Title: Do chief cells of the human stomach possess secretory products other than pepsinogen?

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Abstract

The chief cells of the stomach are those cells classically responsible for the secretion of pepsinogen. Evidence is presented that the cytokine products interleukin 1β (IL-1β), interleukin 8 (IL-8) and interferon gamma (INFδ) can be produced by chief cells. Those changes to chief cells associated with *Helicobacter pylori* infection are described as well as merocrine and holocrine secretion.

Keywords: Stomach, chief cells, secretion, interleukin 1β, interleukin 8, interferon gamma.

Introduction

The classical function of the chief cells of the human stomach is as a producer and secretor of pepsinogens. When the pepsinogens (proenzymes) are exposed to acid they are converted into the active enzyme, pepsin, and activation segment.

During a study of cytokines it became apparent that the chief cells of the gastric glands contained many products other than pepsinogen and these products have significant biological activity particularly with respect to inflammation and infection. It was therefore decided to report on the following cytokines IL-1β, IL-8 and INFδ. This study has involved examining these cytokines in the normal stomach and in the stomach infected with *Helicobacter pylori*.

Material and methods

Endoscopic biopsies have been taken from specific sites in the stomach of 38 normal patients and 54 patients whose stomach has been infected with *Helicobacter pylori*. This infection has been substantiated by a positive CLO test (Kimberley-Clark, Ballard Medical Products, Utah, USA) and immunohistochemical detection of the *Helicobacter pylori* in the biopsies.

The biopsies are processed either for transmission electron microscopic study or for immunohistochemical studies. The transmission electron microscopic study has involved fixing the biopsies in 3% cacodylate buffered glutaraldehyde (pH 7·3) at 4°C for four to twenty four hours. The biopsies are then rinsed in cacodylate buffered 10% sucrose (pH 7·3) at 4°C for twenty four hours. Following postfixing in veronal acetate buffered 1% osmium tetroxide (pH 7·3) at 4°C for two hours, the biopsies are rinsed in chilled tap water at 4°C. Dehydration is carried out in a graded series of ethyl alcohol and the biopsies are cleared in propylene oxide. The biopsies are embedded in epoxy resin. Sections are cut 25nm thick and mounted on copper grids prior to being stained with 1% uranyl acetate and Reynolds lead citrate. The sections are examined with a Philips 7000 electron microscope.

Those biopsies used for immunohistochemical studies have been resin embedded by the technique of Britten, Howarth and Roche (1993). Endoscopic biopsies are immediately placed into ice acetone containing 2mM phenyl methyl sulphonyl fluoride and 20mM iodoacetamide and fixed overnight at -20°C. The fixative is replaced with acetone at room temperature for 15 minutes followed by methyl benzoate at room temperature for 15 minutes. The biopsies are then infiltrated with processing solution consisting of 5% methyl benzoate in glycol methacrylate (GMA solution A) at 4°C with three changes of GMA solution A with two hours in each change of solution. The embedding solution consists of 10 millilitres GMA solution A and 70 millilitres benzoyl peroxide. The embedding solution is freshly prepared by dissolving the benzoyl peroxide in solution A by gently shaking. When dissolved add GMA solution B (250µls). The processed biopsies are embedded in the embedding solution, polymerized at 4°C for 48 hours and stored in airtight boxes at -20°C.

The immunohistochemical studies have been performed using the following antibodies:

Antibody	**Clone**	**Source**
Interleukin-1β (IL-1β)	mouse monoclonal (2805)	R & D Systems
Interleukin 8 (IL-8)	mouse monoclonal (NAP11)	Bender Med Systems
Interferon gamma (INFδ)	mouse monoclonal (25718)	R & D Systems
Helicobacter pylori	rabbit polyclonal	DakoCytomation

The immunochemical staining for monoclonal and polyclonal antibodies has been carried out as described in Steer, 2005.

Ethical approval for the study has been obtained. Permission to obtain the endoscopic biopsies as well as perform the cytochemical analyses have been obtained from the patients. The patients have been undergoing endoscopic examinations as part of the investigation of their presenting symptoms.

Results and discussion

1. Interleukin 1β (IL-1β)

IL-1β production is significantly increased *in vitro* in the gastric mucosal biopsies from patients with *Helicobacter pylori* infection when compared with the normal gastric mucosa (Noach et al 1994; Peek et al 1995). In the present study IL-1β is found in the

mucosa of the normal stomach. There is significant staining for IL-1β in the mucus of the supranuclear portion of the epithelial cells at the luminal surface of the mucosa and at the pit/isthmus of the gastric glands. There is a generalised but weak expression of IL-1β in the connective tissue of the mucosa and patchy staining of the epithelial basement membrane/basal cell membrane in the region of the pit/isthmus of the gastric glands. There is some coarse granular staining in the lumen of the mucosal blood vessels and positive staining of the lining of these blood vessels. In the body of the stomach there is positive granular staining of the cytoplasm of the chief cells (figure 1).

An examination of the gastric mucosa of patients with *Helicobacter pylori* infection reveals that there is a significant upregulation of IL-1β expression in the mucus of the epithelial cells at the gastric surface and the pit/isthmus of the gastric glands. In addition, some of the connective tissue cells are positively stained for IL-1β and there is more intense and generalised staining for IL-1β of the basal cell membrane/basement membrane of the epithelial cells in the region of the pit/isthmus of the gastric glands as well as the luminal epithelium of the stomach. The IL-1β staining in the mucosal blood vessels is similar to that of normal mucosal biopsies but is upregulated.

There is some cytoplasmic staining of the gastric chief cells in patients infected with *Helicobacter pylori* but many of these cells appear to be degranulated.

The effect of *Helicobacter pylori* on IL-1β expression *in vivo* supports the previous *in vitro* evidence and is consistent with the conclusion that this proinflammatory cytokine is associated with the upregulation of IL-8 expression.

IL-1β production is significantly increased *in vitro* in the gastric mucosal biopsies from patients with *Helicobacter pylori* infection when compared with the normal gastric mucosa (Noach et al 1994; Peek et al 1995). There is significant upregulation of IL-1β expression in *Helicobacter pylori* infection (Steer 2005) but the IL-1β expression in gastric chief cells in patients infected with *Helicobacter pylori* gives the appearance of these chief cells being degranulated.

The reason for the localisation of IL-1β in chief cells is uncertain but it is known that IL-1β has an effect on pepsinogen secretion. *In vitro* studies have revealed that IL-1β has an inhibitory effect on pepsinogen secretion caused by db-cAMP and histamine (Serrano et al 1997). The histochemically apparent downregulation of IL-1β chief cell staining in patients infected with *Helicobacter pylori* is consistent with a decrease in the inhibitory effect of IL-1β on pepsinogen secretion. This change would be consistent with the increased serum pepsinogen 1 levels found in patients with *Helicobacter pylori* infection.

2. Interleukin 8 (IL-8)

IL-8 is a cytokine which has a specific chemoattractant property for polymorphonuclear leucocytes.

In the normal stomach the epithelial expression of IL-8 involves that epithelium at the luminal surface of the stomach (Steer 2005). This epithelial expression is both cytoplasmic and membranous. In addition to the intercellular and basal cell membranes, the mucus and basal cytoplasm of these epithelial cells stain for IL-8. In those mucosal biopsies from the body of the stomach there is IL-8 expression in the chief cells (figure 2 and 3) and in those chief cells ‘shed’ into the lumen of the gastric glands. This chief cell expression involves the cytoplasm but is particularly dense at the cytoplasmic granules (figure 3). In some chief cells the granules are absent or infrequent and at such sites the

less densely stained areas for IL-8 appear as vacuoles. The parietal cells are negative for IL-8.

There is occasional IL-8 expression in the lumen of the normal mucosal blood vessels. This appears as very coarse granules and may represent IL-8 expression in platelets. There is no IL-8 expression in the endothelial cells of the normal mucosa.

Infection of the stomach with *Helicobacter pylori* results in an increased expression of IL-8 which is particularly marked in the epithelium (Steer 2005). That IL-8 expression noted in the epithelium at the luminal surface of the normal mucosal biopsies extends to the pit/isthmus area of the gastric glands. Some of the lamina propria connective tissue cells are positively stained for IL-8 with occasional cells having the morphology of plasma cells.

IL-8 is also expressed in the endothelium of mucosal blood vessels. This granular intravascular IL-8 staining may be localised to platelets.

The chief cells in gastric biopsies from patients infected with *Helicobacter pylori* are stained for IL-8 (figure 4) but the IL-8 expression is decreased compared to the normal gastric mucosa. The cytoplasm of these cells contains a significant number of vacuoles with the degranulation of chief cells being consistent with the IL-8 having been secreted by these cells in *Helicobacter pylori* infection.

The effect of IL-8 on adhesion molecules and on polymorphonuclear leucocyte activation and upregulation is discussed in Steer 2005. Analyses of IL-8 in gastric mucosal biopsies from patients with *Helicobacter pylori* infection have revealed an increased expression of this chemokine (Yamaoka et al 1997). This confirms previous *in vitro* research which has revealed a potiential for upregulation of IL-8 on exposure to *Helicobacter pylori*. The upregulation of IL-8 has been demonstrated for whole mucosal biopsies where multiple cell-types could be involved in the production of this chemokine (Crabtree et al 1993; Noach et al 1994). Similar results have been obtained when using epithelial cell lines in culture (Crabtree et al 1994; Crabtree et al 1995; Sharma et al 1995; Crowe et al 1995). This increased expression of IL-8 by either epithelial cell lines (Crabtree et al1994; Crabtree et al 1995) or in mucosal biopsies (Yamaoka et al 1997) is greater if the *Helicobacter pylori* are CagA positive.

This study has shown the chief cells as an extremely rich source of IL-8. This is seen in the normal stomach and the stomach infected with *Helicobacter pylori*. The quantity of IL-8 staining material in chief cells is variable. It may take the form of cells densely packed with granules in the supranuclear region. IL-8 staining granules may be seen in the gastric gland lumen. The presence of such IL-8 concentration in the normal stomach is unexpected in view of the lack of polymorphonuclear leucocyte infiltration in the normal stomach. This raises the question of whether this IL-8 is accessible to the mucosa as a chemokine, acting as a store for IL-8 or alternatively whether additional cell signalling mechanisms are required to initiate polymorphonuclear leucocyte migration. Numerous cell signalling mechanisms in addition to IL-8 have been associated with polymorphonuclear leucocyte migration.

3. Interferon gamma (INFδ)

The expression of INFδ is upregulated by *Helicobacter pylori* infection of the stomach (figure 5 and 6). There is weak or no INFδ expression in the normal gastric mucosa with slight staining in the region of the epithelial mucus and slight granular staining in the mucosal blood vessels. The connective tissue expression is greatly

upregulated particularly in the pit/isthmus regions of the gastric glands. The chief cells are not stained in the normal stomach (figure 7) but there is strong expression of INFδ in the chief cells in *Helicobacter pylori* infection (figure 8). There is strong INFδ expression in those chief cells shed into the lumen of the gastric glands.

4. Secretion by chief cells

Secretion by epithelial cells can take a number of different forms. Two of these forms are merocrine secretion when part (or a fraction) of the cell is shed into the gland lumen. The degranulation of chief cells associated with *Helicobacter pylori* infection resulting in the cells appearing to have a number of vacuoles (figure 4) would be consistent with merocrine secretion.

Another form of epithelial secretion is holocrine secretion (Le Gros Clark 1958) when the whole cell (together with the secretory products) is shed into the gland lumen. Such holocrine secretion is seen in epithelial cell necrosis (Steer 2007) when whole necrosing chief cells are shed into the gastric gland lumen in *Helicobacter pylori* infection of the stomach.

The roles of the cytokine secretory products of chief cells have been discussed in Steer 2005.

Acknowledgements

Grateful acknowledgement is made for the help received from Dr. Susan Wilson, Linda Jackson, Helen Rigden and Jon Ward of the Histochemistry Research Unit, University of Southampton School of Medicine, Anton Page of the Biomedical Imaging Unit, University of Southampton School of Medicine / Southampton University Hospital NHS Trust and Adie Falcinelli of the Learning Media, Southampton University Hospital NHS Trust.

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Figure 1.

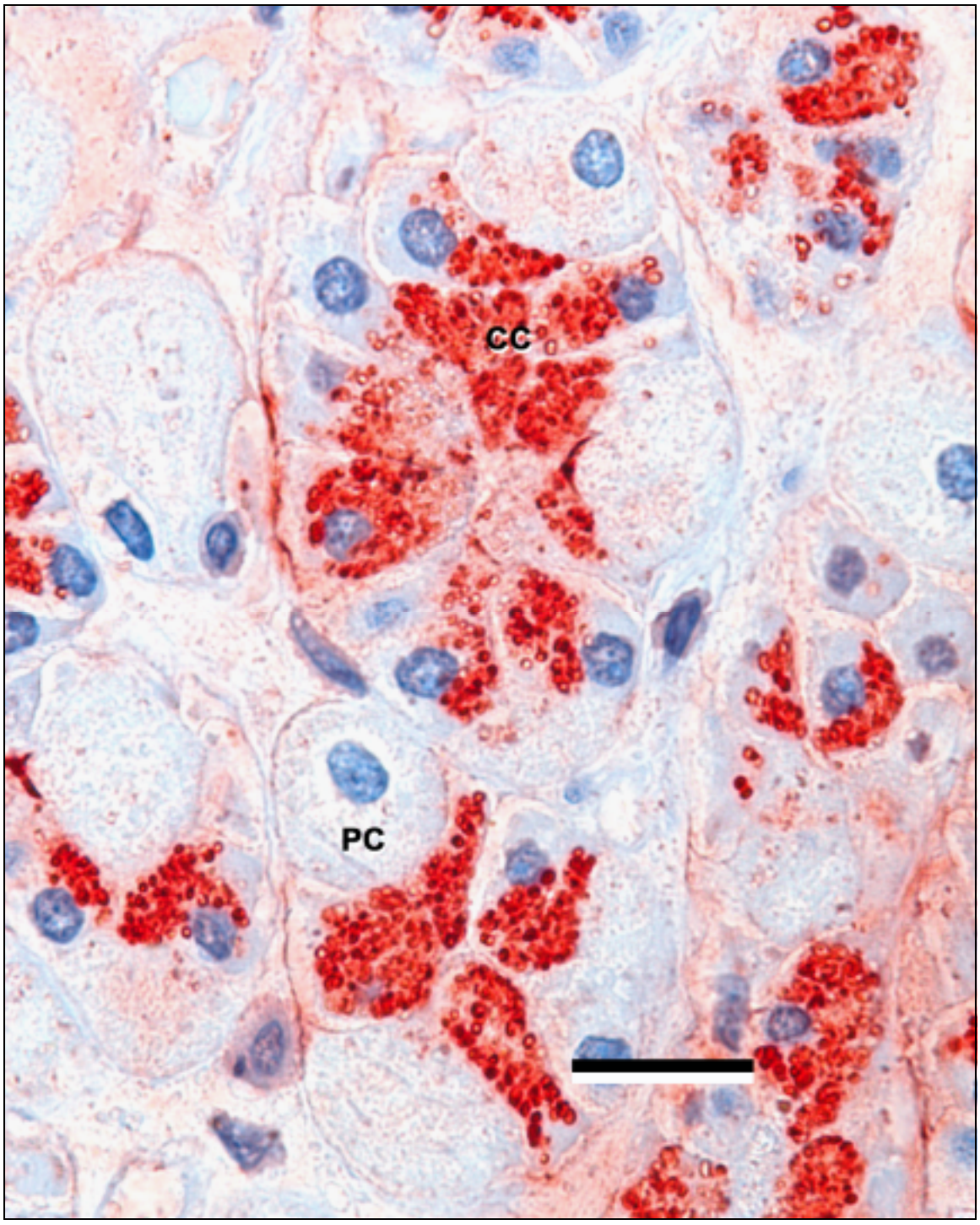

Figure 1. Interleukin 1β. (Chromogen substrate).

Mucosal biopsy from the body of the normal stomach. The chief cells (CC) possess numerous IL-1β positive granules. The parietal cells (PC) lack any IL-1β staining. Scale bar is 20µm.

Figure 2.

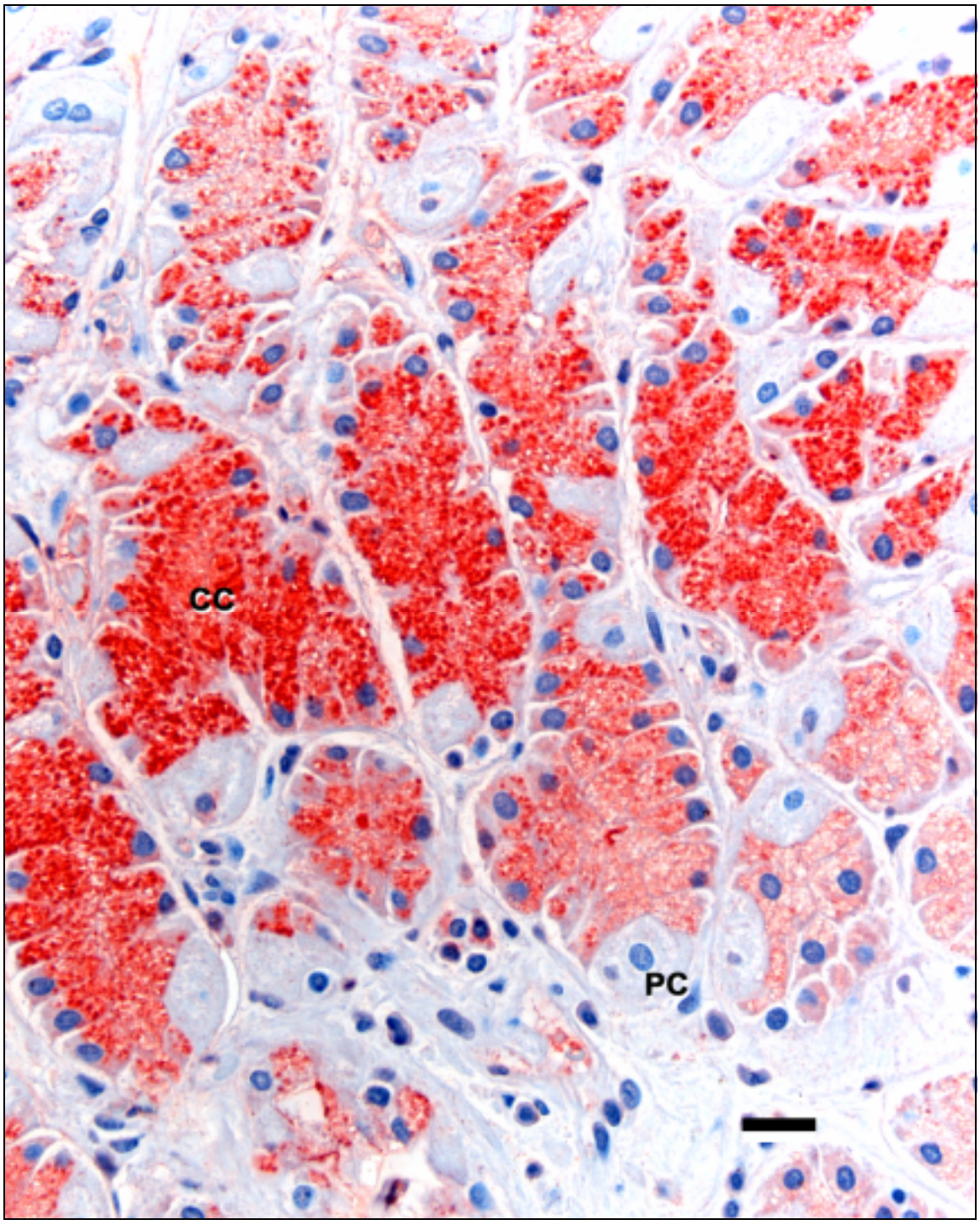

Figure 2. Interleukin 8. (Chromogen substrate).

Mucosal biopsy from the body of a normal stomach. Numerous chief cells (CC) with IL-8 positive granules are seen. The parietal cells (PC) lack any IL-8 staining. Scale bar is 20µm.

Figure 3.

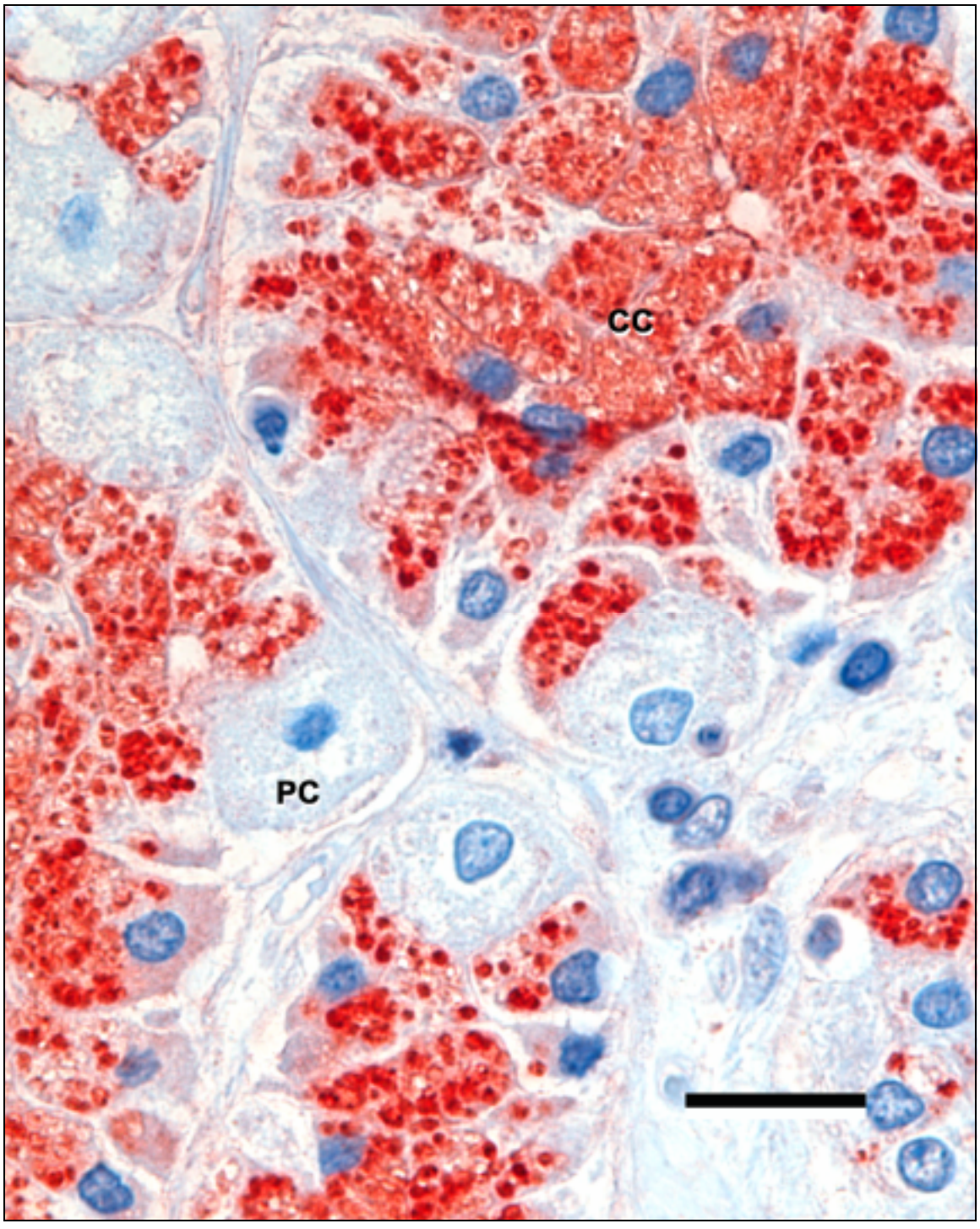

Figure 3. Interleukin 8. (Chromogen substrate).

Mucosal biopsy from the body of the normal stomach. Numerous chief cells (CC) with positive IL-8 granules are seen. The parietal cells (PC) lack any IL-8 staining. Scale bar is 20µm.

Figure 4.

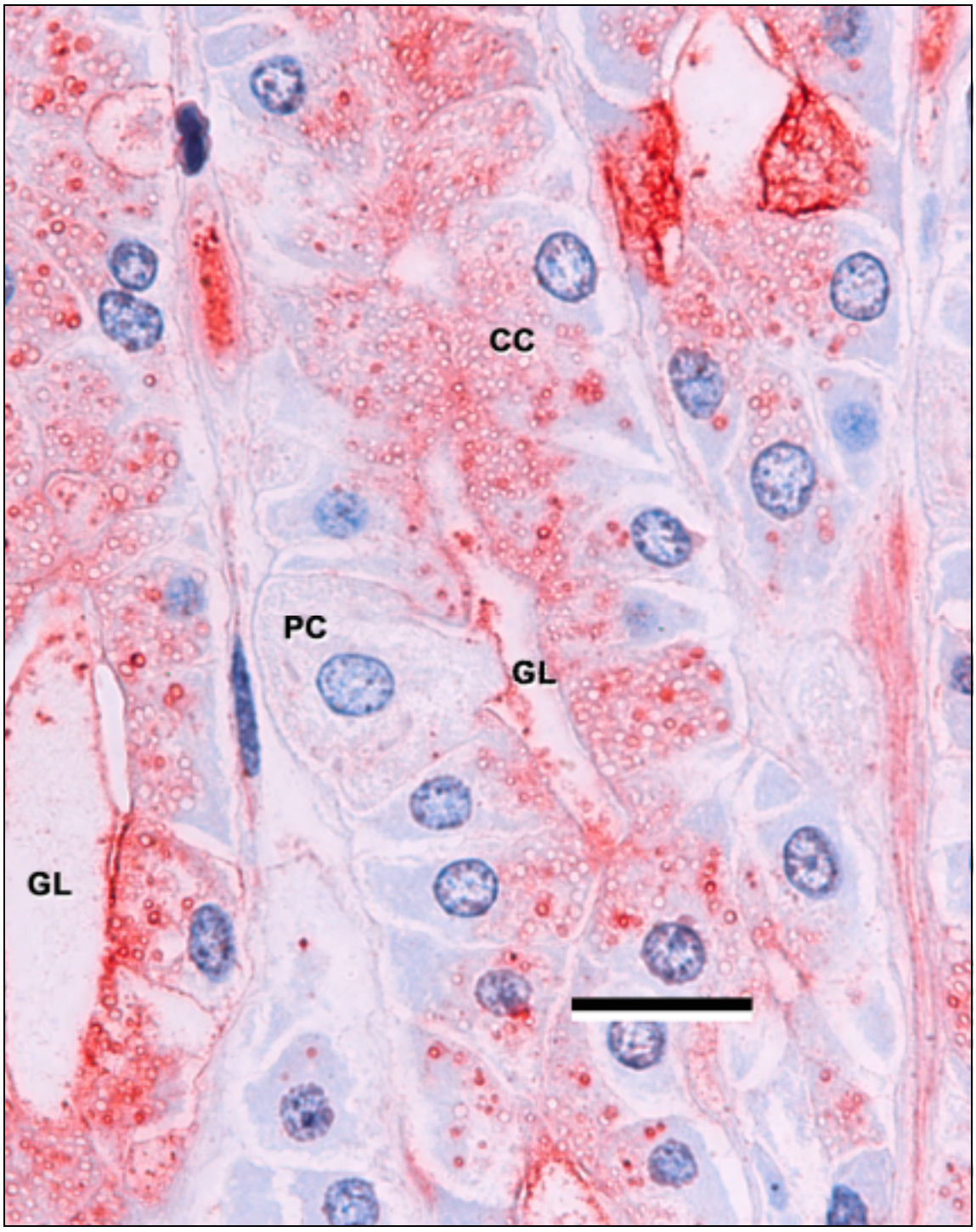

Figure 4. Interleukin 8. (Chromogen substrate).

Mucosal biopsy from the body of a stomach infected with *Helicobacter pylori*. The lumen (GL) of a gastric gland, chief cells (CC) and a parietal cell (PC) are shown. Scale bar is 20µm.

Figure 5.

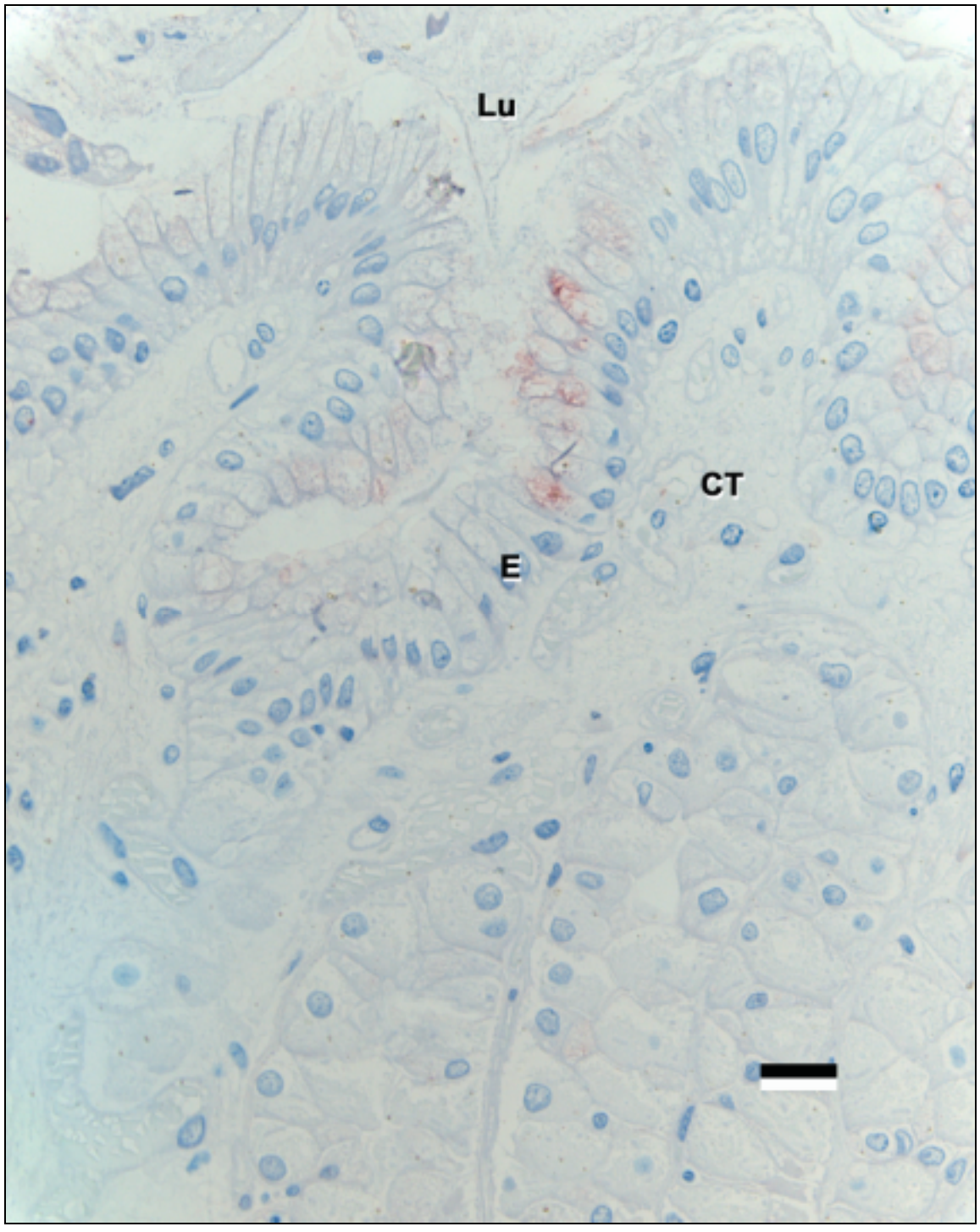

Figure 5. Interferon δ. (Chromogen substrate).

Mucosal biopsy from the body of the normal stomach. The gastric lumen (Lu), gastric epithelium (E) and subepithelial connective tissue (CT) are shown. Scale bar is 20μm.

Figure 6.

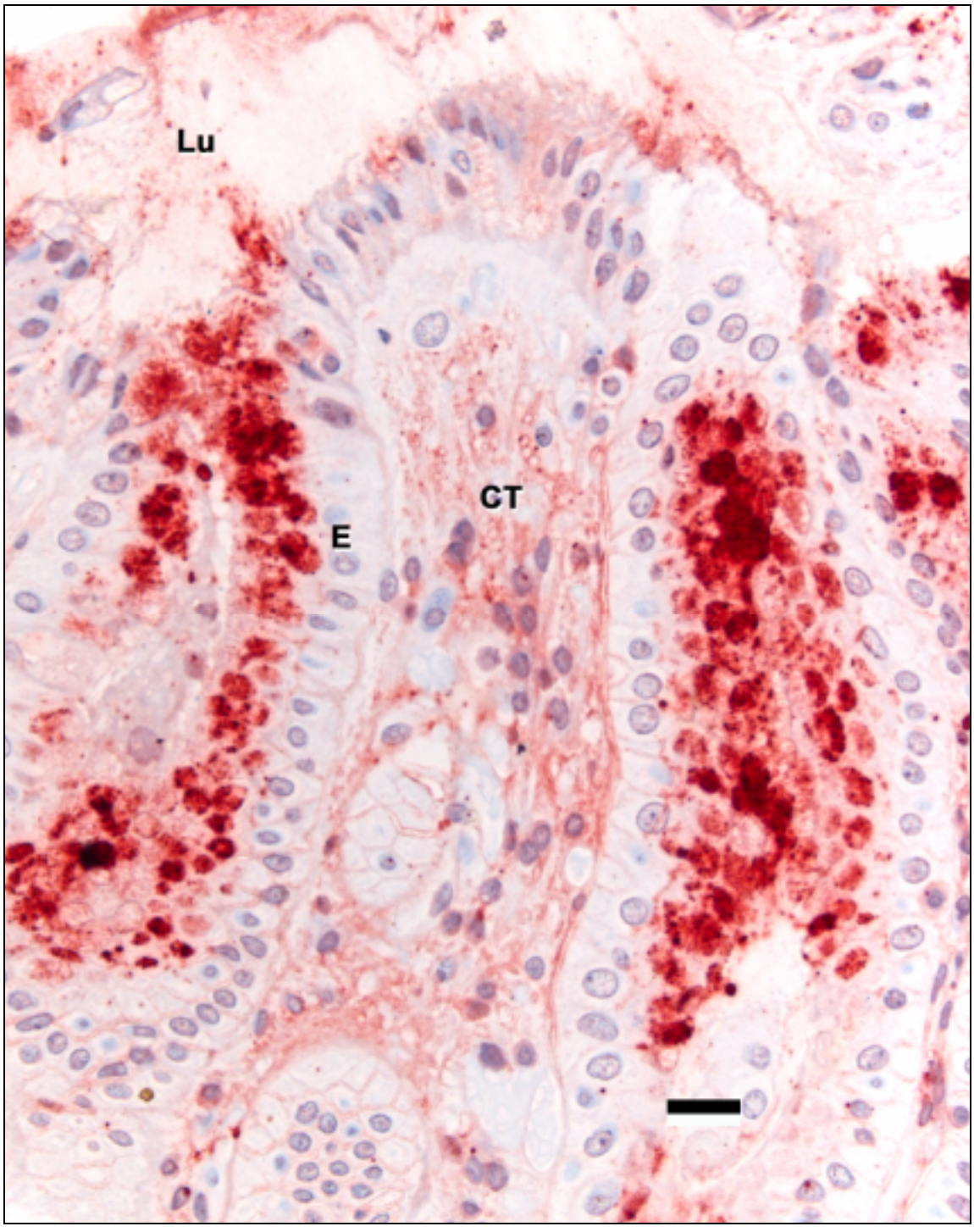

Figure 6. Interferon δ. (Chromogen substrate).

Mucosal biopsy from the body of a stomach infected with *Helicobacter pylori*. The gastric lumen (Lu), gastric epithelium (E) and subepithelial connective tissue (CT) are shown. Scale bar is 20µm.

Figure 7.

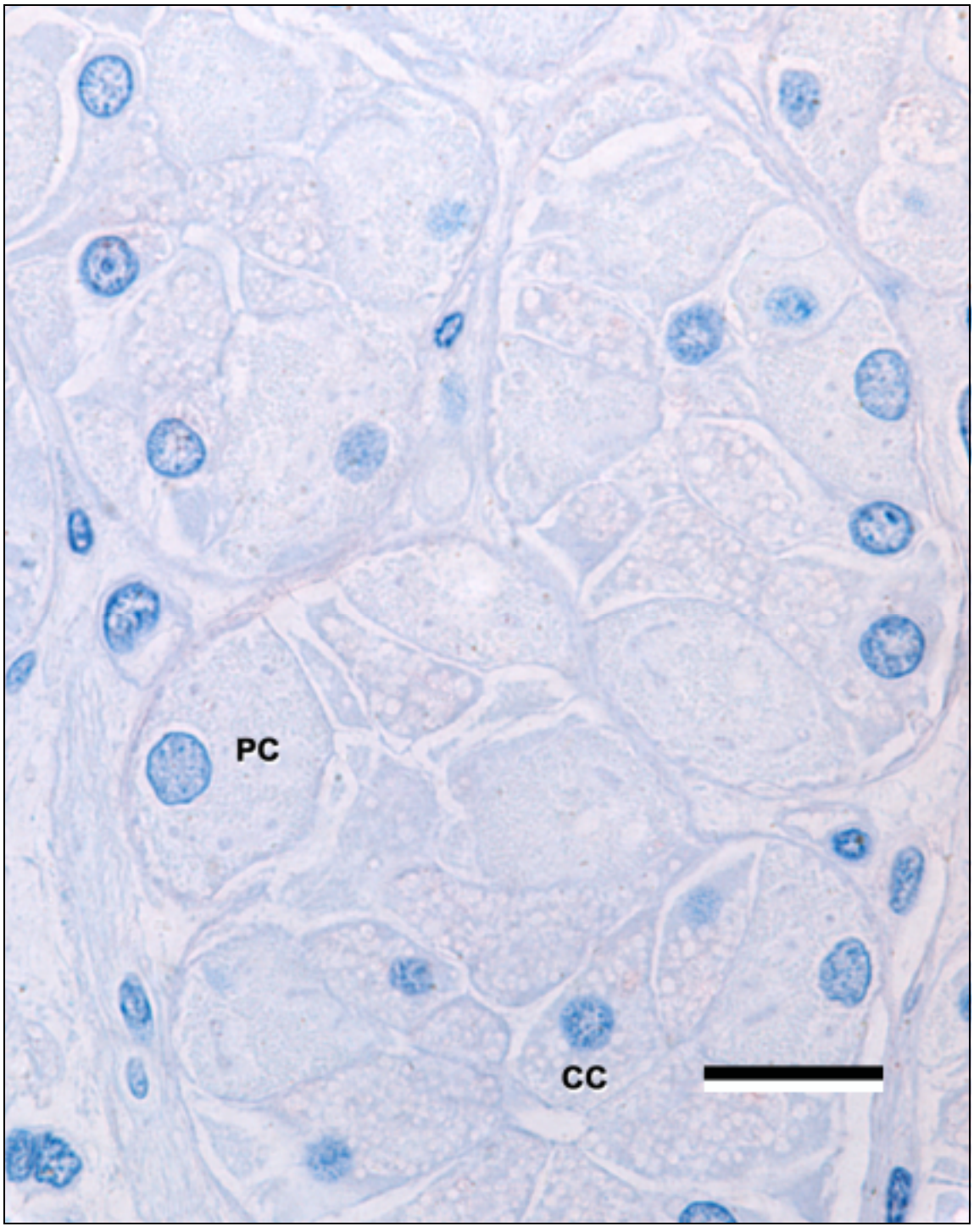

Figure 7. Interferon δ. (Chromogen substrate).

Mucosal biopsy from the body of the normal stomach. The chief cells (CC) and parietal cells (PC) are shown. Scale bar is 20µm.

Figure 8.

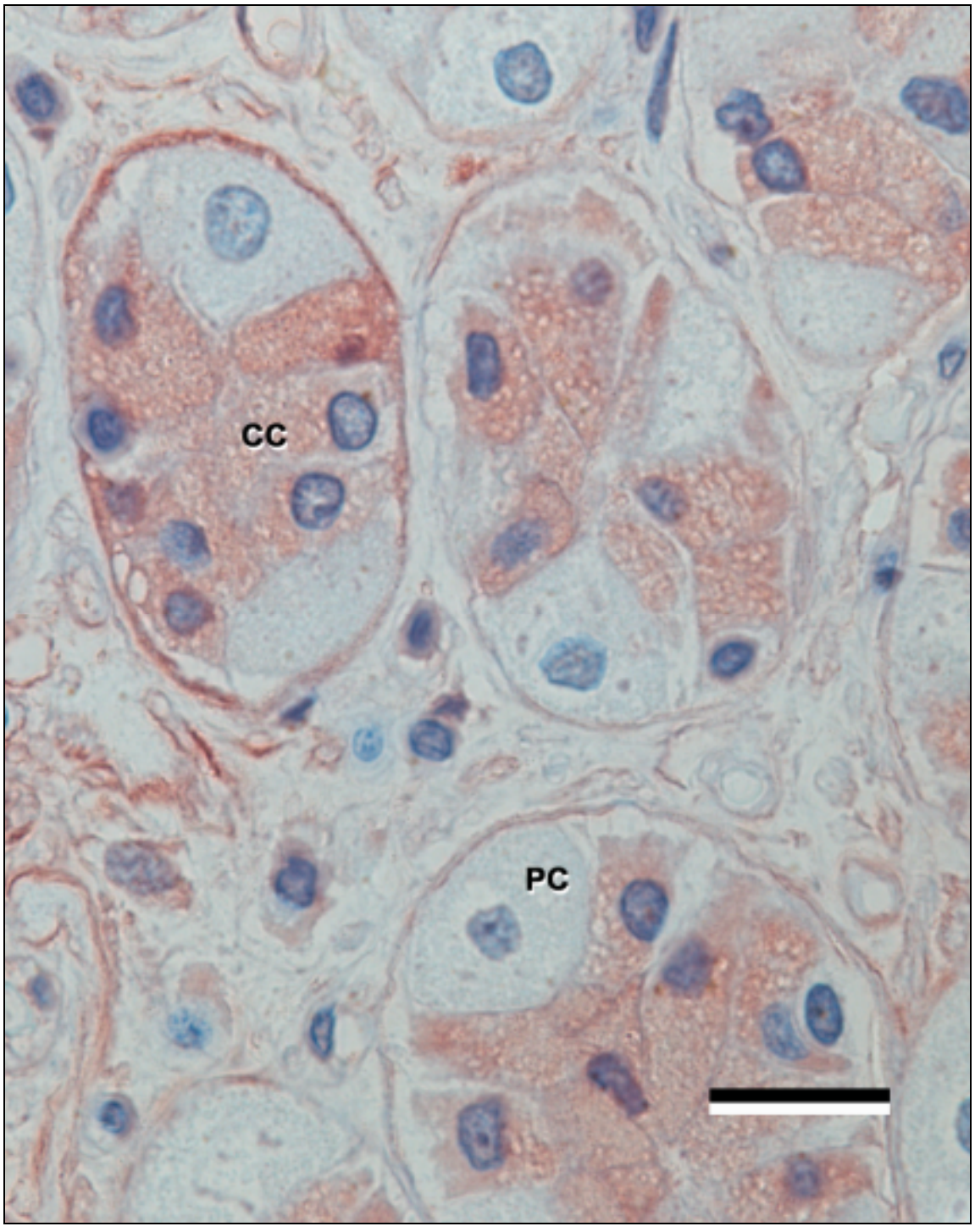

Figure 8. Interferon δ. (Chromogen substrate).

Mucosal biopsy from the body of a stomach infected with *Helicobacter pylori*. The chief cells (CC) and parietal cells (PC) are shown. Scale bar is 20µm.