1448

Microbiologic Surveillance as Part of Human Islet Transplantation: Results of the First 26 Patients

P.B. Carroll, C. Ricordi, P. Fontes, H.R. Rilo, J. Phipps, A.G. Tzakis, J.J. Fung, and T.E. Starzl

THE major risks of islet transplantation described in the past, portal hypertension,[1] portal vein thrombosis and hepatic infarction,[2] and coagulopathy[3] have been solved by the current methods of purification of islet preparations that allow for an infusion of low volume (usually < 2 mL) of highly purified cells. Since patients who receive islet grafts are immunosuppressed, infection is always of concern. Microbiologic quality control samples have been recommended as a mandatory part of any clinical islet cell program to assess the infectious risk of islet cell transplant since contamination of transport solution is not uncommon and contamination of reagents can occur.[4,5] We have performed quality control microbiologic surveillance during human islet isolations from pancreata considered for possible clinical transplantation and found that, while the transport solution is frequently contaminated, the final islet preparations were sterile even when obtained from a pancreas with an infected pseudocyst.

METHODS

Between January 10, 1990 and the time of this report, 26 patients underwent islet cell transplantation with one or more purified islet cell preparations for indications of extensive cancer resection (cluster with liver transplantation),[6] autograft, or type I diabetes with simultaneous liver or kidney transplantation.

Pancreas Procurement

The pancreata were obtained from multiorgan donors.[7] In situ perfusion of the abdominal aorta was with 1500 to 2000 mL of University of Wisconsin solution (UWS).

An additional 500 to 1000 mL of UWS was infused directly into the liver via the portal vein, that was encircled below the catheter tip to prevent retrograde leakage. Venous hypertension of the pancreas was avoided by venting the portal and/or splenic vein. The specimens were immersed in UWS and packed on ice.

Isolation and Purification of Human Islets

Human islets were obtained by a modification[8] of the automated procedure.[9] The islets were purified on discontinuous gradients of Eurocollins Ficoll (Ficoll DL-400, Sigma, St Louis, Mo) at densities of 1.108, 1.096, and 1.037. The gradients were prepared fresh for each human isolation. The cells were purified by centrifugation through the gradients on a Cobe 2991 cell separator (Cobe, Lakewood, Colo).[10,11]

Gradient Preparation

All reagents used in the isolations reported herein were prepared fresh using aseptic technique, under a class II laminar flow hood (Nuaire, model 425-600, Plymouth, Minn). Routine surgical asepsis was used in the laboratory that was not a class 100 clean room. Stock ficoll solution was completed then filtered under the hood using a 0.2 micron filter (Millipore, Milipak 200, Bedford, Mass). After filtering, the final Ficoll stock solution was autoclaved for 13 minutes.

Quality Control Samples

Routine samples were taken from the transport UWS solution, the stock Ficoll solution used to prepare gradients, and the final islet preparation.

In the case of the autografts, a small section of pancreas was also cultured.

The sterile container with the pancreas in UWS was transferred to the hood and opened. The pancreas was removed and placed into a stainless steel pan on ice with sterile Hank's solution for further processing. Approximately 10 mL of UWS was removed from the transfer container with a sterile syringe and 5 mL were injected under sterile conditions into culture vials of soybean-casein digest broth (anaerobic) and tryptic soy broth (aerobic) Bactec (Beckton Dickinson Diagnostic Instrument Systems, Townson, Md). Five milliliters of the stock ficoll used in the isolation were processed similarly. After the purification step was completed, 0.1 mL of the final islet preparation was resuspended in 10 mL of the supernatant from the last wash and 5 mL of this solution were injected into the aerobic and anaerobic culture bottles as described above. The aerobic culture vials have resins that increase the likelihood of culturing organisms even in the presence of antibiotics used during the isolation. All samples were processed for growth of aerobic, anaerobic, and fungal organisms, and were incubated for 5 days. If the vials showed any growth during this time period, they were examined by gram stain and were processed further for identification and sensitivity studies.

RESULTS

The UWS was contaminated with microbes in 8 of 26 isolations (31%). Patients received antibiotic coverage for bacterial contaminants found in UWS until final results of islet preparations were known. The organisms cultured from UWS included aerobic, anaerobic, and fungal organisms. The most common organism isolated was coagulase-negative *Staphylococci*. There was no instance of contamination of stock Ficoll solutions used in clinical

From the Department of Medicine (P.B.C.) and Surgery (P.B.C., C.R., P.F., H.R.R., J.P., A.G.T., J.J.F., T.E.S.), University Health Center of Pittsburgh, University of Pittsburgh, and the Veterans Administration Medical Center, Pittsburgh, Pennsylvania.

Supported by research grants from the Veterans Administration and Project Grant No. DK 29961 from the National Institutes of Health, Bethesda, Maryland.

Address reprint requests to P.B. Carroll, University of Pittsburgh Transplantation Division, 5W Falk Clinic, 3601 5th Avenue, Pittsburgh, PA 15213.

0041-1345/92/$3.00/+0

transplants. The final islet preparations were all free of contamination. This included one isolation for autotransplant in which a pseudocyst from the native pancreas cultured positive for *Pseudomonas maltophilia*, but after being processed using standard techniques, the final islet preparation showed no growth of the organism. This patient received a full course of antibiotics for the infected pseudocyst independent of these results. No patients had a systemic infection related to an islet cell graft.

DISCUSSION

The frequency of contamination of UWS for transport of pancreata are compatible with data obtained by other investigators who reported that between 25% and 68% of transport solutions processed per year were contaminated with one or several organisms.[12] Ficoll stock solution has been a significant source of addition of microbial contaminants in the past. The sterility of the current samples may reflect the introduction of filtering the prepared stock solution through the Millipore Millipak prior to autoclaving.

We have recently moved our basic and clinical facilities into a class 100 laminar air flow clean room for human islet isolation. This is suggested to decrease introduced microbes considerably[13]; however the data reported herein attest to the safety of processing cells using a standard clean room and aseptic operating room techniques if such a facility is not available. Quality control microbial surveillance of transport solutions, reagents, and the final islet preparations used for clinical transplantation must continue to be part of the assessment of materials used for clinical transplantation.

ACKNOWLEDGMENTS

The authors would like to thank Dr A. William Pasculle and Amy Wade for their help with the microbiology, and Katie Murray for secretarial assistance.

REFERENCES

1. Mehigan DG, Bell WR, Zindema GO, et al: Ann Surg 191:287, 1980

2. Walsh TJ, Eggleston JC, Cameron JL: Surgery 91:485, 1982

3. Mittal VK, Toledo-Pereyra LH, Sharma M, et al: Transplantation 31:302, 1981

4. Scharp DW, Lacy PE, In Dubernard JM, Sutherland DER (eds): International Handbook of Pancreas Transplantation. Norwell, Mass: Kluwer, 1989, p 455

5. Corlett MP, Scharp DW, Lacy PE: Transplant Proc 22:894, 1988

6. Tzakis A, Ricordi C, Alejandro R, et al: Lancet 336:402, 1990

7. Starzl TE, Miller C, Broznick B, et al: Surg Gynecol Obstet 165:343, 1987

8. Ricordi C, Tzakis AG, Carroll PB, et al: Transplantation 53:407, 1992

9. Ricordi C, Lacy PE, Finke EH, et al: Diabetes 37:413, 1988

10. Lake SP, Basset PD, Larkins A, et al: Diabetes 38(Suppl): 143, 1989

11. Alejandro R, Strasser S, Zucker PF, et al: Transplantation 50:207, 1990

12. Scharp DW, Lacy PE, McLean M, et al: Transplant Proc (in press)

13. Scharp DW, In Ricordi C (ed): Pancreatic Islet Cell Transplantation. Georgetown, TX, Landes, 1992, p 82