The expression of Lewis^x on carcinoembryonic antigen (CEA)-related glycoproteins of normal and inflamed oesophageal squamous mucosa

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Carcinoembryonic antigen (CEA)-related antigens were detected histologically in normal and inflamed oesophageal squamous mucosa using polyclonal anti-CEA antisera and monoclonal antibodies recognizing CEA or NCAs (non-specific cross-reacting antigens). Expression was limited to the surface of more mature squames. Immunoblotting of detergent extracts of oesophageal mucosa separated on polyacrylamide gels using polyclonal anti-CEA antisera showed a number of CEA-related proteins, of 195, 145, and 80 kDa. CEA-specific monoclonal antibodies recognized only the 195-kDa glycoprotein. The lower molecular weight species were recognized by anti-NCA antibody DD9 and a CD66 antibody. The carbohydrate antigen, Lewis^x (Le^x, CD15), previously shown to be a marker of mature squames, was present predominantly on a subpopulation of the 195-kDa antigen and was demonstrable on the higher molecular weight component of a doublet recognized by the CEA antibodies. Expression of Le^x carbohydrate antigens in inflamed oesophageal squamous mucosa was shown to be significantly reduced relative to the expression seen in normal tissue. A suprabasal layer of CEA-positive, Le^x-negative cells became apparent in inflamed tissue showing altered glycosylation of the CEA under these conditions. It is postulated that CEA plays a role in maintaining the integrity of the squamous mucosa.

Keywords: carcinoembryonic antigen, CD15, Lewis^x, oesophagus, squamous mucosa

Introduction

The oesophageal squamous epithelium plays an important role in prevention of mechanical and chemical damage. The epithelial surface must resist the attack of acid and digestive enzymes from the stomach and duodenum. Oesophagitis and reflux are common conditions affecting 40–50% of the population [1], with increased occurrence associated with overindulgence at certain times of the year. More serious forms of the disease which occur can be treated with H2 blockers. Complications can extend to Barrett’s oesophagus and oesophageal carcinoma [2], which have increased in incidence over the past few years [3]. Relatively little attention has been paid to mechanisms which contribute to the barrier function of the mucosa and the response to regurgitating gastric and duodenal contents.

We have previously demonstrated that different morphological patterns of damage occur with gastric and duodenal juices [4, 5]. A common response, however, was noted in the decrease in number of functional cells and an increase in the basal and prickle cells. The increase in immature cells can be seen histologically as an increase in the basal/prickle cell layers and extension towards the oesophageal lumen of the capillaries [6]. At the same time there is a decrease in the amount of glycogen found in the mature cells.

In the stratified squamous epithelium of normal oesophagus or cervix, carbohydrate structures appear to be added to membrane glycoprotein chains in stepwise fashion as the basal cells mature. The
fucosyl residues which define the Lewis blood group antigens are some of the most peripheral of these structures [7]. In contrast to the cervix, there is little sialylation of the carbohydrate chains in the oesophagus [7]. During inflammation, the expression of the related carbohydrate structures, Lewis\textsuperscript{a} (Le\textsuperscript{a}, CD15) and Lewis\textsuperscript{b} (Le\textsuperscript{b}), found only on mature cells, decreases with the decrease in the proportion of mature cells [6].

CEA is a heavily glycosylated protein which has been used widely as a serum marker of neoplasia. In tumours, CEA is frequently associated with a number of carbohydrate 'tumour markers', including those related to the Lewis blood group antigens. Recent work has shown that CEA is one member of a large family of proteins found in normal as well as neoplastic tissues. We have shown that, in the cervix, Le\textsuperscript{a} and sialylated Le\textsuperscript{a} are coexpressed on CEA-related glycoprotein [8]. We have also recently demonstrated the presence of CEA-related glycoproteins in the oesophagus [9]. The oesophagus represents a more accessible tissue to study the differentiation of wet squamous epithelium. We have therefore investigated the expression of CEA-related molecules on normal and inflamed oesophagus. We have found a surprising complexity in the expression of CEA-related molecules. A subpopulation of the higher molecular weight CEA expressed all of the Le\textsuperscript{a}. The known role of CEA family members as adhesion molecules suggests that they may play a role in the formation of the oesophageal permeability barrier, supplementing the desmosomes which have been shown to hold the squames together.

Materials and methods

Antibodies used in this study were:

(a) Polyclonal rabbit anti-CEA (Dako, High Wycombe, UK) used at 1:500 dilution.

(b) Monoclonal anti-CEA 'A5B7' (Dako), which does not recognize NCAs when used at 1:300 dilution [10].

(c) Monoclonal anti-CEA '198' (a gift from Dr M. R. Price, CRC Laboratories, University of Nottingham, UK) tissue culture supernatant used neat [11].

(d) Monoclonal anti-CEA IM10 6.2 and IM10 13.1 (Scottish Antibody Production Unit, Carluke, UK) ascites used at 1:40 and 1:80 dilution respectively.

(e) Monoclonal anti-NCA 50/90 'DD9' (a gift from Dr A. Grant, Department of Clinical Biochemistry, St. Georges Hospital and Medical School, London, UK) used as neat tissue culture supernatant [12].

(f) CD66-classified rat monoclonal antibody YTH-71.3 (a gift from H. Waldmann, Department of Pathology, Cambridge University, UK) raised against leucocytes, used at 1:100 dilution [13].

(g) Anti-Lewis\textsuperscript{b}, CD15-classified mouse IgM monoclonal antibody MC2, raised in this laboratory, used at 1:250 [14].

For controls in immunohistochemistry or immunoblotting, normal rabbit serum, normal rabbit IgG, tissue culture supernatant or ascitic fluid containing an irrelevant monoclonal antibody were used at the same dilution as the specific primary antibody.

Immunohistochemistry

Staining of 5-μm sections of routinely paraffin-processed oesophageal biopsies was carried out using antibodies against CEA or related antigens. Binding of rabbit antibodies was detected using goat anti-rabbit second antibody with an avidin-biotin complex (ABC) as described by the manufacturers (Vectastain Kit, Vector Laboratories, Peterborough, UK). Binding of mouse or rat monoclonal antibodies was detected using rabbit anti-mouse second antibody with a streptavidin ABC (Dako) as described by the manufacturers.

Immunoblotting

Endoscopically and histologically normal or inflamed oesophageal mucosa was used, obtained from organ donors or pinch biopsies. The contamination rate of mesenchyme in these biopsies is about 5% [15]. The specimen was chopped finely, resuspended in phosphate-buffered saline (PBS) at a concentration of 100 mg wet tissue/ml and then homogenized thoroughly using a Polytron homogenizer at half-speed for 2 min. Triton X-100 was added to a final concentration of 1% (v/v) and the extract rotated for 1 h at 4°C. After centrifugation at 13,000 g for 3 min (MSE microCentaur) the supernatant was removed and used for further study.

Proteins from the oesophageal mucosal extracts were separated by SDS–polyacrylamide gel electrophoresis on 10% polyacrylamide slab gels by the method of Laemmli [16]. For immunoblotting, approximately 100 μg of protein was run per lane, except when subsequent immunoblotting was