Short Communications

Human Pancreatic B Cells in Vitro: Microtubule and Insulin Immunofluorescence

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Summary. Primary monolayer cultures of human B cells established using collagenase digestion, assume a characteristic epithelial morphology which is ideal for indirect immunofluorescence studies. Using double label indirect immunofluorescence, it was possible to identify an extensive cytoplasmic microtubule complex and numerous insulin antigen positive granules within the human B cells. The microtubules are discrete rod-like structures which terminate at, or near, the plasma

membrane and are observed sometimes in close association with insulin antigen positive granules. This culture system is well-suited for studying the role of the microtubules in human B cell function.

Key words: Human pancreas; microtubules; insulin; immunofluorescence.

Although the literature contains numerous techniques for the culture of B cells, the culture of human B cells is reported less frequently. Human monolayer pancreatic cultures have been used to show that Cocksackie B3 viruses will infect the human B cell [1, 2]. Prince et al. found that cultures of human B cells were susceptible also to mumps virus [3]. In this study, we describe a method to establish human pancreatic cultures which are enriched for B cells and are suitable for studies involving indirect immunofluorescence. Using a double label indirect immunofluorescence technique, it is possible to observe the relationship between the microtubular complex and the insulin antigen positive granules.

Materials and Methods

Isolation and Culture of Pancreatic Epithelial Cells

After informed consent, pancreatic tissue was obtained from five patients undergoing operation for acute trauma. The monolayer cultures were established using a modification of a previously reported technique [4]. Immediately after removal, the tissue was placed in Medium 199 containing penicillin (100 U/ml) and streptomycin (100 µg/ml). Fat was removed with scissors and the tissue minced into fine pieces approximately 1 mm³. Any residual fat which floated to the surface of the medium was removed by pipette. The medium was discarded and the tissue resuspended in Medium 199 buffered with NaHCO₃, and containing bovine serum albumin (20 mg/ml) and collagenase type III (2 mg/ml). The tissue was then agitated in a sterile centrifuge tube in a water bath at 37 °C for 30 min, the cells were spun for 4 min at 500 × g

and the pellet rinsed with Medium 199. The cells were again centrifuged, resuspended in Medium 199 (10 ml), supplemented with 20% fetal calf serum (growth medium) and plated into 100 mm tissue culture dishes. After 4–6 h of incubation (95% air and 5% CO₂) at 37 °C, many of the fibroblastic cells attached to the plate and the floating cells were carefully removed and plated into 60 mm tissue culture dishes containing growth medium (5 ml). In 24–48 h after plating, the epithelial cells attached to the bottom of the dish. The cultures were tested for mycoplasma contamination [5–6] and found to be negative.

Antibodies

The procedures for raising antibodies to tubulin and insulin, for their purification by affinity chromatography and use in a double label immunofluorescence technique have been published elsewhere [7–10].

Results

Monolayer Culture of Pancreatic Cells

Monolayer cultures were established from five human pancreases. The yield and quality of B cells was increased by: (a) removal of as much fat as possible from the pancreas before digestion, and (b) careful observation to prevent overdigestion of the tissue. The digestion rate was noted to vary between different batch lots of the enzyme and had to be established by frequent observation of the tissue under a dissecting microscope. Following 4 days in culture, the cells had an epithelial appearance with an oval shape, prominent nuclei and the viabil-

Fig. 1 A and B. Indirect immunofluorescence ($\times 150$) of pancreatic monolayer cultures using a monospecific antibody to insulin followed by the addition of a fluorescein labelled second antibody to guinea pig IgG. A group of insulin positive B cells is surrounded by insulin antigen negative cells (A). Magnification ($\times 300$) of a single insulin positive B cell (B) shows discrete fluorescent particles (arrow) that may be insulin secretory granules

ity as measured by dye exclusion [11] was $> 90\%$. The B cells maintained differentiated function and released insulin promptly following glucose stimulation (data not shown).

Insulin Detection

B cells within the monolayer cultures were identified by indirect immunofluorescence using a guinea pig IgG anti-insulin first antibody and a fluorescein conjugated

anti-guinea pig second antibody. Figure 1A illustrates a group of B cells surrounded by insulin antigen negative cells. The insulin positive cells were brightly fluorescent and easily distinguishable from the surrounding negative cells. Preadsorption of the anti-insulin sera with excess insulin resulted in loss of immunofluorescence. Quantitation of B cells in monolayer culture revealed that 10%–20% of the cell population were insulin antigen positive. When a single B cell was observed at higher magnification, discrete insulin antigen positive granules were visible (Figure 1 B).

Fig. 2 A and B. Indirect immunofluorescence ($\times 300$) using two different fluorescent probes was used to identify the cytoplasmic microtubular complex in B cells. The B cells were identified with a monospecific antibody to insulin prepared in guinea pig followed by a fluorescein labelled anti-guinea pig IgG. Secretory granules (indicated by arrows in **A**) are numerous throughout the cytoplasm. The microtubules (arrows in **B**) were identified with a monospecific antibody to tubulin prepared in rabbit and a rhodamine labelled anti-rabbit IgG

Identification of Microtubules Within B Cells

Double label indirect immunofluorescence using a fluorescein labelled second antibody to guinea pig IgG (insulin) and a rhodamine labelled second anti-rabbit IgG (tubulin) enabled us to identify insulin granules and the cytoplasmic microtubule complex in the same B cells. Figure 2A illustrates a B cell that contains a large number of insulin antigen positive granules. When this same cell is observed for the tubulin antibody marker, an intricate microtubular network is revealed (Figure 2B). Not all of the B cells lend themselves to this technique, since cells that are full of granules sometime obscure the microtubular complex.

Discussion

Prince et al. enriched the human B cell population to 1%–5% of the monolayer culture using a discontinuous Ficoll gradient [3]. The procedure we describe is simpler and enriches the B cell population by 10%–20% of the monolayer culture. The techniques reported by Yoon [1] will yield up to 30% B cells. However, we found B cells obtained by this method attach poorly to glass coverslips and are unsuitable for immunofluorescence studies.

These pancreatic cells, like other epithelial cells in culture, contain an extensive microtubular network. The microtubules do not usually occur in bundles, but are identified as individual rod-like structures approximate-

ly 25 nm in diameter. Using this monolayer culture technique in mouse B cells, we disrupted the microtubules with colchicine and showed they are not necessary for the immediate release of insulin, but are required for its sustained release [7].

The microtubular-microfilament complex has been implicated as an integral part of the insulin secretory process [12–15]. Culture techniques for growing human pancreatic cells yield mixed cell populations. Using a double label indirect immunofluorescence technique, it is possible to identify the B cells by the insulin antigen positive granules; however, we cannot rule out the possibility that the insulin antigen positive granules in these cells are composed of multiple antibodies, coalesced around either insulin secretory granules or other forms of insulin in the cell. The lack of immunofluorescence after preadsorption of the first antisera with an excess of insulin argues for the specificity of this technique. Using an antibody to tubulin raised in a different species it is possible to identify the cytoplasmic microtubular complex. Since the microtubule network in these B cells radiates from the perinuclear region of the cell to the plasma membrane and insulin antigen positive granules are frequently seen along the microtubule, the cytoplasmic microtubular complex may be involved in guiding the insulin secretory granules from the site of synthesis in the rough endoplasmic reticulum through the Golgi complex to the site of secretion at the plasma membrane. This culture technique is a general one which can be used to identify other endocrine cell types, or the increasing number of cytoskeletal and contractile proteins important in the processing and secretion of hormones from pancreatic cells.

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