using gene therapy or protein transduction technologies [16,17], which would also rely on pretransplant islet culture.

The possibility of culturing the islets prior to transplantation offers a key opportunity to implement strategies to overcome the challenges raised by the low engrafted islet mass. This study by Ihm *et al.* shows that short-term islet culture *per se* may in fact be beneficial to islet vitality and functionality, and identifies certain noxious mechanisms that should be overcome to improve its potential further [6].

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