

## Cellular Composition of Islet Cell Suspensions for Transplantation

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A SIGNIFICANT number of nonislet cells still remains in cell suspensions from human islet cell preparations obtained by our previously described method.<sup>1,2</sup> Nonislet components such as acinar cells or immunogenic hemolymphoid cells could have adverse effects on initial graft taking as well as long-term graft survival through mechanisms of enzymatic injury and initiation of rejection.<sup>3</sup> In this study, we have examined in detail the cellular composition of islet cell suspensions using paraffin-embedded cell pellets of the isolates immediately after purification and after 1 week in culture medium.

### MATERIALS AND METHODS

Human islets obtained from six different multiorgan donors were separated and purified by the automated method and Euro-Collins-Ficoll gradient separation using the COBE 2991 cell processor.<sup>1,2</sup> A portion of the suspension was fixed in buffered formaldehyde, and cell pellets were obtained by centrifugation at 800 g for 3 minutes. Additional pellets were prepared after the remaining suspensions were maintained in CMRL-1066 culture medium at 30°C in 5% CO<sub>2</sub> for 1 week. All formalin-fixed pellets were paraffin embedded, sectioned, and stained with the avidin-biotin-complex (ABC) immunoperoxidase method, as described by Hsu.<sup>4</sup> A panel of antibodies was used with antibodies directed against insulin, glucagon, chromogranin, actin (all Biogenex, San Ramon, CA), AE-1, AE-3 (Boehringer Mannheim, Indianapolis, IN), vimentin (Sigma, St Louis, Mo), factor VIIIIR-Ag, S100, LCA/CD45, MAC-387, lysozyme, UCHL-1, L26/CD20 (all DAKO, Santa Barbara, Calif), LN3/HLA-DR (Biotest Diagnostics, Denville, NJ), and Leu-22/CD43 (Becton-Dickinson, Mountain View, Calif). Small fractions from two preparations were fixed in 2% glutaraldehyde, postfixated in 1% osmium tetroxide, embedded in Epon-Araldite resin, stained with 4% uranyl acetate followed by lead citrate, and examined with a Phillips EM 300 transmission electron microscope.

### RESULTS AND DISCUSSION

Immediately after separation, the isolates contained 30% to 80% islet cells; however, all other cell types found in normal pancreas were also present. This included HLA-DR and S100 positive dendritic cells, which are known as potent stimulators of immune reactions, including rejection.<sup>5</sup> The difference in purity of the various

preparations was mainly due to a different content of islet and acinar cells; whereas ductal, soft tissue, and hemolymphoid cells comprised approximately 10% to 20% in all preparations. One week of culture had different effects on moderately and highly purified preparations: In less purified isolates (30% to 40% islets), the islet cell content dropped to as low as 5%. In addition, remaining islets cells weakly expressed HLA-DR. Remaining cells were almost exclusively acinar cells; however, scattered endothelial cells and a few lymphoid aggregates as well as very rare HLA-DR positive macrophages could still be detected. In contrast, highly purified isolates (70% to 80% islets) mainly showed decreased insulin content without significant loss of islet cells or new HLA-DR expression. Epithelial, soft tissue, and hemolymphoid elements decreased only minimally. It appears that reduction of immunogenic cells in islet cell suspensions cannot be achieved solely by primarily mechanical purification procedures but requires additional measures. Short-term culture only reduces but does not eliminate immunogenic cells and is deleterious to islet cells if the initial content of acinar cells is high.

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