Brief Report

Limited Capacity of Human Adult Islets Expanded In Vitro to Rediferentiate Into Insulin-Producing β-Cells

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Limited organ availability is an obstacle to the widespread use of islet transplantation in type 1 diabetic patients. To address this problem, many studies have explored methods for expanding functional human islets in vitro for diabetes cell therapy. We previously showed that islet cells replicate after monolayer formation under the influence of hepatocyte growth factor and selected extracellular matrices. However, under these conditions, senescence and loss of insulin expression occur after >15 doublings. In contrast, other groups have reported that islet cells expanded in monolayers for months progressed through a reversible epithelial-to-mesenchymal transition, and that on removal of serum from the cultures, islet-like structures producing insulin were formed (1). The aim of the current study was to compare the two methods for islet expansion using immunostaining, real-time quantitative PCR, and microarrays at the following time points: on arrival, after monolayer expansion, and after 1 week in serum-free media. At this time, cell aliquots were grafted into nude mice to study in vivo function. The two methods showed similar results in islet cell expansion. Attempts at cell differentiation after expansion by both methods failed to consistently recover a β-cell phenotype. Redifferentiation of β-cells after expansion is still a challenge in need of a solution. *Diabetes* 56: 703–708, 2007

Expansion of human β-cells in monolayer culture leads to a progressive loss of insulin expression. We previously showed that epithelial cells derived from adult human islets can be expanded on human tumor bladder-9 (HTB-9) matrix in the presence of hepatocyte growth factor (2). Despite the loss of insulin expression after about five doublings, these epithelial cells persistently expressed pancreatic duodenal homeobox 1 (Pdx1). Several groups have reported varying degrees of success at expanding and subsequently redifferentiating islet cells in vitro (3,4), but in only one report (1), cell expansion up to 10^{12}-fold was obtained after growing the cells in tissue culture dishes without a matrix or exogenous growth factors by a process resembling epithelial-to-mesenchymal transition. These results, coupled with the capacity of the de-differentiated cells to regain insulin production on serum withdrawal, made this protocol particularly appealing for its potential for new β-cell output. In this study, we compared the islet expansion protocol of the Gershengorn (National Institutes of Health [NIH]) group (1) to ours (Whittier Institute [WHI]) and attempted to redifferentiate the expanded cells by the use of the same conditions.

RESULTS AND DISCUSSION

Comparison of the morphology of cells expanded from human adult islets. Six islet preparations from six different donors (supplementary Table 1, which can be found in an online appendix [available at http://diabetes.diabetesjournals.org]) were expanded. At all times, the cells propagated using the WHI protocol are strikingly more epithelial than the NIH cells (supplemental Fig. 1). Regardless of the method of expansion, there is a progressive decrease in the insulin content of the cells with each passage, and by passage 3 or 4, insulin becomes virtually undetectable (Fig. 1).

The doubling time of the NIH cells increased throughout the expansion phase. At passages 0, 1, 2, 3, and 4, the doubling times were 52.2, 95.86, 198.45, 208.13, and 290 h, respectively. Beyond that, the cells had to be passed 1:1. This is in contrast to the sustained 60-h doubling time previously reported (1). Ouziel-Yahalom et al. (4), who also expanded human adult islets using the same medium and culture conditions, with the difference that they thoroughly trypsinized the islets before culture, have reported 7-day doubling times. We previously reported a 25-h doubling time for the WHI cells (2).

Comparison of the phenotypes of the expanding cells. Insulin expression in both protocols at the end of 1 week is shown in Fig. 2*A* and *B*, respectively. Coexpression of insulin and Ki67, a marker of proliferation, was rare.

We argued previously (2) that replicating Pdx1-positive cells represent β-cells that no longer express insulin and are not ductal cells because they are panCK (pan-cytokeratin) positive and CK-19 (cytokeratin-19) negative. Therefore, we compared Pdx1 expression in the NIH and WHI protocols. At 1 week, in the NIH protocol, cells expressed Pdx1 mostly inside islet remnants, and only ~1% displayed costaining for Ki67 (Fig. 2*C*). In the WHI cells, 14% of the Pdx1-positive cells costained for Ki67, indicating that

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DKK1, Dickkopf 1; HTB, human tumor bladder; NIH, National Institutes of Health; Pdx, pancreatic duodenal homeobox; WHI, Whittier Institute.

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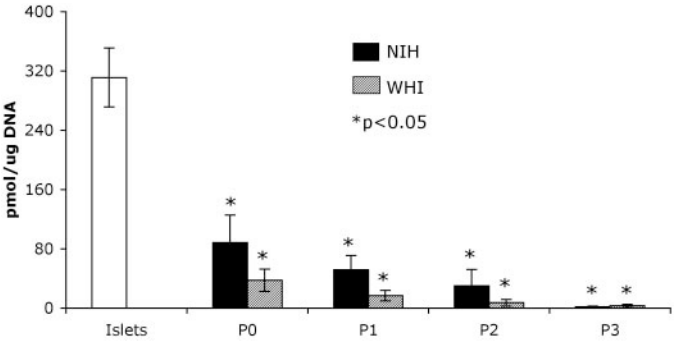

FIG. 1. Insulin content diminishes with each passage in human islets expanded in monolayer. Insulin content of human islets and the NIH and WHI expanded cells at the end of each passage are the means ± SE. Each bar represents at least five samples. Insulin was determined in acid-ethanol–extracted cell samples using a Mercodia human insulin enzyme-linked immunosorbent assay kit (Alpco Diagnostics). After serum withdrawal, C-peptide was undetectable in clusters from cells expanded using both protocols. P, passage.

Pdx1-positive cells were replicating (Fig. 2*D*). At the end of three or four passages, though, the Pdx1-positive cells were few in number for either protocol (data not shown).

Vimentin, a type III intermediate protein normally found in mesenchymal cells, has been used as a molecular marker of epithelial-to-mesenchymal transition in epithelial cells (rev. in 5). Vimentin is always expressed in physiological or pathological situations requiring epithelial migration, such as embryological and organogenesis processes (6). The cells propagated by the NIH protocol had been shown to express vimentin (1). Despite the epithelial morphology of the cells expanded on HTB-9 matrix in the presence of hepatocyte growth factor (a generous gift from Genentech, San Francisco, CA), these cells also exhibited vimentin (Fig. 3*B*). Vimentin expression in the NIH cells at the end of passage 1 was robust in cells with elongated fibroblast-like morphology and filaments stained brightly with the vimentin antibody (Fig. 3*A*).

To further characterize the cells that stain weakly for vimentin, we stained for other epithelial markers in WHI cells. Figure 3*C* and *F* shows the coexpression of cytokeratin and vimentin in three passages of the WHI cells. The expression of vimentin filaments along the preexisting filament network has been reported in the epithelial-to-mesenchymal transition of neonatal rat hepatocytes in culture (7). In the NIH cells, the majority of the cells expressed only vimentin, whereas there were some epithelial cells that expressed only cytokeratin (Fig. 3*I*).

A defining feature of epithelial-to-mesenchymal transition is a reduction of E-cadherin expression (5). Immunohistochemical studies confirmed that during the first 8 days of expansion, some of the WHI cells maintained their

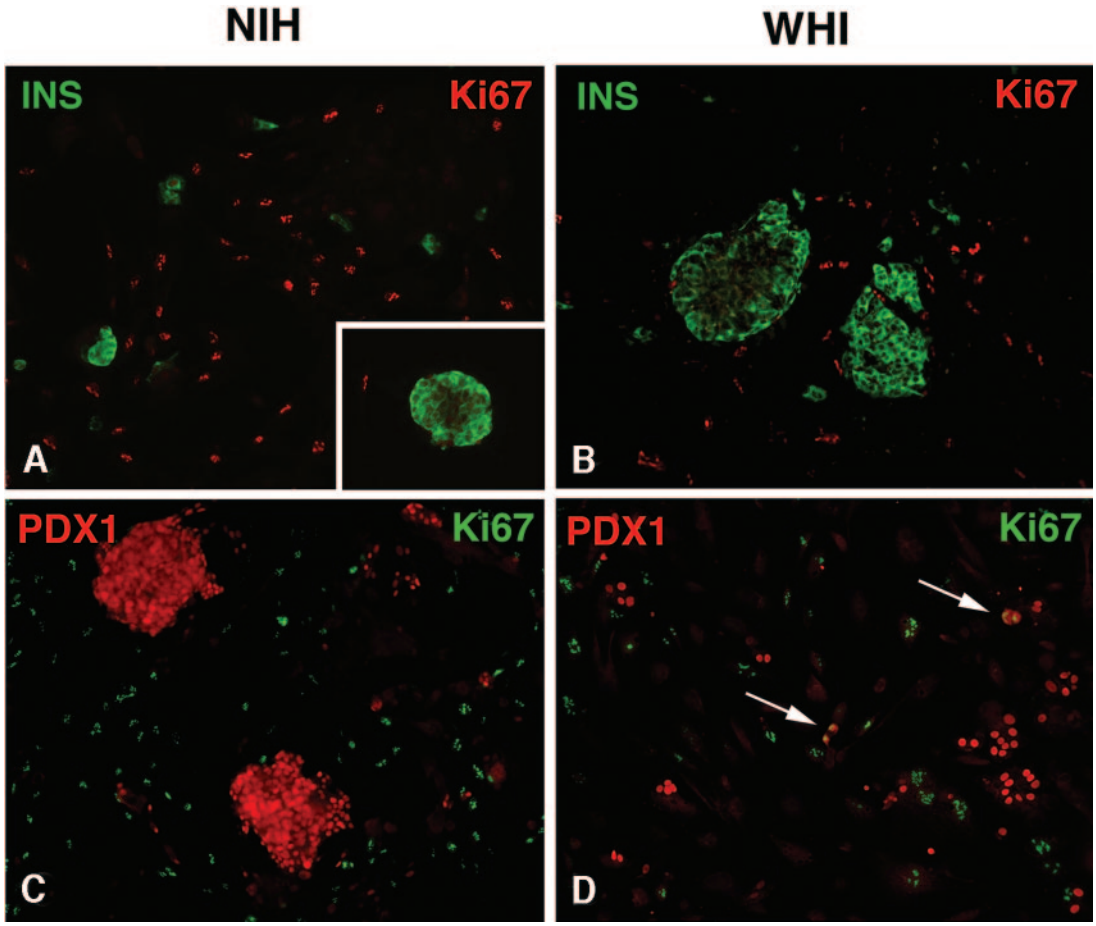

FIG. 2. Insulin and Pdx1 expression in cells expanded from human adult islets. *A* and *B*: Fluorescein isothiocyanate staining (green) shows cells stained with insulin. Rhodamine-staining (red) cells are Ki67 positive. Cells expanded by the NIH and WHI protocols for 1 week express insulin in the islets that are still intact. Replicating cells display Ki67 staining. Colocalization of insulin and Ki67 was not observed in these experiments (*A* and *B*). *C* and *D*: Fluorescein isothiocyanate staining (green) shows cells stained with Ki67. Rhodamine staining (red) shows Pdx1-positive cells. At the end of 1 week, most Pdx1-positive cells in the NIH protocol were in islet remnants, and no colocalization was seen (*C*). At 1 week WHI expanded cells display many Pdx1-positive clusters (*D*). A number of cells display colocalization of Pdx1 and Ki67 (arrows, *D*).

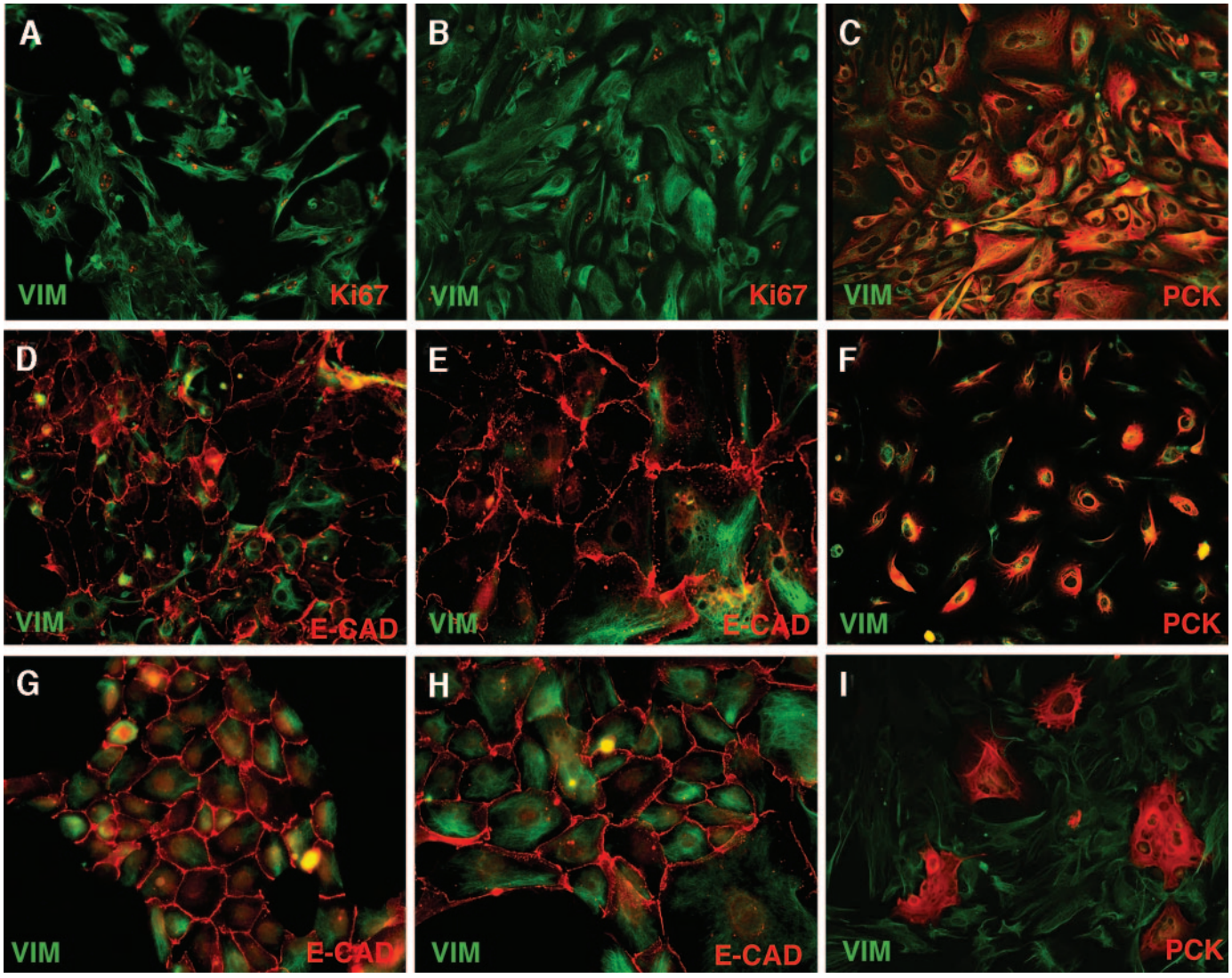

FIG. 3. Expression of epithelial and mesenchymal markers. *A* and *B*: Fluorescein isothiocyanate–staining (green) cells are positive for vimentin. Rhodamine-staining (red) cells are Ki67 positive. In NIH expanded cells, at the end of passage 1, robust vimentin expression is observed in elongated fibroblast-like cells, many of which were dividing (*A*). WHI cells at the end of passage 1 exhibit faint vimentin staining in cells that look morphologically epithelial (*B*). Note that a few of the cells do show stronger vimentin expression. *C*, *F*, and *I*: Fluorescein isothiocyanate–stained (green) cells are positive for vimentin, and rhodamine-staining (red) cells are cytokeratin positive. *C* and *F*: WHI cells expanded after one (*C*) and three passages (*F*) and NIH cells expanded after one passage (*I*). During the three passages, WHI cells display colocalization of vimentin and pan-cytokeratin. NIH cells display two populations of cells; many of the cells express vimentin, and only a few express cytokeratin. *D*, *E*, *G*, and *H*: WHI cells expanded for 2 days (*D*), 4 days (*E*), 7 days (*G*), and 8 days (*H*) were stained with anti–E-cadherin (rhodamine [red]) and anti-vimentin (fluorescein isothiocyanate [green]) antibodies. Colocalization of E-cadherin and vimentin is observed throughout the week of expansion by cells with epithelial morphology.

epithelial characteristics while expressing E-cadherin and vimentin simultaneously (Fig. 3*D*, *G*, and *H*). Other cells lost their columnar shape and E-cadherin expression on the membrane and displayed strong vimentin staining (Fig. 3*E*). These findings suggest that some of the WHI cells had undergone an epithelial-to-mesenchymal–like transition, whereas others were in a transitional state.

Morphology of differentiating cells. After expansion, the cells were kept in serum-free medium to induce redifferentiation (1). In this report, we refer to this serum-free culture period when the cells no longer expand as the "differentiation" phase. Supplemental Fig. 2 (*top panel*) shows the cells at the beginning of the differentiation step. After 12–24 h in the differentiation medium, the NIH cells looked mesenchymal (supplemental Fig. 2*C*), and within the next 12 h they formed clusters spontaneously (supplemental Fig. 2*E*). When we attempted to differentiate the WHI cells in the absence of HTB-9, spontaneous clusters formed (supplemental Fig. 2*D*), but the cells did not survive. Therefore, the cells were kept on HTB-9 matrix. Under these conditions, they displayed a mixture of epithelial and fibroblastic morphology (supplemental Fig. 2*F*). To obtain clusters at the end of the differentiation stage, the cells were reaggregated in vitro after dissociation from the matrix. The islet-like clusters formed by the two protocols are shown in supplemental Fig. 2*G* and *H* (*bottom panel*).

Hormone expression of expanded versus differentiated cells. Insulin and glucagon message levels in the islets and at the end of expansion and differentiation phases were assessed by quantitative RT-PCR. In both protocols, at the end of expansion and after serum withdrawal, insulin was profoundly downregulated (Fig. 4*A*).

In one experiment with islets from a donor (no. 5) with

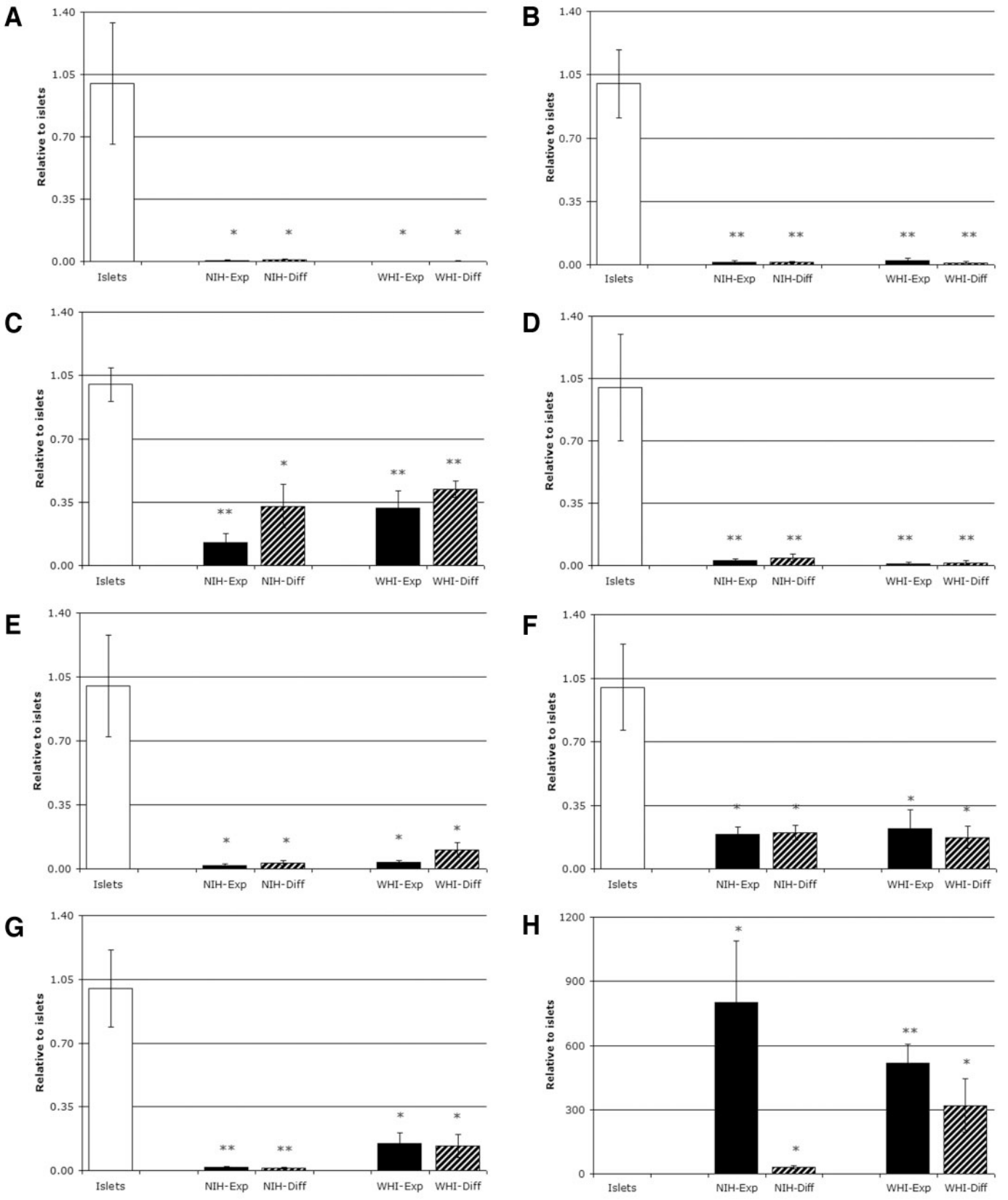

FIG. 4. Comparison of mRNA levels in adult human islets and cells expanded by the NIH and WHI protocols by quantitative RT-PCR. *A–G*: Each bar represents the means ± SE of samples from five donors. mRNA levels are expressed as relative values with respect to the amount found in islets, set as 1. *$P < 0.05$, **$P < 0.005$ vs. islets. Insulin message is downregulated in islet cells expanded by the NIH and WHI protocols ($P < 0.05$) (*A*). Glucagon message follows the same pattern ($P < 0.005$) (*B*). The differentiation attempts do not result in an increase in insulin or glucagon

a BMI of 48, insulin expression increased at the end of the differentiation phase, but to only 6% of that measured in normal islets. The same was seen with glucagon (Fig. 4*B*).

Transcription factors in expanded versus differentiated cells. A comparison of PDX1 expression at the end of the expansion phase for the two protocols revealed that PDX1, although decreased significantly compared with islets (NIH, $P < 0.005$; WHI, $P < 0.05$), was somehow better maintained in the hepatocyte growth factor–treated WHI cells (Fig. 4*C*), confirming our earlier report. In cells expanded by both the NIH and the WHI protocols, the expression of PDX1 appeared to increase on differentiation, but even with this increase, PDX1 message levels were <30% of that seen in islets. Islet1 (ISL1), a transcription factor of the LIM homeodomain family, was also reduced dramatically ($P < 0.05$) compared with islets and appeared to modestly increase after serum withdrawal (possibly because of an increase in the level of ISL1-expressing mesenchymal elements) (Fig. 4*E*) (8).

Mesenchymal and epithelial marker expression in the expanded versus differentiated cells. In general, the E-cadherin expression of NIH expanded cells was low in comparison to islets (NIH, $P < 0.005$; WHI, $P < 0.05$) (Fig. 4*G*). We did not observe a consistent shift in E-cadherin message expression in response to serum withdrawal, suggesting either that the epithelial-to-mesenchymal transition is not reversed or that regulation is not occurring at the message level.

A gene that was regulated most prominently in both protocols was Dickkopf 1 (DKK1) (Fig. 4*H*). DKK1 is a canonical Wnt/β-catenin signaling pathway inhibitor secreted by mesenchymal stem cells that can stimulate the expansion of these cells (9). DKK1 expression was upregulated >300-fold in expanded cells compared with the islets. On serum withdrawal, DKK1 levels were downregulated in NIH cells ($P < 0.05$).

Microarray analysis. The microarray expression profiles of expanded and redifferentiated samples were highly different from fresh islets and to some extent within themselves, probably reflecting donor variability. Differential expression analysis of NIH expanded and differentiated versus normal islets identified 2,429 and 2,465 genes, respectively (false discovery rate [FDR] of 0.001). We observed downregulation of insulin expression and other islet hormones as well as dramatic downregulation of exocrine-enriched genes that probably contaminated all of the islet preparations (data not shown). Correspondence analysis did not show any significant gene expression changes in expanded versus differentiated cells, but their gene profiles showed significant differences compared with those found in fresh islets (supplemental Fig. 3). Thus, combining the microarray results with quantitative PCR and protein expression data, it seems clear that the gene profile of the expanded cells does not revert to the original phenotype.

Serum withdrawal does not bring back insulin expression. A principal finding for both protocols is that withdrawal of serum after expansion in monolayer showed no measurable return of insulin expression (Fig. 1), as shown by the lack of insulin content in the cells analyzed. In only one preparation (donor no. 5), a few cells stained positive for C-peptide (supplemental Fig. 4), but in this experiment, residual cell clusters persisted, as visualized by phase contrast microcopy (supplemental Fig. 2*G*). The insulin-positive cells may have resulted from carryover from the last passage of the expansion.

Experiments with human islets show an inherent variability because of multiple factors such as the conditions of the islets after isolation and shipping, the age and metabolic status of the donor, and other variables. Obesity causes compensatory growth in the endocrine pancreas (10) and results in larger and more robust islets (rev. in 11). Quantitative PCR and microarray results from cells derived from donor no. 5 (BMI of 48 kg/m^2) showed some, though still much reduced, response to serum withdrawal (data not shown).

Transplant experiments. At 2 months after transplantation of the islets or expanded cells into athymic mice, serum C-peptide in response to a glucose challenge was 454.3 ± 117.7 pmol/l (means ± SE) in control mice. There was no human C-peptide detected in any of the mice receiving the expanded cells. In accordance with these findings, only control animals showed viable transplants after kidney removal (supplemental Fig. 5).

These studies show that epithelial-to-mesenchymal–like transition of islet-derived cells appears to occur in monolayer culture. We cannot, however, rule out that the cells that we propagated were not contaminating (passenger) mesenchymal cells from the start, especially in the NIH protocol, where the islets were not handpicked or enriched by removing the outer layer of the islet. This question can only be answered by lineage tracing studies. The spontaneous formation of clusters and quantitative PCR results showing minimal shifts in RNA expression suggest that the NIH expanded cells are phenotypically different from the WHI cells. However, the microarray data suggests that the islet-like clusters are closer in phenotype to mesenchymal cells than to the endocrine cells from which they are derived. Taken together, these data demonstrate that a process by which functional islets can be generated from expanded primary islet sources using these and further culture manipulations remains to be achieved.

In summary, in neither protocol were we able to expand the cells as vigorously as reported with the original NIH protocol. We were also unable to consistently obtain cells resembling the unexpanded islets that robustly express multiple end-stage pancreatic markers in vitro or in vivo.

RESEARCH DESIGN AND METHODS

Human adult islets. Human adult islets were provided by the Islet Cell Resource Center Basic Science Human Islet Distribution Program and the Islet Transplant Program at the University of Illinois at Chicago. We used preparations from six donors, aged 25–55 years (supplementary Table 1), that were 60–90% pure in mature islets and with a viability of 80–90%.

Expansion phase

NIH protocol. As previously described (1), 2,000 islet equivalents enriched by retention on a 40-μm filter were seeded onto tissue culture–treated dishes in CMRL-1066 medium (Invitrogen, Carlsbad, CA) containing 2 mmol/l L-glu-

message (*A* and *B*). Expression of Pdx1 is also decreased to ~10% compared with the islets in the NIH expanded cells ($P < 0.05$) (*C*). There is a trend toward an increase in PDX1 message with differentiation, but only up to 30% of the islet expression (*C*). In WHI expanded cells, PDX1 expression appears higher compared with the NIH cells (*C*). Transcription factor PAX6 expression is profoundly diminished in both protocols pre- and postdifferentiation ($P < 0.005$) (*D*). Both expansion protocols display reduced ISL1 expression ($P < 0.05$) (*E*). There is a trend toward increased ISL1 expression in WHI cells with differentiation. NKX6.1 message levels are reduced in all expanded and differentiated cells ($P < 0.05$) (*F*). E-cadherin message is significantly reduced in cells expanded by both protocols (NIH, $P < 0.005$; WHI, $P < 0.05$) (*G*). DKK1 expression is highly induced in the expansion phase of both protocols (NIH, $P < 0.05$; WHI $P < 0.005$) (*H*). In the NIH protocol, differentiation results in a significant decrease in DKK1 message ($P < 0.05$). Diff, differentiation; Exp, expansion.

tamine and 10% fetal bovine serum. Cells were cultured for 2 weeks (passage 0), and after that cells were harvested every 4–7 days with trypsin and subcultured (1:2) for up to 2 months.

Whittier protocol. A total of 1,000 islets of 50–150 μm in diameter purified by handpicking after dithizone staining (12) were partially dissociated using Versene (Invitrogen) (13) to separate "outer" and "inner" populations (2). Cell clusters from the inner population were plated on HTB-9 matrix–coated dishes (14) in RPMI-1640 medium (Mediatech, Herndon, VA) supplemented with 2 mmol/l L-glutamine, 10% fetal bovine serum, and 25 ng/ml hepatocyte growth factor. After confluence, cells were harvested, using Versene containing 0.025% trypsin, and subcultured (1:2).

Differentiation phase

NIH protocol. Cells expanded for up to eight passages (2 months) were dispersed with trypsin and cultured in serum-free CMRL-1066 medium supplemented as previously described (1). Clusters formed after 1 week in culture were handpicked for further analysis.

Whittier protocol. After four passages (~1 month expansion), cells were dispersed with Versene and cultured in the same serum-free media as NIH cells on tissue culture–treated 6-well plates, with or without HTB-9 matrix. After 1 week of culture on HTB-9 coated plates, cells were harvested and forced to reaggregate overnight (15).

Insulin, C-peptide, and DNA content. Insulin content of islets and expanded cells at the end of each passage and C-peptide content of clusters postdifferentiation phase were assayed with Mercodia enzyme-linked immunosorbent assay kits (Alpco Diagnostics, Windham, NH). Results were expressed as picomoles per microgram of DNA (assayed by fluorometric method).

Microarray analysis. RNA was purified using an RNEasy kit (Qiagen, Chatsworth, CA) and treated with RNase-free DNaseI (Promega, Madison, WI) for 15 min at room temperature during isolation. Integrity and quality of the isolated total RNA were verified with Agilent BioAnalyzer nanochips (data not shown), and microarray assays were performed with Affymetrix GeneChip HG U133 Plus 2.0 arrays. Following the standard Affymetrix protocol, 2 μg of total RNA was used to perform one-cycle cDNA synthesis. Biotin-labeled and fragmented cRNA was used for microarray hybridizations.

Data analysis. Low-level processing, normalization, and statistical analysis was performed using R/Bioconductor packages (16). Annotations from R-package hs133pEntrezGene (version 7) were used to obtain expression values for 17,814 genes represented on the array (17). Microarray data from different samples were filtered based on intensity and variation, and statistical analysis was performed with the shrunken variance estimates using linear model–fitted data. Correspondence analysis was used as an explorative computational method for the study of associations between variables.

Real-time quantitative PCR. RNA was isolated from samples with a 6,100–nucleic acid extractor (Applied Biosystems), and 100–500 ng was used for reverse transcription with an iScript cDNA synthesis kit (Bio-Rad). PCRs were run in duplicate using 1/40th of the cDNA per reaction and 400 nmol/l forward and reverse primers with QuantiTect SYBR Green master mix (Qiagen). Alternatively, QuantiTect primer assays (Qiagen) were used according to the manufacturer's instructions. Real-time PCR was performed using the Rotor Gene 3000 (Corbett Research). Relative quantification was performed in relation to a standard curve. The standard curve was created using a mixture of total RNA samples from various human fetal and adult endoderm tissues (including whole-organ pancreatic RNA) and differentiated human embryonic stem cells. Quantified values for each gene of interest were normalized against the input determined by two housekeeping genes (CYCG [cyclophilin-G] and GUSB [glucuronidase-β] or TBP [TATA binding protein]). After normalization, the samples were plotted relative to the standard curve for that gene. Primers used in this study are shown in supplementary Table 3.

Immunohistochemistry. Fluorescent staining of cells expanded in monolayer and of paraffin sections of islets or reaggregated clusters previously embedded into fibrin clots was performed and analyzed as previously described (2,18,19). Antibodies used are shown in supplementary Table 3.

Transplantation. After the serum withdrawal phase, 500 cell clusters from four different experiments were placed under the kidney capsule of five athymic nude mice for each protocol as previously described (20). Three mice used as controls were grafted with 500 fresh human adult islets. Then, 2–3 months later, fasted animals were given 3 g/kg glucose i.p., and after 30 min blood samples were taken for the assay of circulating human C-peptide with an enzyme-linked immunosorbent assay kit (Mercodia; Alpco Diagnostics), with no cross-reactivity to the mouse C-peptide. Serial sections of the kidney-bearing grafts were examined histologically for insulin-containing cells as described above.

Statistical analysis. Student's *t* test was used to analyze the data in Figs. 1 and 4.

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REFERENCES

1. Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B, Raaka BM: Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. *Science* 306:2261–2264, 2004
2. Beattie GM, Itkin-Ansari P, Cirulli V, Leibowitz G, Lopez AD, Bossie S, Mally MI, Levine F, Hayek A: Sustained proliferation of PDX-1+ cells derived from human islets. *Diabetes* 48:1013–1019, 1999
3. Lechner A, Nolan AL, Blacken RA, Habener JF: Redifferentiation of insulin-secreting cells after in vitro expansion of adult human pancreatic islet tissue. *Biochem Biophys Res Commun* 327:581–588, 2005
4. Ouziel-Yahalom L, Zalzman M, Anker-Kitai L, Knoller S, Bar Y, Glandt M, Herold K, Efrat S: Expansion and redifferentiation of adult human pancreatic islet cells. *Biochem Biophys Res Commun* 341:291–298, 2006
5. Lee JM, Dedhar S, Kalluri R, Thompson EW: The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 172:973–981, 2006
6. Gilles C, Polette M, Zahm JM, Tournier JM, Volders L, Foidart JM, Birembaut P: Vimentin contributes to human mammary epithelial cell migration. *J Cell Sci* 112 (Pt. 24):4615–4625, 1999
7. Pagan R, Martin I, Alonso A, Llobera M, Vilaro S: Vimentin filaments follow the preexisting cytokeratin network during epithelial-mesenchymal transition of cultured neonatal rat hepatocytes. *Exp Cell Res* 222:333–344, 1996
8. Ahlgren U, Pfaff SL, Jessell TM, Edlund T, Edlund H: Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. *Nature* 385:257–260, 1997
9. Horwitz EM: Dkk-1-mediated expansion of adult stem cells. *Trends Biotechnol* 22:386–388, 2004
10. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110, 2003
11. Prentki M, Nolan CJ: Islet beta cell failure in type 2 diabetes. *J Clin Invest* 116:1802–1812, 2006
12. Latif ZA, Noel J, Alejandro R: A simple method of staining fresh and cultured islets. *Transplantation* 45:827–830, 1988
13. Beattie GM, Cirulli V, Lopez AD, Hayek A: Ex vivo expansion of human pancreatic endocrine cells. *J Clin Endocrinol Metab* 82:1852–1856, 1997
14. Langhofer M, Hopkinson SB, Jones JC: The matrix secreted by 804G cells contains laminin-related components that participate in hemidesmosome assembly in vitro. *J Cell Sci* 105 (Pt. 3):753–764, 1993
15. Rouiller DG, Cirulli V, Halban PA: Differences in aggregation properties and levels of the neural cell adhesion molecule (NCAM) between islet cell types. *Exp Cell Res* 191:305–312, 1990
16. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J: Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 5:R80, 2004
17. Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, Bunney WE, Myers RM, Speed TP, Akil H, Watson SJ, Meng F: Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. *Nucleic Acid Res* 33:e175, 2005
18. Beattie GM, Rubin JS, Mally MI, Otonkoski T, Hayek A: Regulation of proliferation and differentiation of human fetal pancreatic islet cells by extracellular matrix, hepatocyte growth factor, and cell-cell contact. *Diabetes* 45:1223–1228, 1996
19. Otonkoski T, Cirulli V, Beattie M, Mally MI, Soto G, Rubin JS, Hayek A: A role for hepatocyte growth factor/scatter factor in fetal mesenchyme-induced pancreatic beta-cell growth. *Endocrinology* 137:3131–3139, 1996
20. Hayek A, Beattie GM: Experimental transplantation of human fetal and adult pancreatic islets. *J Clin Endocrinol Metab* 82:2471–2475, 1997