Received: 19 October 2019 | Revised: 26 December 2019 | Accepted: 29 January 2020

DOI: 10.1111/ahe.12541



ANATOMIA HISTOLOGIA EMBRYOLOGIA

WILEY

ORIGINAL ARTICLE

# Immunoreactivity of cytotoxic T-lymphocyte antigen 2 alpha in mouse pancreatic islet cells

**Claudius Luziga**

Department of Veterinary Anatomy & Pathology, Sokoine University of Agriculture, Morogoro, Tanzania

**Correspondence**
Claudius Luziga, Department of Veterinary Anatomy & Pathology, Sokoine University of Agriculture, P.O. Box 3016 Morogoro, Tanzania.
Email: luziga@sua.ac.tz

**Funding information**
Cabinet Office, Government of Japan, Grant/Award Number: 15H04428

## Abstract

Cells of the pancreatic islets produce several molecules including insulin (beta cells), glucagon (alpha cells), somatostatin (delta cells), pancreatic polypeptide (PP cells), ghrelin (epsilon cells), serotonin (enterochromaffin cells), gastrin (G cells) and small granules of unknown content secreted by the P/D1 cells. Secretion mechanism of some of these molecules is still poorly understood. However, Cathepsin L is shown to regulate insulin exocytosis in beta cells and activate the trypsinogen produced by the pancreatic serous acini cells into trypsin. The structure of the propeptide region of Cathepsin L is homologous to Cytotoxic T-lymphocyte antigen-2 alpha (CTLA-2 alpha) which is also shown to exhibit selective inhibitory activities against Cathepsin L. It was thought that if CTLA-2 alpha was expressed in the pancreas; then, it would be an important regulator of protease activation and insulin secretion. The purpose of this study was, therefore, to examine by immunohistochemistry the cellular localization and distribution pattern of CTLA-2 alpha in the pancreas. Results showed that strong immunoreactivity was specifically detected in the pancreatic islets (endocrine pancreas) but not in the exocrine pancreas and pancreatic stroma. Immunostaining was further performed to investigate more on localization of Cathepsin L in the pancreas. Strong immunoreactivity for Cathepsin L was detected in the pancreatic islets, serous cells and the pancreas duct system. These findings suggest that CTLA-2 alpha may be involved in the proteolytic processing and secretion of insulin through regulation of Cathepsin L and that the regulated inhibition of Cathepsin L may have therapeutic potential for type 1 diabetes.

**KEYWORDS**

Cathepsin L, CTLA-2 alpha, mouse, pancreas, pancreatic islets

## 1 | INTRODUCTION

The mouse pancreas is diffusely distributed within the mesentery of the proximal small intestine in a dendritic manner (Bunnag, Bunnag, & Warner, 1963). Glossily, three major lobes can be distinguished: duodenal, splenic and gastric, separated by patches of adipose, connective and lymphatic tissues. In the exocrine pancreas of the mouse, each lobe consists of several lobules measuring 0.5–1.5 mm in diameter (Murakami, Hitomi, Ohtsuka, Taguchi, & Fujita, 1997). Each lobule is composed of several pancreatic acini, the clusters of pyramidal serous cells which form dome-like structures. The serous cells direct secretions from their apical domains into the lumen of intercalated ducts that drain into intralobular ducts, which then form interlobular ducts that drain into branches of the main pancreatic duct (Longnecker, 2014). Centroacinar cells are located at the junctions established between the serous acini and the intercalated ducts. The solution of digestive enzymes produced by serous cells and the bicarbonate-rich secretions produced by the centroacinar and ductal cells flows through the pancreas duct system to the duodenum (Korc, 1993).

  

The pancreatic islets also appear as clusters of cells that vary greatly in size ranging from 50 to 250 μm in diameter in humans (Hellman, 1959; Pfeifer et al., 2015). The shape of the pancreatic islets may be spherical, ellipsoid or irregular (Longnecker, 2014). Smaller islets are found scattered throughout the pancreatic lobules. In mice, the larger islets are located along the interlobular ducts while in humans, they are frequently observed at intralobular ducts and at the level of the lobules (Brissova et al., 2005; Merkwitz et al., 2013; Murakami et al., 1997).

The major cell types found in the pancreatic islets include alpha cells that secrete glucagon and represent 10%–20% of total number of islet cells in mice (Gromada, Franklin, & Wollheim, 2007; Lee, Wang, Du, Charron, & Unger, 2011); beta cells which synthesize and secrete insulin and they are most numerous, representing 60%–80% of islet cells in mice (Huang, Shen, Atkinson, & Kennedy, 1995; Rorsman & Ashcroft, 2017; Rorsman & Renström, 2003; Rutter, Pullen, Hodson, & Martinez-Sanchez, 2015); delta cells which produce somatostatin (Francis, Baskin, Saunders, & Ensinck, 1990; Marcinkiewicz, Ramla, Seidah, & Chretien, 1994) and gamma or pancreatic polypeptide (PP) cells that secrete pancreatic polypeptide; each one represents <5% of the islet cells in mice (Rahier et al., 1983; Tan & Bloom, 2013). Other cell types include the epsilon cells which produce ghrelin (Wierup, Svensson, Mulder, & Sundler, 2002); the enterochromaffin cells which produce serotonin (Capella, Hage, Solcia, & Usellini, 1978); G cells which produce gastrin (Suissa et al., 2013) and the P/D1 cells that secrete small granules of unknown content (Solcia et al., 1989). Each of the aforementioned cells represents less than 1% of the islet cells in mice. In addition to the pancreatic islets, isolated islet cells are also found dispersed in the pancreatic lobules or in association with the ducts (Korc, 1993).

Physiologically, most digestive proteases are secreted as proenzymes and only acquire activity after cleavage and activation by proteolytic processing (Rawlings & Salvesen, 2013). Notably, trypsinogen reaches the small intestine as inactive and is activated by striated-border enterokinase, and then activates other digestive proteases (Hadorn et al., 1974; Semrad, 2012). In the absence of enterokinase, trypsinogen is activated by Cathepsin B in the pancreas and this does not happen normally on the pancreas territory, but during pathology (Halangk et al., 2000; Hirshkowitz & Shoichet, 1959). The endoproteolytic activity of Cathepsin B is less strong compared to that of Cathepsin L, which is also reported to be an important regulator of protease activation in pancreatitis (Kirschke & Barrett, 1985). Cathepsin L is also a very effective activator of trypsinogen into trypsin in isolated acini in vivo and in vitro and it is secreted into pancreatic juice. (Wartmann et al., 2010). The transformation of trypsinogen into trypsin at the level of serous cells of the acini which constitute the pancreatic lobules instantly triggers auto-digestion, and thus, the beginning of acute pancreatitis.

Expression of Cathepsin L has also been demonstrated in beta cells where it is involved in proenzyme sorting in secretory granules and remained as precursor in a compartment to regulate exocytosis of insulin (Kuliawat, Klumperman, Ludwig, & Arvan, 1997). Like other cathepsins, the enzymatic activity of Cathepsin L is regulated by changes in pH and interaction with inhibitors (Kos & Lah, 1998; Levičar et al., 2002). One of the potent and specific inhibitor of Cathepsin L is the Cytotoxic T-lymphocyte antigen-2 alpha (CTLA-2 alpha). The structure of CTLA-2 alpha is homologous to the proregion of Cathepsin L (Kurata et al., 2003). However, little is known regarding the cellular localization, distribution pattern and physiological function of CTLA-2 alpha in the pancreas. It was thought that if CTLA-2 alpha was expressed in the pancreas, it would be an important regulator of protease activation and insulin secretion. Therefore, the purpose of this study was to examine by immunohistochemistry the cellular localization and distribution pattern of CTLA-2 alpha in the pancreas.

## 2 | MATERIALS AND METHODS

### 2.1 | Tissue preparation

All experiments conformed to the ethics for animal experimentation of Sokoine University of Agriculture. Five male and five female adult mice aged twelve months were used in this study. They were kept in separate compartments in small animal laboratory house under controlled conditions of light (12-hr light–dark cycles) and temperature (20–25°C), and they were fed standard laboratory chow and water ad libitum. The mice were anaesthetized with sodium pentobarbital (60 mg/kg body weight) by intraperitoneal injection. After sacrificing the mice pancreases were dissected, followed by fixation in 4% paraformaldehyde for 2 hr at 4°C. The histopathology procedure was done as previously described by Slaoui and Fiette (2011) with minor modifications. Tissue blocks were prepared and cut at 5 μm thick to produce tissue sections. Some sections were used for Haematoxylin and Eosin (H & E) staining and others for immunohistochemistry (Bolam, 1994) using 3, 3′-diaminobenzidine tetra-hydrochloride (DAB) for detection of binding sites.

### 2.2 | Routine haematoxylin and eosin staining

Tissue sections were deparaffinised in xylene followed by rehydration through a descending ethanol series to phosphate-buffered saline (0.01M PBS-pH7.4). The sections were stained using H&E in order to examine the histological organization and cellular components of the pancreas.

### 2.3 | Generation of antibodies

Recombinant CTLA-2 alpha and Cathepsin L were purified using methods described previously by other authors (Camenisch et al., 1999; Takahashi, Zhao, Kageyama, & Yamamoto, 1992) with minor modifications. Affinity-purified rabbit anti-CTLA-2 alpha IgG and anti-Cathepsin L IgY were obtained. In brief, antiserum against CTLA-2 alpha was obtained by immunizing rabbit against

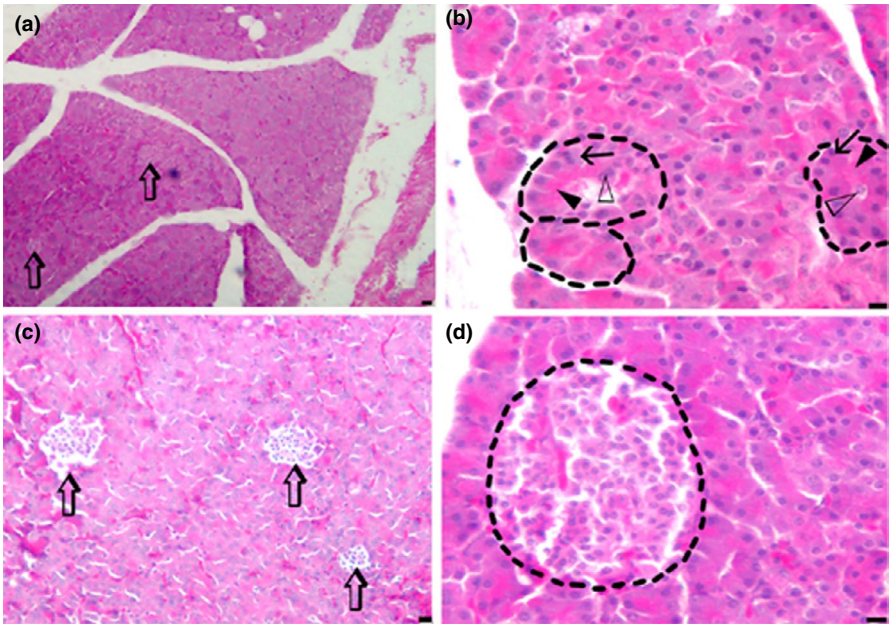


**FIGURE 1** Haematoxylin and Eosin stained sections demonstrating histological organization of the pancreas. (a) Low power section showing distinct lobes of the pancreas with pancreatic islets (open arrows) and anastomosing tubular network of interlobular ducts. (b) Shows pancreatic acini (dashed circles) of serous cells stained basophilic at the cells' basal domain (solid arrow) due to the high content of RNA and the presence of nuclei, and they are acidophilic at their apical domain (solid arrowhead) where there is high content of zymogen proteins (digestive enzymes). The nuclei of centroacinar cells (open arrowhead) are also seen within an acinus. (c) Low power section showing three, round-shaped pancreatic islets (open arrows). (d) Shows islet cells in pancreatic islets (in dashed circles). Note that the islet cells are smaller in size, and they have paler cytoplasm than the surrounding serous cells. Original magnification, scale bar: 5 μm

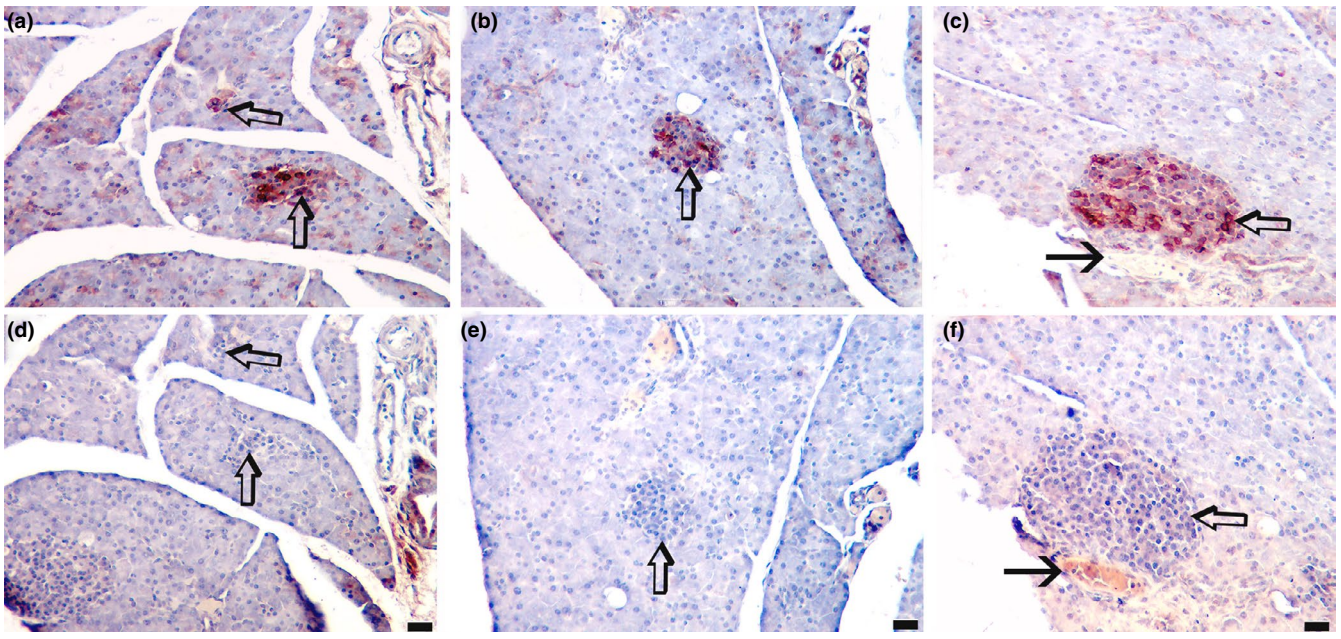


**FIGURE 2** Immunohistochemical localization of CTLA-2 alpha in the pancreas using peroxidase chromogen DAB. Note the positive immunoreactivity of CTLA-2 alpha in cells of the pancreatic islets (open arrows) in small pancreatic islets seen scattered in the pancreatic lobules (a, b) and large pancreatic islet (c) located along interlobular duct (arrow). Immunoreactivity is not observed in the control serial sections (d–f) incubated with PBS in the place of anti-CTLA-2 alpha antibody. Original magnification, scale bar: 5 μm

recombinant CTLA-2 alpha and egg yolk immunoglobulin fractions were prepared from eggs laid by hens immunized against recombinant Cathepsin L. The polyclonal anti-CTLA-2 alpha antibody against CTLA-2 alpha protein and the chicken anti-Cathepsin L IgYs were affinity-purified through columns with recombinant CTLA-2 alpha or Cathepsin L protein-conjugated resins. The specificity of the purified antibodies was characterized by Western blot (Luziga et al., 2008; Nga, Luziga, Yamamoto, Kusakabe, & Yamamoto, 2015).

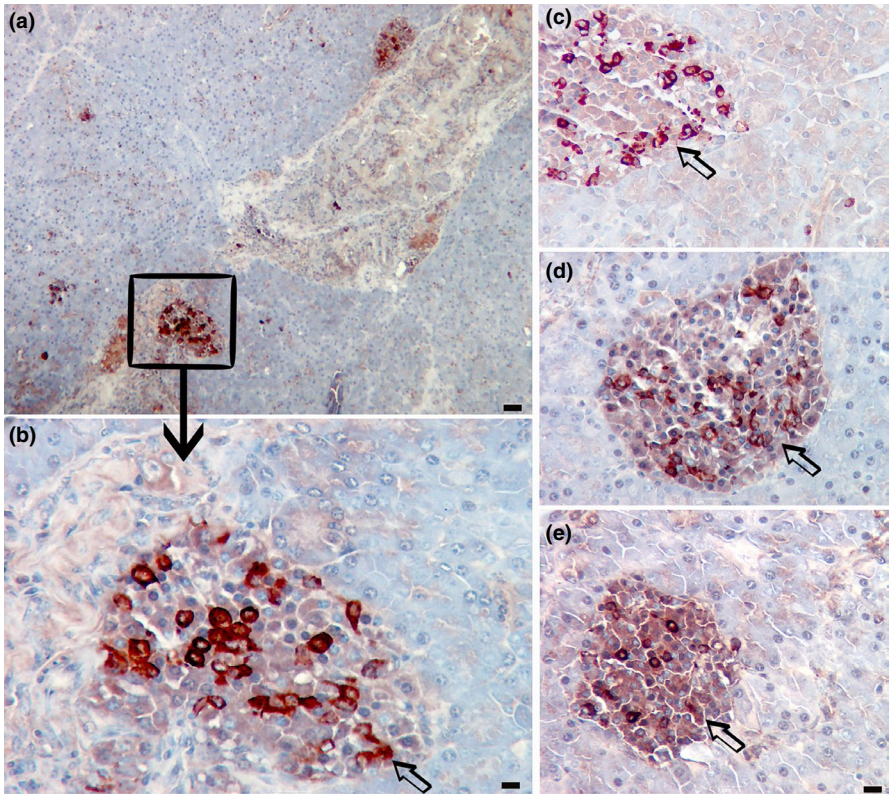


**FIGURE 3** Specific localization of CTLA-2 alpha in cells of the pancreatic islets using peroxidase chromogen DAB. (a) Low power section showing positive immunoreactivity of CTLA-2 alpha in the pancreatic islets. (b) is a higher magnification of the rectangle in figure (a) and (c–e) are various pancreatic islets showing clearly the localization of CTLA-2 alpha in the cytoplasm of islet cells. Original magnification, scale bar: 5 μm

### 2.4 | Immunohistochemical examination using diaminobenzidine tetra-hydrochloride (DAB) chromogen

Tissue sections were deparaffinised as described previously followed by immersion in a solution of 0.3% hydrogen peroxide (v/v) in distilled water for 30 min at room temperature (RT) to inhibit endogenous peroxidase activity, followed by washing (3 × 5 min) in PBS. Sections were then incubated with 10% goat normal serum for 30 min at RT to block non-specific binding. Tissue sections were then incubated with primary antibodies overnight in a dark, humid chamber at 4°C. The antibodies used were the CTLA-2 alpha antibody and Cathepsin L antibody both diluted at a ratio of 1:500 in PBS. For negative control, PBS was applied in place of the primary antibodies. Sections were then washed (3 × 5 min) in PBS followed by incubation with biotinylated goat anti-rabbit IgG (BIORAD STAR 5B Lot 1803-1) and goat anti-chicken IgY (abcam-ab97131) for 30 min at RT. Sections were then washed (3 × 5 min) in PBS before incubation with streptavidin–peroxidase conjugate for 30 min at RT. The tissue sections were then incubated for 3–5 min with a medium containing 0.05% DAB (BIORAD), 0.01% hydrogen peroxide and 0.05 M Tris–HCl, pH 7.6 to visualize binding sites followed by counterstaining with haematoxylin for 30 s in order to stain the nuclei. The tissue sections were then rinsed for 15 min in running tap water followed by dehydration through a graded ethanol series, clearance and mounting by a mixture of distyrene (a polystyrene), a plasticiser (tricresyl phosphate), and xylene (DPX). Binding sites were assessed using Olympus BH-2 microscope fitted with Olympus camera for image capturing.

## 3 | RESULTS

### 3.1 | Histological structure of the mouse pancreas

Haematoxylin and Eosin stained sections showed distinct lobules containing pancreatic acini and anastomosing tubular network of interlobular and intralobular ducts. The serous cells of pancreatic acini appeared pyramidal in shape surrounding a narrow lumen. Their basal domains contained spherical nuclei and they appeared basophilic due to high content of RNA, with their apical domains being acidophilic due to their high content of zymogen. The nuclei of centroacinar cells were also encountered within an acinus. Pancreatic islets were found dispersed throughout the pancreatic lobules and they were spherical or ellipsoid in shape. Some of them were seen along the interlobular ducts. The islet cells were generally smaller and showed paler cytoplasm than the surrounding serous cells (Figure 1a–d).

### 3.2 | Localization of CTLA-2 alpha in pancreatic islets

In both male and female mice, strong immunoreactivity for CTLA-2 alpha was detected in small islets positioned into the pancreatic lobules and at the level of the larger islets, localized along the interlobular ducts. Positive cells appeared dispersed throughout the islets (Figures 2a–c and 3a–e). Immunoreactivity was not observed in the control serial sections incubated with the PBS in the place of anti-CTLA-2 alpha antibodies (Figure 2d–f). However, it was not possible

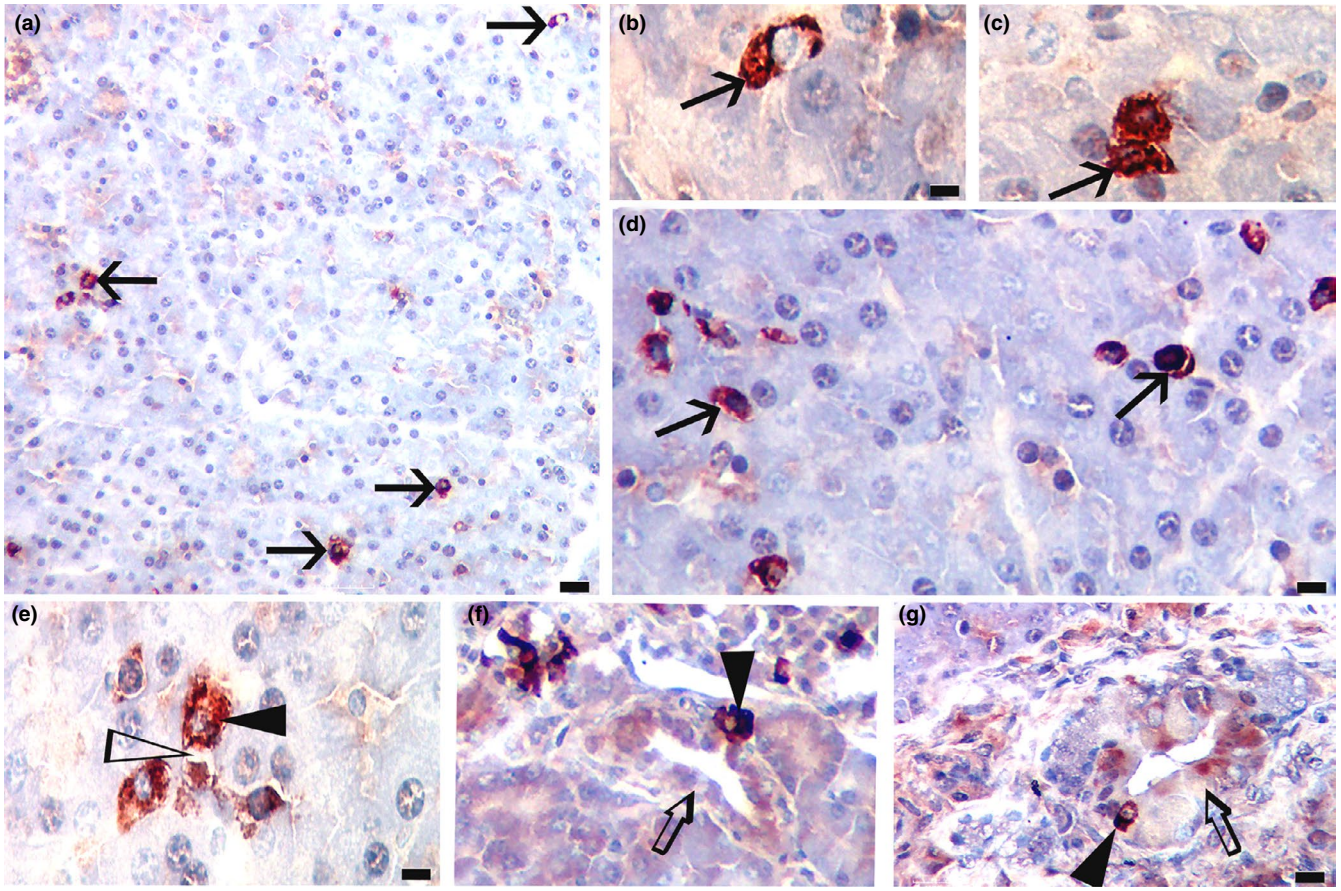


**FIGURE 4** CTLA-2 alpha immunoreactivity in cells other than those of the pancreatic islets. (a) Low power section showing CTLA-2 alpha immunoreactive islet cells (solid arrows) dispersed in pancreatic lobules. (b–d) show the immunoreactivity at higher magnification. (e–g) show other positive cells (solid arrowhead) in association with intralobular (open arrowhead) and interlobular (open arrows) ducts. Original magnification, scale bar: 5 μm

to identify the specific islet cell types expressing CTLA-2 alpha in the pancreatic islets. Such experiment would need double or triple immunostaining using a set of other antibodies specific to insulin, glucagon pancreatic polypeptide, ghrelin, serotonin or gastrin.

### 3.3 | Immunoreactivity of CTLA-2 alpha in cells other than those of the pancreatic islets

Apart from the pancreatic islets, other isolated CTLA-2 alpha immunoreactive islet cells were found dispersed inside the pancreatic lobules, among the serous acini, as well as in association with intralobular and interlobular ducts (Figure 4a–g).

### 3.4 | Localization of Cathepsin L in the pancreatic islets, pancreatic lobules and duct system

Since CTLA-2 alpha was observed exclusively in pancreatic islet cells in both male and female mice, interest was developed to know the cellular localization of its cognitive enzyme, the Cathepsin L. Therefore, immunostaining was further performed using antibody against Cathepsin L to investigate more the localization of Cathepsin L in the pancreas. Interestingly, Cathepsin L was also found to be highly expressed in pancreatic islet cells, serous cells of pancreatic acini and at the level of the pancreas duct system (Figures 5a,b and 6a,b).

## 4 | DISCUSSION

The objective of the present study was to examine by immunohistochemistry the cellular localization and distribution pattern of CTLA-2 alpha in the pancreas and to deduce its functional implications in the gland. Results showed that in both male and female mice, CTLA-2 alpha is specifically localized at high levels in the pancreatic islet cells. However, additional studies involving double or triple immunostaining are required in order to identify specific islet cell types that synthesize and secrete CTLA-2 alpha.

A remarkable finding regarding the distribution pattern of CTLA-2 alpha in the mouse pancreas was its significant correlation with the distribution of Cathepsin L in pancreatic islets. Taking into consideration that beta cells are most numerous in the mouse pancreatic islets (60%–80%), they synthesize and secrete insulin (Huang

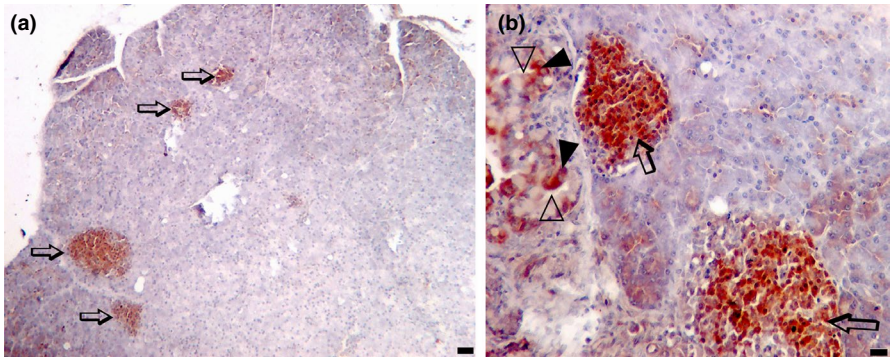


**FIGURE 5** Localization of Cathepsin L in cells of pancreatic islets using peroxidase chromogen DAB. (a) Shows positive immunoreactivity for Cathepsin L (open arrows) in the pancreatic islets. (b) Higher magnification, showing clearly the localization of Cathepsin L in the cytoplasm of islet cells (open arrows) and in ductal cells (solid arrowheads) surrounding a ductal lumen (open arrowhead). Original magnification, scale bar: 5 μm

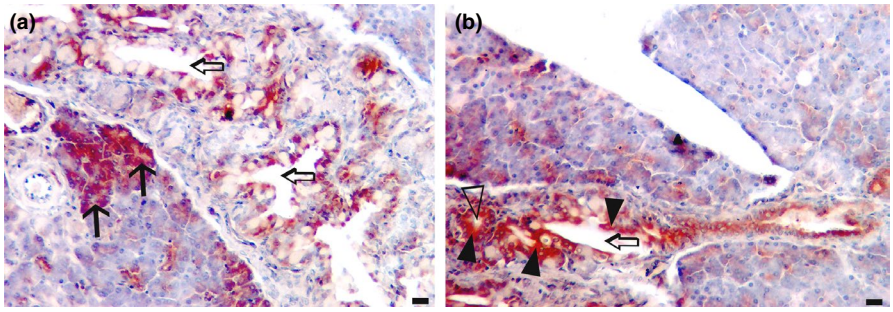


**FIGURE 6** Localization of Cathepsin L in serous cells of pancreatic acini and ductal cells using DAB chromogen. (a, b) Localization of Cathepsin L in the cytoplasm of serous cells of pancreatic acini (solid arrows); ductal cells (solid arrow head) of small interlobular (open arrowhead) and large interlobular (open arrows) ducts. Original magnification, scale bar: 5 μm

et al., 1995; Rorsman & Ashcroft, 2017; Rorsman & Renström, 2003; Rutter et al., 2015) and that Cathepsin L is intensely expressed in beta cells where it is involved in proenzyme sorting in secretory granules and regulate exocytosis of insulin (Kuliawat et al., 1997) and further that CTLA-2 alpha is a potent and selective inhibitor of Cathepsin L (Kurata et al., 2003). These data provide some clues for the possible role of CTLA-2 alpha with regard to the physiological control of insulin secretion in the endocrine pancreas through regulation of Cathepsin L activity.

Unlike Cathepsin L, CTLA-2 alpha was not detected in serous cells and the pancreas duct system, suggesting that CTLA-2 alpha is not involved in proteolytic processing of zymogens (digestive enzymes) and regulation of protease activation but rather in insulin processing pathways. Indeed, Cathepsin L is shown to be abundantly expressed in the exocrine pancreas sorted into lysosomal and secretory pathway of serous cells and it is secreted into pancreatic juice (Wartmann et al., 2010). Certainly, the localization of Cathepsin L in serous cells and at the level of pancreas duct system was also confirmed in this study.

So far, several cell types have been identified in the pancreatic islets that secrete various molecules including insulin (beta cells), glucagon (alpha cells), somatostatin (delta cells), pancreatic polypeptide (PP cells), ghrelin (epsilon cells), serotonin (enterochromaffin cells), gastrin (G cells) and small granules of unknown content secreted by the P/D1 cells. The present immunohistochemical findings on the localization of CTLA-2 alpha in pancreatic islet cells expand previous information regarding the molecules secreted by islet cells and that their major function remains to be established.

In conclusion, this study demonstrates that CTLA-2 alpha is specifically localized in mouse pancreatic islet cells. This observation is suggestive of a specialized function of CTLA-2 alpha in relation to proteolytic processing of insulin through regulation of Cathepsin L activity. However, the identity of specific cell types secreting CTLA-2 alpha warrant additional investigation and opens ways to new studies on the function of CTLA-2 alpha in the endocrine system.

## ACKNOWLEDGEMENTS

The author is grateful to the Japanese Ministry of Education, Culture, Sports, Science and Technology for financial support. Acknowledgements also go to the Emeritus Professor Yoshimi Yamamoto for his contribution in antibody production.

## CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

## ORCID

*Claudius Luziga* https://orcid.org/0000-0002-5596-7732

## REFERENCES


Bolam, J. P. (1994). Immunohistochemistry II. In A. C. Cuello (Ed.), *IBRO handbook series: Methods in the neurosciences* (vol. *14*, 1993, £ 45.00 (xv+ 456 pages)). Hoboken, NJ: J. Wiley and Sons.

Brissova, M., Fowler, M. J., Nicholson, W. E., Chu, A., Hirshberg, B., Harlan, D. M., & Powers, A. C. (2005). Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *Journal of Histochemistry & Cytochemistry*, *53*(9), 1087–1097. https://doi.org/10.1369/jhc.5C6684.2005

Bunnag, S. C., Bunnag, S., & Warner, N. E. (1963). Microcirculation in the islets of Langerhans of the mouse. *The Anatomical Record*, *146*(2), 117–123. https://doi.org/10.1002/ar.1091460205

Camenisch, G., Tini, M., Chilov, D., Kvietikova, I., Srinivas, V., Caro, J., … Gassmann, M. (1999). General applicability of chicken egg yolk antibodies: The performance of IgY immunoglobulins raised against the hypoxia-inducible factor 1α. *The FASEB Journal*, *13*, 13–81. https://doi.org/10.1096/fasebj.13.1.81

Capella, C., Hage, E., Solcia, E., & Usellini, L. (1978). Ultrastructural similarity of endocrine-like cells of the human lung and some related cells of the gut. *Cell and Tissue Research*, *186*, 25–37. https://doi.org/10.1007/BF00219652

Francis, B. H., Baskin, D. G., Saunders, D. R., & Ensinck, J. W. (1990). Distribution of somatostatin-14 and somatostatin-28 gastrointestinal-pancreatic cells of rats and humans. *Gastroenterology*, *99*(5), 1283–1291. https://doi.org/10.1016/0016-5085(90)91151-U

Gromada, J., Franklin, I., & Wollheim, C. B. (2007). α-Cells of the endocrine pancreas: 35 years of research but the enigma remains. *Endocrine Reviews*, *28*(1), 84–116. https://doi.org/10.1210/er.2006-0007

Hadorn, B., Hess, J., Troesch, V., Verhaage, W., Götze, H., & Bender, S. W. (1974). Role of bile acids in the activation of trypsinogen by enterokinase: Disturbance of trypsinogen activation in patients with intrahepatic biliary atresia. *Gastroenterology*, *66*(4), 548–555. https://doi.org/10.1016/S0016-5085(74)80043-0

Halangk, W., Lerch, M. M., Brandt-Nedelev, B., Roth, W., Ruthenbuerger, M., Reinheckel, T., … Deussing, J. (2000). Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *The Journal of Clinical Investigation*, *106*(6), 773–781. https://doi.org/10.1172/JCI9411

Hellman, B. (1959). Actual distribution of the number and volume of the islets of Langerhans in different size classes in non-diabetic humans of varying ages. *Nature*, *184*(4697), 1498–1499.

Hirshkowitz, A., & Shoichet, I. (1959). The activation of trypsinogen by cathepsin B. *Journal of Biological Chemistry*, *234*, 2885–2890.

Huang, L., Shen, H., Atkinson, M. A., & Kennedy, R. T. (1995). Detection of exocytosis at individual pancreatic beta cells by amperometry at a chemically modified microelectrode. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(21), 9608–9612. https://doi.org/10.1073/pnas.92.21.9608

Kirschke, H., & Barrett, A. J. (1985). Cathepsin L–a lysosomal cysteine proteinase. *Progress in Clinical and Biological Research*, *180*, 61–69.

Korc, M. (1993). Normal function of the endocrine pancreas. Chapter 38. In V. L. W. Go et al. *The pancreas: Biology, pathobiology, and disease* (2nd ed., pp. 751–758). New York, NY: Raven Press Ltd.

Kos, J., & Lah, T. T. (1998). Cysteine proteinases and their endogenous inhibitors: Target proteins for prognosis, diagnosis and therapy in cancer. *Oncology Reports*, *5*(6), 1349–1410.

Kuliawat, R., Klumperman, J., Ludwig, T., & Arvan, P. (1997). Differential sorting of lysosomal enzymes out of the regulated secretory pathway in pancreatic β-cells. *The Journal of Cell Biology*, *137*(3), 595–608. https://doi.org/10.1083/jcb.137.3.595

Kurata, M., Hirata, M., Watabe, S., Miyake, M., Takahashi, S. Y., & Yamamoto, Y. (2003). Expression, purification, and inhibitory activities of mouse cytotoxic T-lymphocyte antigen-2α. *Protein Expression and Purification*, *32*(1), 119–125. https://doi.org/10.1016/S1046-5928(03)00222-5

Lee, Y., Wang, M. Y., Du, X. Q., Charron, M. J., & Unger, R. H. (2011). Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice. *Diabetes*, *60*(2), 391–397. https://doi.org/10.2337/db10-0426

Levičar, N., Strojnik, T., Kos, J., Dewey, R. A., Pilkington, G. J., & Lah, T. T. (2002). Lysosomal enzymes, cathepsins in brain tumour invasion. *Journal of neuro-oncology*, *58*(1), 21–32.

Longnecker, D. S. (2014). Anatomy and histology of the pancreas. *Pancreapedia: The Exocrine Pancreas Knowledge Base*. https://doi.org/10.3998/panc.2014.3

Luziga, C., Nakamura, O., Deshapriya, R. M. C., Usui, M., Miyaji, M., Wakimoto, M., … Yamamoto, Y. (2008). Dendritic and axonal localization of cytotoxic T-lymphocyte antigen-2 alpha protein in mouse brain. *Brain Research*, *1204*, 40–52. https://doi.org/10.1016/j.brainres.2008.01.067

Marcinkiewicz, M., Ramla, D., Seidah, N. G., & Chretien, M. (1994). Developmental expression of the prohormone convertases PC1 and PC2 in mouse pancreatic islets. *Endocrinology*, *135*(4), 1651–1660. https://doi.org/10.1210/endo.135.4.7925129

Merkwitz, C., Blaschuk, O. W., Schulz, A., Lochhead, P., Meister, J., Ehrlich, A., & Ricken, A. M. (2013). The ductal origin of structural and functional heterogeneity between pancreatic islets. *Progress in Histochemistry and Cytochemistry*, *48*(3), 103–140. https://doi.org/10.1016/j.proghi.2013.09.001

Murakami, T., Hitomi, S., Ohtsuka, A., Taguchi, T., & Fujita, T. (1997). Pancreatic insulo-acinar portal systems in humans, rats, and some other mammals: Scanning electron microscopy of vascular casts. *Microscopy Research and Technique*, *37*(5–6), 478–488. https://doi.org/10.1002/(SICI)1097-0029(19970601)37:5/6<478:AID-JEMT10>3.0.CO;2-N

Nga, B. T. T., Luziga, C., Yamamoto, M., Kusakabe, K. T., & Yamamoto, Y. (2015). Identification and characterization of the interactive proteins with cytotoxic T-lymphocyte antigen-2α. *Bioscience, Biotechnology, and Biochemistry*, *79*(4), 587–597. https://doi.org/10.1080/09168451.2014.991686

Pfeifer, C. R., Shomorony, A., Aronova, M. A., Zhang, G., Cai, T., Xu, H., … Leapman, R. D. (2015). Quantitative analysis of mouse pancreatic islet architecture by serial block-face SEM. *Journal of Structural Biology*, *189*(1), 44–52. https://doi.org/10.1016/j.jsb.2014.10.013

Rahier, J., Wallon, J., Loozen, S., Lefevre, A., Gepts, W., & Haot, J. (1983). The pancreatic polypeptide cells in the human pancreas: The effects of age and diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *56*(3), 441–444. https://doi.org/10.1210/jcem-56-3-441

Rawlings, N. D., & Salvesen, G. (Eds.) (2013). *Handbook of proteolytic enzymes* (vol. *135*, p. 141). Amsterdam, The Netherlands: Academic Press.

Rorsman, P., & Ashcroft, F. M. (2017). Pancreatic β-cell electrical activity and insulin secretion: Of mice and men. *Physiological Reviews*, *98*(1), 117–214. https://doi.org/10.1152/physrev.00008.2017

Rorsman, P., & Renström, E. (2003). Insulin granule dynamics in pancreatic beta cells. *Diabetologia*, *46*(8), 1029–1045. https://doi.org/10.1007/s00125-003-1153-1

Rutter, G. A., Pullen, T. J., Hodson, D. J., & Martinez-Sanchez, A. (2015). Pancreatic β-cell identity, glucose sensing and the control of insulin secretion. *Biochemical Journal*, *466*(2), 203–218. https://doi.org/10.1042/BJ20141384

Semrad, C. E. (2012). Approach to the patient with diarrhea and malabsorption. In L. Goldman (Ed.), *Goldman's cecil medicine* (pp. 895–913). Philadelphia, PA: WB Saunders.

Slaoui, M., & Fiette, L. (2011). Histopathology procedures: From tissue sampling to histopathological evaluation. In G. Jean-Charles (Ed.), *Drug safety evaluation* (pp. 69–82). New York, NY: Humana Press.

Solcia, E., Usellini, L., Buffa, R., Rindi, G., Villani, L., Aguzzi, A., & Silini, E. (1989). Endocrine cells producing regulatory peptides. In J. M. Polak (Ed.), *Regulatory peptides* (pp. 220–246). Basel, Switzerland: Birkhäuser.

Suissa, Y., Magenheim, J., Stolovich-Rain, M., Hija, A., Collombat, P., Mansouri, A., … Glaser, B. (2013). Gastrin: A distinct fate of neurogenin3 positive progenitor cells in the embryonic pancreas. *PLoS ONE*, *8*(8), e70397. https://doi.org/10.1371/journal.pone.0070397

Takahashi, S. Y., Zhao, X., Kageyama, T., & Yamamoto, Y. (1992). Acid cysteine proteinase from the eggs of silkmoth, Bombyx mori: Tissue distribution, developmental changes and the sites of synthesis for the enzyme. *Insect Biochemistry and Molecular Biology*, *22*(4), 369–377. https://doi.org/10.1016/0965-1748(92)90075-P

Tan, T. M., & Bloom, S. R. (2013). *Pancreatic polypeptide* (2nd ed.). Cambridge, MA: Academic Press.

Wartmann, T., Mayerle, J., Kähne, T., Sahin-Tóth, M., Ruthenbürger, M., Matthias, R., … Lerch, M. M. (2010). Cathepsin L inactivates human trypsinogen, whereas Cathepsin L-deletion reduces the severity of pancreatitis in mice. *Gastroenterology*, *138*(2), 726–737. https://doi.org/10.1053/j.gastro.2009.10.048

Wierup, N., Svensson, H., Mulder, H., & Sundler, F. (2002). The ghrelin cell: A novel developmentally regulated islet cell in the human pancreas. *Regulatory Peptides*, *107*(1–3), 63–69. https://doi.org/10.1016/S0167-0115(02)00067-8

**How to cite this article:** Luziga C. Immunoreactivity of cytotoxic T-lymphocyte antigen 2 alpha in mouse pancreatic islet cells. *Anat Histol Embryol*. 2020;00:1–8. https://doi.org/10.1111/ahe.12541