

UNIMPAIRED SURVIVAL OF NON-ENDOCRINE CELLS IN 22°C-CULTURED HUMAN ISLETS

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Introduction

Pre-transplant low-temperature culture is an established method to reduce the immunogenicity of rodent pancreatic islets. After 10-14 days culture at 22°C, mouse or rat pancreatic islets can survive in allogeneic hosts without (or with only minimal) immunosuppression. However, from the clinical islet transplantation results it can be concluded that low temperature culture may be much less effective to reduce the immunogenicity of human islets. In accordance with this assumption, we demonstrated that APCs in low temperature-cultured human islets remained fully active, whereas rodent islet APCs were inactivated. In these experiments, the activity of intra-islet APCs was monitored by their capacity to support the proliferation of lectin-incubated highly purified T cells.

Aim of the Study

To see whether the proposed presence of immunogenic non-endocrine islets in low temperature-cultured human islets can directly be demonstrated by immunohistochemistry.

Materials and Methods

Islets

Human pancreatic islets were isolated by collagenase digestion and density gradient centrifugation from the pancreata of four multi-organ donors. The islets were collected into culture flasks containing CMRL-1066/10% FCS, and stored at 22°C. Islets were removed from the culture flasks either within the first day after preparation (freshly isolated islets), or after 14 days culture at 22°C (low temperature-cultured islets).

Fixation and staining

Islet suspensions were fixed for 18 hours at 4°C in Zamboni's solution, washed and embedded in OCT, before freezing in liquid nitrogen. Insulin was labelled by guinea-pig insulin antibodies (Dako) combined with an AP-conjugated rabbit anti guinea-pig antibody (Rockland). Alkaline phosphatase activity was then visualised with Vector-blue. Non-endocrine cells were labelled by monoclonal mouse antibodies (Dako) combined with a HRP-conjugated rabbit anti-mouse antibody (Dako). Peroxidase activity was visualised (red) by AEC.

Summary

This study demonstrated the excellent survival of non-endocrine cells during 22°C-culture of human islets in serum-containing medium. This may explain the persistent immunogenicity of those islets when tested in vitro, or when transplanted clinically. Therefore, approaches other than low temperature culture must be used to diminish the capacity of isolated human islets for "direct" antigen presentation.

Results

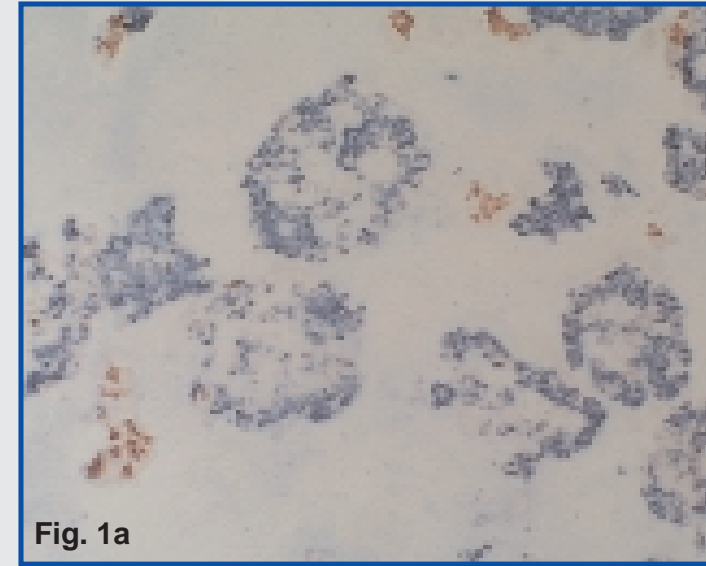


Fig. 1a

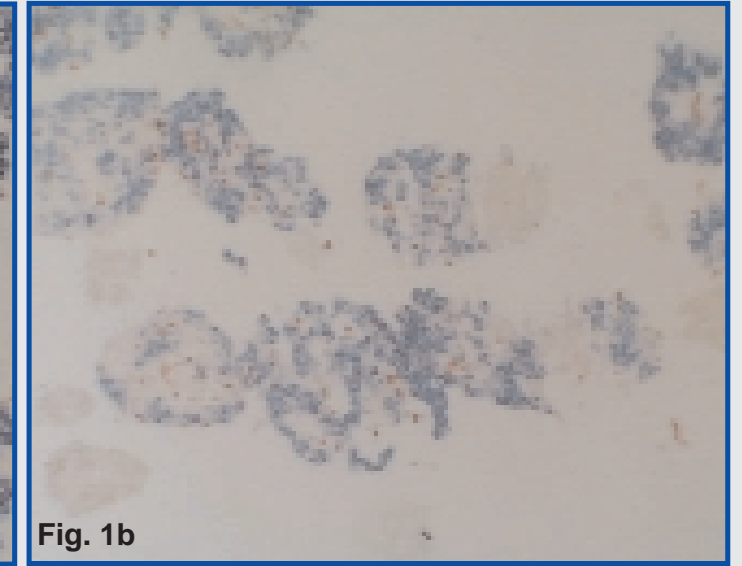


Fig. 1b

Abundant epithelial cells (anti-cytokeratin 19) were found in the exocrine tissue, and closely adhering to the islets in freshly prepared (Figure 1a) and cultured (Figure 1b) islet preparations.

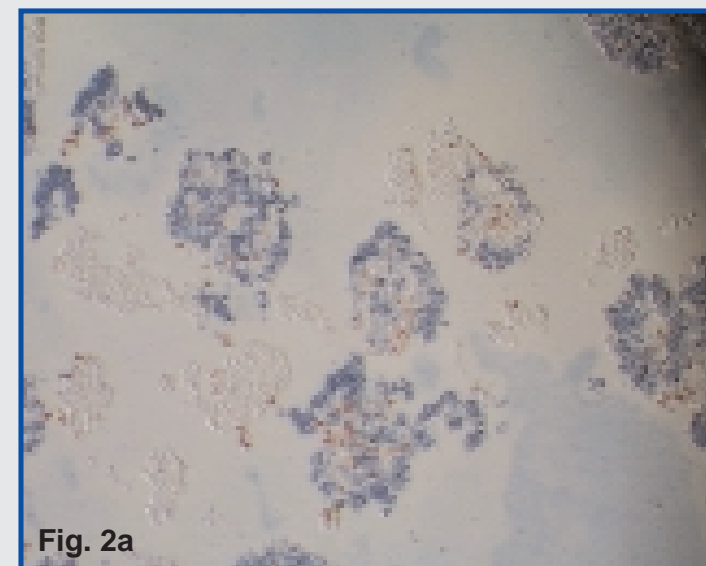


Fig. 2a

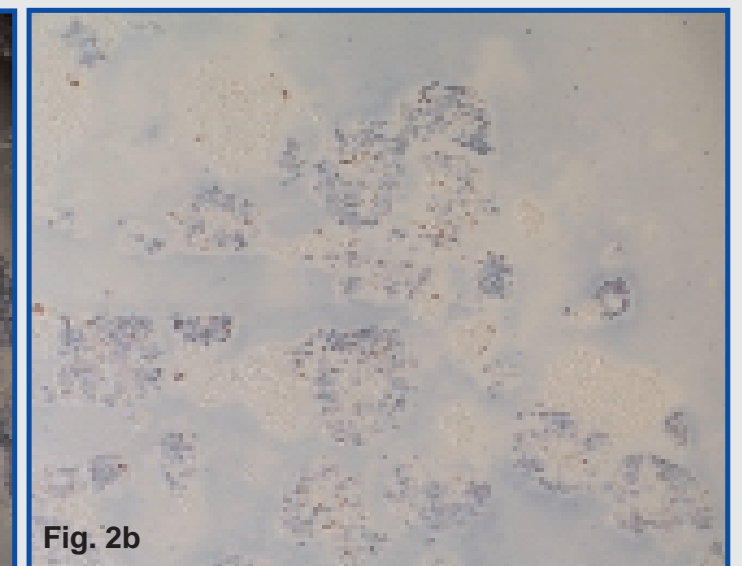


Fig. 2b

Endothelial cells (anti-CD31, PECAM-1) in islets and exocrine tissue fragments were present in both freshly prepared (Figure 2a) and cultured (Figure 2b) tissue. However, the vascular structure was lost and the cells showed a scattered pattern. Similar pictures were obtained after staining for CD34 or von Willebrand factor.

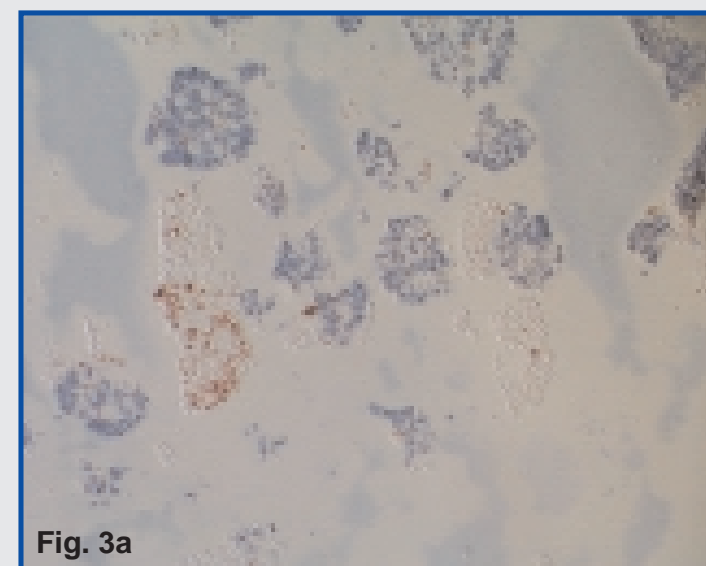


Fig. 3a

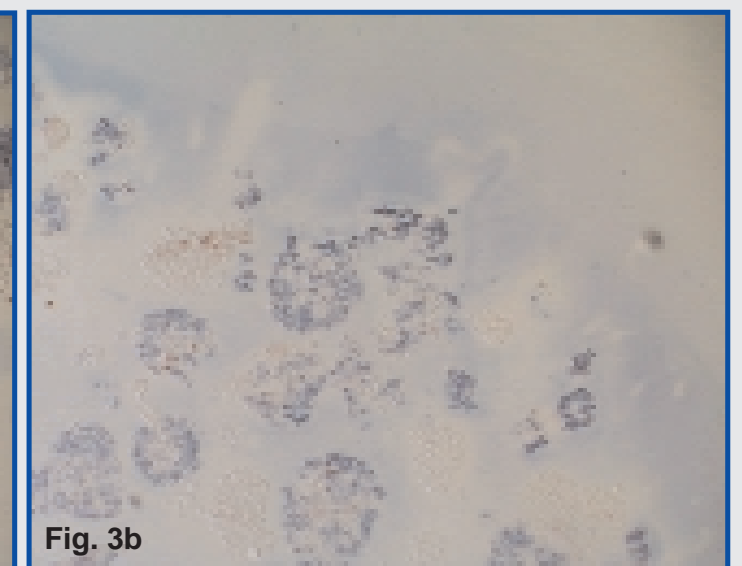


Fig. 3b

Macrophages (anti-CD 68) were also found in both freshly prepared (Figure 3a), and cultured human islets (Figure 3b).

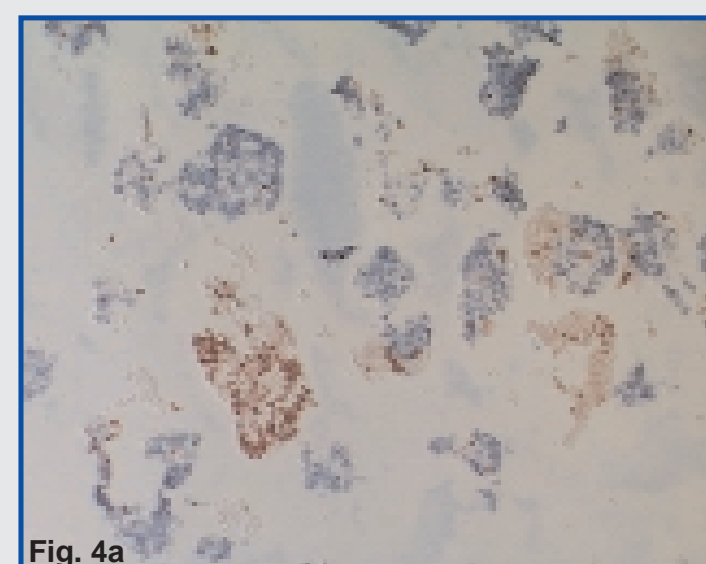


Fig. 4a

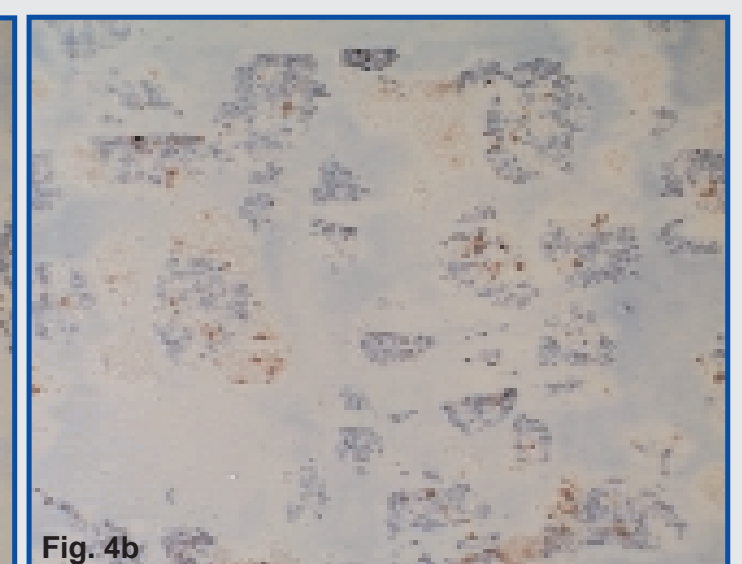


Fig. 4b

MHC class II molecules were stained in both freshly prepared (Figure 4a) and cultured (Figure 4b) islets.