Chemical and histochemical studies of normal and diseased human gastrointestinal tract. II. A comparison between histologically normal small intestine and Crohn’s disease of the small intestine

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Summary

Comparative chemical and histochemical studies were performed on formalin-fixed, surgical specimens of human small intestine from cases of Crohn’s disease and normal controls. The sialic acids of the crude glycoproteins isolated from normal ileum were significantly less neuraminidase-susceptible and more C4 substituted (P < 0.01) than those of the glycoproteins isolated either from normal upper small intestine (duodenum and jejunum) or from cases of Crohn’s disease of the ileum. Fractionation yielded two major sialic acid-containing fractions, eluting from DEAE-cellulose with 0.2 M or 0.3 M sodium chloride. Both fractions contained fucose, galactose, glucosamine and galactosamine in addition to sialic acids both with and without O-acyl substituents at position C4 and/or in the side-chain (side-chain O-acylated sialic acids were also detected by histochemical procedures). The fractions differed significantly from one another with respect to the neuraminidase susceptibility of their sialic acids (P < 0.01), the percentage of C4 (P < 0.01) and side-chain substituted sialic acids (P < 0.05), and the molar fucose–sialic acid ratio (P < 0.05). The O-acyl substitution patterns of the sialic acids of both the 0.2 M and 0.3 M fractions of the upper small intestine glycoproteins differed significantly from those of the corresponding fractions from normal ileum, while the sialic acids of the 0.2 M fractions from Crohn’s disease of the ileum differed significantly from normal with respect to neuraminidase susceptibility (P < 0.01) and percentage C4 substitution (P < 0.01); the 0.3 M fractions differed only in the percentage of sialic acids substituted at C4. The differences between the sialic acids from the normal and Crohn’s disease specimens were shown to be independent of either the anatomical origin of the specimen or the histopathological sub-group of the Crohn’s disease specimens; no significant differences were noted between the sub-groups but all the sub-groups differed from normal.
Introduction

As part of a continuing chemical and histochemical investigation of the role of the epithelial glycoproteins in human gastrointestinal disease (Culling et al., 1975, 1977a,b, 1979; Reid et al., 1980, 1984), we have described methods for the isolation of such molecules from formalin-fixed tissue specimens (Reid et al., 1978, 1980, 1983) and their subsequent fractionation (Ramey et al., 1979) and microchemical analysis (Reid et al., 1978, 1980, 1983). In previous communications, we have reported the results of comparative studies of glycoproteins isolated from histologically normal large intestine, colonic tumours and cases of ulcerative colitis and diverticular disease of the colon (Reid et al., 1980, 1983). In this paper, we report the results of a comparative study of the epithelial glycoproteins isolated from cases of Crohn's disease of the ileum and from histologically normal controls.

Materials and methods

The materials and methods used in this investigation are described in detail in previous publications (Reid et al., 1973, 1975a,b, 1977, 1978, 1980, 1984; Culling et al., 1976, 1977c; Ramey et al., 1979).

TISSUES

Surgical specimens of normal and diseased human small intestine fixed in 10% formalin–calcium were obtained from the surgical pathology departments of the Vancouver General and Shaughnessy hospitals. The tissues examined are listed in Table 1. A representative sample of each tissue specimen was processed for histopathological and histochemical studies. The remainder of the tissue was used for the extraction of glycoproteins.

METHODS

Histological and histochemical procedures

Formalin-fixed tissues were processed through ethanol and chloroform, embedded in Paraplast and sectioned at 5 μm intervals. Sections were stained with the following:

1. Haematoxylin and Eosin (Culling, 1974): diseased specimens were classified into the following three sub-groups: (a) uninvolved, or showing (b) active disease or (c) inactive disease. The criteria for establishing active versus inactive disease were essentially as outlined by Morson (1975) and Whitehead (1979).

2. Histochemical procedures known to stain side-chain substituted sialic acids: the PBT–KOH–PAS (Reid et al., 1973) and the PAT–KOH–PAS (Culling et al., 1976) procedures. The theoretical basis of these methods have been reviewed in detail elsewhere (Culling & Reid 1980; Culling et al., 1981a,b). Staining with the PBT–KOH–PAS procedure was graded as 0, 1, 2, 3, 4 while sections stained with the PAT–KOH–PAS were graded as blue, blue-purple, purple, red-purple and red.

Chemical procedures

Glycoproteins were extracted from formalin-fixed tissues as described previously (Reid et al., 1978, 1980, 1983) and fractionated by micro DEAE-cellulose chromatography (Ramey et al., 1979). The procedures and calculations used for the determination of (a) the percentage of sialic acids