The Cronkhite-Canada syndrome: an ultrastructural study of pathogenesis

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SUMMARY Electron microscopical and cytochemical studies of intestinal biopsies from a patient with typical features of the Cronkhite-Canada syndrome show that the primary process affects the crypts. This results in cystic dilatation associated with expansion and focal degeneration of the crypt compartment of the intestinal epithelium. The villous epithelium compartment is reduced but ultrastructurally normal. Inflammation and oedema of the lamina propria follows from leakage of mucin through breaks in the abnormal crypts.

The syndrome of gastrointestinal polyposis, alopecia, and nail dystrophy now known as the Cronkhite-Canada syndrome was first described in 1955.¹ Although about 80 cases have now been reported there is little information about the aetiology and pathogenesis. The main clinical effects of the gastrointestinal disease are protein losing enteropathy, hypoproteinaemia, and a range of abnormalities in the absorption of carbohydrates, proteins, and fats.^{2,3} Radioisotope and scanning electron microscopical studies have suggested that the protein loss results from heavy secretion of mucous substances containing protein rather than exudation or rupture of vessels.³ Acquired disaccharidase deficiency and intestinal bacterial overgrowth have been shown in a few cases. There is no familial pattern of incidence. Remission may occur,⁴ either spontaneously or after partial surgical resection, corticosteroid treatment, or nutritional support. Surprisingly, there are no detailed descriptions of the transmission electron microscopical appearances of the gastrointestinal lesions. We describe the electron microscopy and cytochemistry of a typical case.

Case report

A 66 year old man presented with a four month history of scalp hair loss; a three month history of diarrhoea, abdominal pain, and weight loss; and loose nails and ankle swelling for two weeks. Results of investigations were as follows. Haemoglobin concentration 13.4 g/dl; blood film was normal; ery-

throcyte sedimentation rate 55 mm in the first hour; serum B₁₂ and folate were normal; prothrombin time was normal; serum albumin 33 g/l; serum alkaline phosphatase 299 units (normal less than 250); serum calcium 1.88 mmol/l; faecal fat 41 mmol/24 h. A lactose tolerance test using breath hydrogen was normal; small bowel enema suggested malabsorption; sigmoidoscopy showed a normal mucosa; upper gastrointestinal endoscopy showed a strikingly polypoid antrum, pylorus, and duodenum.

He was given only calciferol and folic acid and spontaneously improved over the next six months. He regained his weight, his diarrhoea ceased, his scalp hair regrew, but his nails remained dystrophic.

METHODS

Light microscopy

Endoscopic biopsies were obtained from the stomach, duodenum, and rectum. Sections of formalin fixed, paraffin embedded tissue were stained with haematoxylin and eosin, by the periodic acid Schiff (PAS) method with and without diastase digestion, by Hale's dialysed iron technique, with Alcian blue at pH 2.5 and 1.0 and after sialidase treatment by the high iron diamine technique, and by the combined high temperature methylation saponification technique.⁵ Sections were also stained for IgA, IgM, IgG, IgE, and muramidase by the peroxidase-antiperoxidase immunoperoxidase technique.⁶

Electron microscopy

Some endoscopic biopsies of polypoid mucosa from the duodenum were orientated, processed through glutaraldehyde fixation and postfixation in osmium

tetroxide, and stained with uranyl acetate before embedding in Araldite.⁷ Semithin sections were stained with haematoxylin and eosin⁸ for light microscopy, and ultrathin sections from blocks showing no evidence of biopsy trauma were further stained with lead citrate and examined in a JEOL 100S transmission electron microscope. Specimens for ultrastructural cytochemistry were fixed in paraformaldehyde and embedded in Araldite resin. Ultrathin sections were stained by the periodic acid thiocarbonylhydrazide silver proteinate (PATSP) method.⁹

Results

LIGHT MICROSCOPY

Stereomicroscopical examination of duodenal mucosa showed some normal villi but also flat areas with prominent crypt openings. In sections (Fig. 1) there was patchy partial villous atrophy with elongated and irregular crypts and glandular cysts. The dilated crypts and cysts were lined by columnar epithelium and contained mucin, granular debris, degenerate epithelial cells, and foamy histiocytes. Extensive areas of the epithelium were pale staining, attenuated, and flattened. In places the attenuated epithelium was lost exposing the lamina propria, which showed oedema and was infiltrated by many histiocytes and some plasma cells and eosinophils. Biopsies from the gastric antrum showed that the

Fig. 1 Light micrograph of duodenal mucosa in Cronkhite-Canada syndrome. There is irregular partial villous atrophy, with elongated, irregular, and cystic crypts. The cysts are lined by attenuated epithelium. There is a mixed inflammatory cell infiltrate in the lamina propria. Haematoxylin and eosin. Original magnification $\times 125$.

pits and mucous glands were irregular, branched, and often cystic. The rectal mucosa showed slight irregularity and dilatation of the crypts.

Abundant PAS positive mucinous material was found within intestinal goblet cells and gastric mucous cells and within the dilated crypts and cysts in intestinal and gastric mucosa. PAS positive material extended across breaches in the epithelium into the lamina propria and was also present in the cytoplasm of histiocytes surrounding such areas. No significant qualitative histochemical abnormality of mucin was detected compared with controls. Immunohistochemical staining for muramidase showed a few Paneth cells at the bases of small-intestinal crypts. The counts of IgA, IgM, IgG, and IgE plasma cells were within normal limits.

Electron microscopy

The villi and the polypoid areas in the duodenal mucosa were covered by columnar absorptive

Fig. 2 Electron micrograph of apical part of villous absorptive cell from the surface of the duodenum in Cronkhite-Canada syndrome. Microvilli, lysosomes, endoplasmic reticulum, mitochondria, and intercellular junctions show no pathological changes. Lead citrate and uranyl acetate. Original magnification $\times 12\,500$.

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epithelium (Fig. 2). The cells appeared normal and showed no abnormalities of microvilli or lysosomes or other degenerative changes. The predominant cell type lining both crypts and the cysts was the undifferentiated crypt cell (Figs. 3 and 4). In crypts and most parts of the cyst walls these cells were columnar with basal nuclei. Round, membrane bound granules up to $1.5\ \mu\text{m}$ across were identified in the apical regions of otherwise undifferentiated cells. The PATSP method strongly stained glycoprotein in the glycocalyx on the apical surface, as a rim in the granules, and in the Golgi cisternae. Goblet cells were widespread among the undifferentiated cells as in the lateral walls of normal crypts. Paneth cells were not seen in the walls of cysts, but they were present in the bases of the less abnormal crypts and showed the characteristic large, membrane bound secretory granules in the cytoplasm towards the apex of the cell. Endocrine cells (Fig. 5) were present as in normal small intestinal mucosa.

Throughout the lining of the cysts and crypts small groups of cells or single cells showed degenerative changes with vacuolation and florid dilatation of the endoplasmic reticulum and mitochondria. In some cells the changes amounted to focal necrosis with, in addition, loss of cytoplasmic ground substance and empty nuclei. In some areas dilatation of endoplasmic reticulum and vacuolation were associated with separation of the cells from the basement membrane and from each other, and luminal contents were seen beneath the epithelium (Fig. 5). The underlying basement membrane appeared attenuated. In the adjacent lamina propria there was pronounced oedema with collagen fibres separated by abundant pale staining amorphous material (Fig. 3). Numerous histiocytes, many with large cytoplasmic vacuoles containing amorphous light staining material, were present (Figs. 4 and 5). This material stained black with PATSP, which confirms that these cells were muciphages. Lymphocytes,

Fig. 3 Electron micrograph of cells lining a cyst and the underlying lamina propria from the duodenum in Cronkhite-Canada syndrome. Most of the cells are undifferentiated crypt cells (c). An oligomucous cell is also present (o). A cell shows vacuolar degeneration (v). The lamina propria shows abundant lightly staining intercellular material (ic). Lead citrate and uranyl acetate. Original magnification $\times 2400$.

Fig. 4 Electron micrograph of crypt epithelium and lamina propria. Three necrotic crypt cells (n) show pale cytoplasm and empty nuclei. The lamina propria contains inflammatory cells including several vacuolated macrophages (m). Lead citrate and uranyl acetate. Original magnification $\times 2400$.

eosinophils, and polymorphonuclear leucocytes showing no appreciable abnormalities were also seen.

Discussion

In the initial description the gastrointestinal lesions were said to be adenomatous polyps, but in most recent reports they are described as inflammatory pseudopolyps⁴ resembling juvenile polyps of the

colon.¹⁰ Ultrastructural study shows no epithelial changes resembling those seen in large bowel adenomas or metaplastic polyps.¹¹ Unlike juvenile polyps the lesions of the Cronkhite-Canada syndrome are small and diffuse. They do not usually show surface ulceration, blockage of the necks of crypts by inflammation, or qualitative histochemical abnormalities of mucin pattern which are typical of juvenile polyps,¹² although an occasional case may show extensive ulceration of numerous polyps (H

Fig. 5 Electron micrograph of cyst wall. Undifferentiated crypt cells (c) and an enterochromaffin cell (ec) show vacuolation and dilatation of endoplasmic reticulum. Part of the epithelium is separated from the basement membrane by amorphous material (a). Vacuolated muciphages (m) are present in the underlying lamina propria. Lead citrate and uranyl acetate. Original magnification $\times 2500$.

Thompson, personal communication). It has been suggested that juvenile polyps result from epithelial regeneration in granulation tissue following local inflammation and ulceration.¹²⁻¹⁴ This explanation appears inappropriate for the Cronkhite-Canada syndrome, however, because of the structural and histochemical differences from juvenile polyps listed above.

The distinctive structural features of the polypoid lesions of the Cronkhite-Canada syndrome as studied here in the small intestine have to be interpreted in relation to the functional anatomy of the mucosa. The small-intestinal epithelium can be divided into two functionally and structurally distinct compartments: the crypt and the villus. In the Cronkhite-Canada mucosa the villous compartment is reduced, which results in patchy villous atrophy, but the cells appear structurally normal. This is unlike coeliac disease and many diseases associated with malabsorption, in which the villous absorptive cells show striking ultrastructural abnormalities.¹⁵ The crypt compartment is considerably expanded with elongated, irregular, and cystic crypts lined mostly by undifferentiated crypt cells which are ultrastructurally normal.¹⁶ These and the goblet cells which they produce¹⁷ have a physiological function of secreting mucin,¹⁸ ions, and water. Ultra-

cytochemical study shows that mucin production is preserved in the Cronkhite-Canada mucosa. The cystic change is unlikely to result solely from excess mucin production; even massive stimulation of mucin release such as accompanies acetylcholine stimulation does not result in cystic alteration to the crypts of normal small-intestinal mucosa.¹⁹ A previous scanning electron microscopical study reported horn like plugs of highly viscous mucus protruding from crypts.³ Our studies do not support the suggestion that an abnormally viscous mucus is involved. The mucin is histochemically normal and the lesions do not show the wide necked open crypts plugged by mucus and the distortion of villi which are known to result from production of viscous mucin in cystic fibrosis.²⁰

The reduced but ultrastructurally normal villous compartment and expanded crypt compartment suggest a block in the normal maturation of undifferentiated crypt cells to villous epithelial cells. The degeneration and detachment of lining cells in the abnormal crypts and cysts may indicate selective damage to crypt epithelium by an exogenous agent, as has been shown in vitro to result from bathing the serosal surface of ileal mucosal sheets with solutions depleted of calcium.²¹ Alternatively, the epithelial degeneration may result simply from pressure on the

wall of distended and cystic crypts. The oedema and inflammation in the lamina propria appear to be secondary to leakage of mucin and possibly other secretions through the damaged wall, as reflected by the muciphages shown in this study.

The remission of some cases⁴ supports the possibility that an exogenous agent or nutritional deficiency might be responsible. No infectious agent was found in this or any other case.²² The lesions have been compared to the cystic lesions described in the colons of patients with pellagra,²³ but cystic changes have not been described elsewhere in the gut in pellagra. The relation of those lesions to Cronkhite-Canada syndrome is doubtful.

Ultrastructural study provides further evidence that the characteristic protein loss is due to excess mucus secretion by crypt cells and that the minor and variable degree of malabsorption results from a reduction in absorptive surface area and not from damage to the absorptive epithelium. Reduction in Paneth cells, for which a role in controlling bacterial growth has been suggested,¹⁰ may be connected with the bacterial overgrowth which has been described.

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