Rapid Publication

Use of Reflected Green Light for Specific Identification of Islets in Vitro After Collagenase Isolation

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SUMMARY

A simple technique is described that permits specific identification in vitro of isolated islets as contrasted with small lymph nodes. DIABETES *28*:612–613, June 1979.

Passenger leukocytes (lymphocytes and macrophages) in tissue transplants may play a significant role in the initiation of immune rejection.[1] Lafferty et al.[2] reported that organ culture of the thyroid prior to transplantation prolonged the survival of mouse thyroids transplanted across a major histocompatibility barrier. The prolongation of survival was apparently due to removal of passenger leukocytes during in vitro culture since injection of peritoneal exudate cells from the donor strain initiated rejection of the transplanted, cultured thyroids.[3]

Studies in our laboratory have shown that islet allograft survival can be prolonged by using pretreatment regimens of donor rats to diminish the passenger leukocyte content of the islet tissue in conjunction with the use of clean, handpicked islets for transplantation.[4] If islet tissue was removed directly from a Ficoll gradient after collagenase digestion and used for transplantation without handpicking the islets, then prolongation of survival was not obtained. Recent studies have shown that in vitro culture of handpicked islets at 24 °C for 1 wk before transplantation in conjunction with one injection of rat antilymphocyte serum into the recipient produced allograft survival longer than 100 days across a major histocompatibility barrier.[5] Since clean islets were required for successful allografts, a simple method was sought that would permit positive identification of islets in vitro as contrasted to small lymph nodes, acinar tissue, ducts, and vascular tissue. The purpose of this communication is to describe such a procedure.

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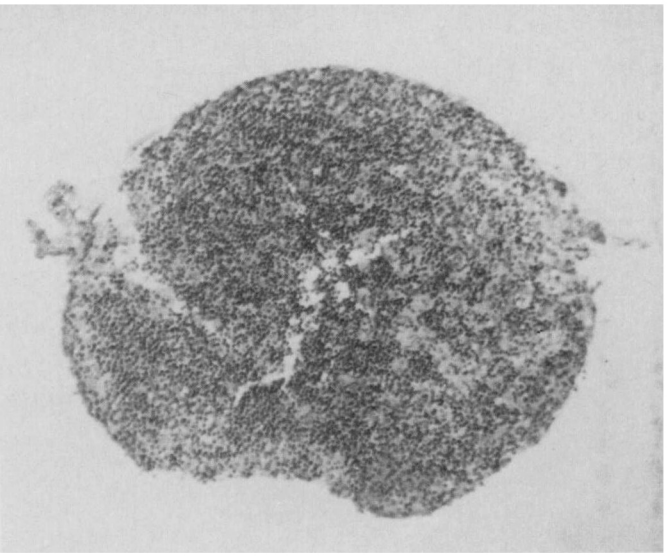
METHODS AND RESULTS

Islets were isolated from the pancreases of male rats (300–350 g) by the collagenase technique[6,7] and separated on a Ficoll gradient by centrifugation.[8] Islet tissue was removed from the interface of the 11 and 20.5% layers of the Ficoll gradient, washed twice with Hank's solution containing phenol red (20 mg/L), and transferred to a flat-bottom, glass crystallizing dish (60 × 40 mm) in 20 ml of tissue culture medium CMRL-1066 containing fetal calf serum (10%), penicillin (100 U/ml), streptomycin sulfate (100 μg/ml), D-glucose (1.0 mg/ml), and phenol red (20 mg/L). A dissecting microscope (Olympus) was used, which provided illumination through the bottom of the crystallizing dish by a white bulb (20 W). The mirror of the microscope was covered with black paper and rotated so that the black surface was

FIGURE 1. Photomicrograph of a lymph node that was identified in an islet preparation using the "green-light" technique. (Magnification ×100.)



parallel to the stage of the microscope, thus providing a black background for observing the islets. A green gelatin filter (Kodak no. 58) was placed between the bottom of the crystallizing dish and the glass stage of the microscope. When the preparation was examined under these conditions of illumination, the islets were pink, whereas lymph nodes, acinar tissue, ducts, and vascular tissue were a bright green against a black background. The optimum depth of the medium in the dish was 2.0 cm. If a larger amount of fluid was used all of the structures appeared pink, and with a small amount of fluid (depth, 0.5 cm) all of the tissue was green.

The "green-light" technique was used to screen islets that had been handpicked under a dissecting microscope by our usual procedure of illuminating the dish from above with an adjustable microscope lamp without light filters. Figure 1 illustrates a small lymph node that was identified as an islet by our usual technique but was green in color when the preparation was screened by the green-light method.

Approximately 70 years ago, Bensley[9] used neutral red as an intravital stain for the specific demonstration of islets in the pancreas. The islets accumulated neutral red, and thus appear dark red in contrast to the surrounding pale pink acinar tissue. Presumably, the phenol red used in the culture medium is accumulated by the islets similar to neutral red thus imparting a pink color which is evident when the islets are viewed with a diffuse, low-intensity, green light.

This simple procedure for the specific identification of islets in vitro is of great importance to the field of islet transplantation and may be of assistance in biochemical and metabolic studies requiring clean preparations of only islet tissue.

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