

E-cadherin expression in intestinal epithelium

A Doğan, Z D Wang, J Spencer

Abstract

Aims—To investigate E-cadherin expression in normal and inflamed intestine, in the colonic adenocarcinoma cell line HT29, in normal fetal intestine, and in a fetal gut organ culture model where a T cell mediated enteropathy can be generated; to determine whether expression of E-cadherin changes in intestinal inflammation.

Methods—Immunohistochemistry was used to determine E-cadherin expression in following tissues: frozen and paraffin wax sections of normal and inflamed intestine; HT29 colonic adenocarcinoma cell line cultured on coverslips in the presence or absence of cytokines; frozen sections of fetal small intestinal tissue (gestational age 11–22 weeks); and frozen sections of cultured fetal gut in which a T cell mediated enteropathy had been induced.

Results—E-cadherin was strongly and evenly expressed by the epithelium in all specimens of intestine studied. Although there was no change in inflammation generally, in some cases of Crohn’s disease groups of glands with the characteristic morphology of “ulcer associated cell lineage” showed lower expression of E-cadherin. In fetal gut organ cultures epithelial expression of E-cadherin was lower when local T cells were activated with mitogens, compared with control explants. By contrast, the HT29 cell line showed low levels of expression which increased after treatment with conditioned medium from activated tonsil cells.

Conclusions—E-cadherin is strongly and evenly expressed by epithelium in normal and inflamed intestine, although an increase in E-cadherin expression in cytokine treated HT29 cells was observed. E-cadherin expression is reduced in the epithelium adjacent to ulcers (ulcer associated cell lineage), possibly to assist regeneration.

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E-cadherin is a calcium dependent cell adhesion receptor expressed by epithelial cells.¹ It is a member of the cadherin family of cell adhesion molecules which is characterised by the presence of an intracytoplasmic and a transmembrane region, and three homologous extracellular domains. The N-terminus is responsible for homotypic adhesion. The C-terminus is associated with the cytoskeleton via a group of molecules known as catenins. E-cadherin is a critical morphogenetic regulator during embryogenesis and recent evidence

strongly suggests that downregulation of E-cadherin expression in cancers is associated with a high rate of invasion and metastasis.¹ As yet, no role in inflammation has been reported.

In response to mucosal inflammation several functional and morphological changes occur in the intestinal epithelium. T cell activation in the mucosal microenvironment causes the crypt epithelial cell proliferation rate and villous atrophy to increase.² In Crohn’s disease disorganisation or complete loss of tight junctions in the involved ileal epithelium has been reported.³ This suggests that compensatory changes in the expression of E-cadherin may occur; either a decrease which would permit increased mobility of cells and the ability to cover the areas of epithelial loss or an increase which would increase epithelial stability.

In this study we used immunohistochemistry to examine E-cadherin expression in human intestinal epithelium in normal and inflamed intestine, in the cytokine treated colonic adenocarcinoma cell line HT29, and in a fetal gut organ culture model where a T cell mediated enteropathy can be generated.⁴

Methods

POSTNATAL NORMAL AND INFLAMED INTESTINE
All tissues used in this study were obtained from the paraffin wax and frozen tissue banks of Department of Histopathology, UCL Medical School and Department of Paediatric Gastroenterology, Medical College of St. Bartholomew’s Hospital, London. The following snap frozen specimens were studied: normal small intestine (n=3), normal colon (n=3), small intestine with Crohn’s disease (n=3), colon with Crohn’s disease (n=3), and ulcerative colitis (n=3). The following paraffin wax embedded specimens were studied: small intestine with Crohn’s disease (n=9) and colon with Crohn’s disease (n=4).

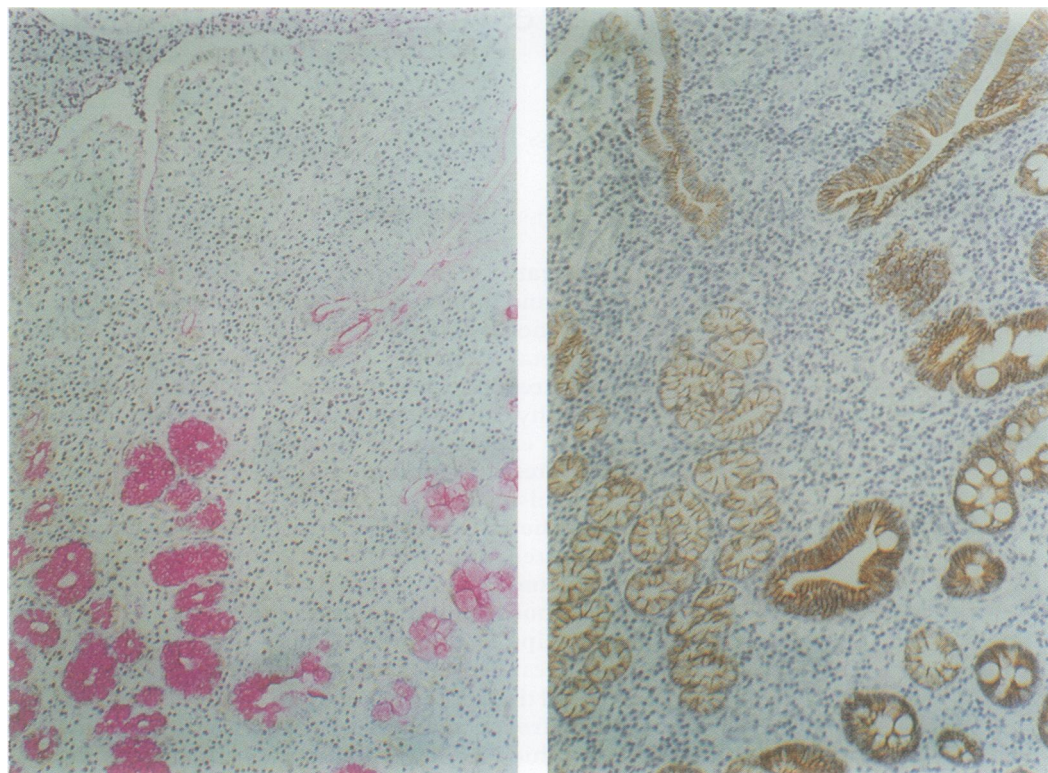
FETAL INTESTINE AND FETAL GUT ORGAN CULTURE

Fetal intestine was obtained from Medical Research Council fetal tissue bank. Ethical committee approval was received for all experiments using fetal tissue. Fetal small intestinal specimens (n=10; gestational age 11–22 weeks) were either snap frozen for immunohistochemistry or in three cases explants were cultured as described previously.⁵ In experimental cultures T cells were activated with pokeweed mitogen; control cultures contained no T cell stimulus. In this model in situ activation of T cells can cause a range of changes in the small intestinal explants, the severity of which is directly related to the number of T

Department of Histopathology, University College London Medical School, University Street, London WC1E 6JJ
A Doğan
Z D Wang
J Spencer

Correspondence to: Dr A Doğan.

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(A)

(B)

Figure 1 (A) Paraffin wax section of Crohn's disease with fissuring ulceration, stained with diastase/PAS, tubules of UACL are strongly positive. (B) Serial section of the same area stained with the monoclonal antibody HECD-1. Reactivity with the UACL tubules is significantly weaker than that of the crypts (immunoperoxidase technique; original magnification $\times 40$).

cells present in each explant.⁶ In 11–14 week old explants, which contain few T cells, adaptive changes such as crypt hyperplasia and villous atrophy are observed. In explants from 16 to 20 week old fetuses, which are rich in T cells, destructive changes with shedding of epithelium are observed. Control and activated fetal gut cultures were snap frozen at zero, four, eight, 16, 24, 36, 60, and 96 hours after initiation of culture. Frozen sections were cut and stained as described below.

HT29 CELL LINE

Cells from the HT29 colonic adenocarcinoma cell line were cultured on gelatin coated coverslips in RPMI 1640 tissue culture medium (Imperial Laboratories, Andover, Hampshire, UK), supplemented with 10% fetal bovine serum (Imperial Laboratories). In some experiments cultured cells were treated for 48 hours with conditioned medium from phytohaemagglutinin (PHA; Sigma, Poole, Dorset, UK) activated tonsil mononuclear cells as a cytokine source. Treated and control cultures were then washed, fixed, and stained as described below.

IMMUNOHISTOCHEMISTRY

Frozen and paraffin wax sections and coverslips were stained with an E-cadherin antibody (HECD-1; R&D Systems Europe, Abingdon, Oxon, UK) using an indirect immunoperoxidase technique. A microwave antigen

retrieval technique was used to enhance sensitivity in paraffin wax sections.

Results

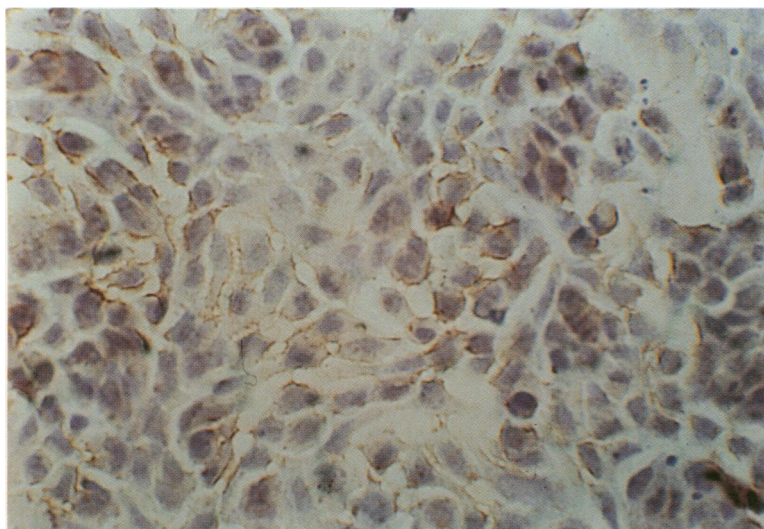
EXPRESSION OF E-CADHERIN IN POSTNATAL NORMAL AND INFLAMED INTESTINE

In frozen sections E-cadherin was strongly expressed in normal small intestine and colon. There was no obvious change in the level of epithelial E-cadherin expression in Crohn's disease or ulcerative colitis sections.

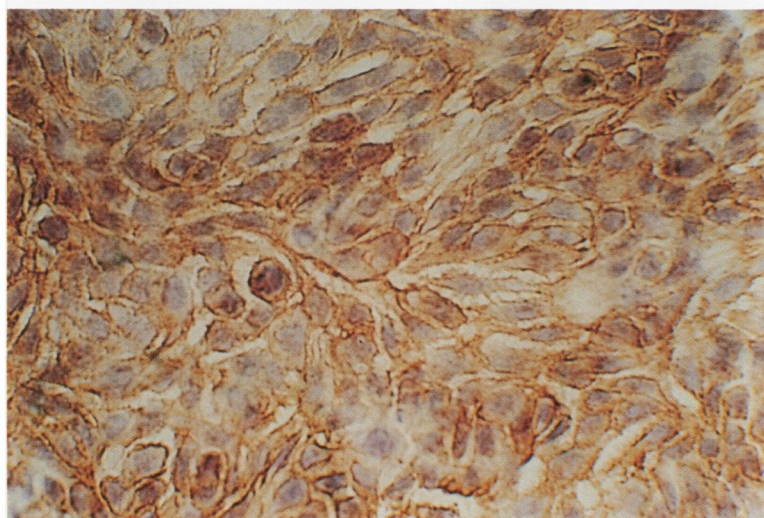
In paraffin wax sections E-cadherin was strongly expressed in morphologically uninvolved areas of small intestine and colon. The level of expression in the intestinal crypts or surface epithelium in areas showing the classic histological changes of Crohn's disease was similar to that in uninvolved mucosa. However, groups of glands adjacent to fissuring ulcers, which showed the characteristic morphology and diastase/periodic acid Schiff (PAS) reactivity of "ulcer associated cell lineage (UACL)",⁷ exhibited considerably lower levels of staining for E-cadherin (fig 1).

EXPRESSION OF E-CADHERIN IN THE FETAL INTESTINE AND CHANGES IN THE LEVEL OF EXPRESSION IN FETAL GUT ORGAN CULTURE AFTER ACTIVATION

E-cadherin was strongly and uniformly expressed by epithelial cells in fetal gut (gestational age 11–22 weeks). In fetal gut organ



(A)



(B)

Figure 2 E-cadherin expression in the HT29 cell line. (A) HT29 cell line grown on coverslips and (B) after treatment with conditioned medium from PHA activated tonsil mononuclear cells (immunoperoxidase technique; original magnification $\times 40$).

culture E-cadherin expression was slightly reduced in both control and activated explants when compared with uncultured (zero hour) fetal gut. There was no obvious change in the level of expression between control and activated cultures up to 60 hours of incubation. By 96 hours, however, where severe damage to the explant and loss of epithelium was apparent in activated explant cultures, the expression of E-cadherin was lower in the remaining epithelium compared with control explant epithelium.

INDUCTION OF E-CADHERIN EXPRESSION IN HT29 CELL LINE

When cells from the HT29 colonic adenocarcinoma cell line were grown on coverslips and treated with conditioned medium, E-cadherin expression was increased compared with control coverslips (fig 2). Although this increase was very marked in small groups of cells, the overall pattern was patchy, some areas having stained stronger than others.

Discussion

The intestinal epithelium forms a barrier between the antigenic load of the lumen and immunocompetent cells of the lamina propria. Disruption of epithelial integrity leads to an extensive inflammatory reaction in the lamina propria against faecal antigens. An increase in epithelial expression of the integrin VLA-1 (laminin/collagen receptor) in active inflammatory bowel disease is thought to contribute to the maintenance of the epithelial barrier during longstanding inflammatory response.⁸ In this study, however, we observed that E-cadherin expression in the intestinal epithelium was consistently high in normal fetal intestine and in postnatal intestine whether inflamed or not.

Significantly, E-cadherin expression in UACL was lower than other intestinal epithelial cells. Cells of this lineage are observed adjacent to ulcers in the gastrointestinal tract, and are believed to be important for the regeneration of the epithelium. UACL has a characteristic mucin profile and secretes epidermal growth factor, a mitogen for epithelial cells.⁷ As UACL buds from the bases of existing crypts and migrates to the mucosal surface through the lamina propria, lower levels of E-cadherin expression would be compatible with reduced cohesion and higher mobility.

In the fetal gut organ cultures both control and pokeweed mitogen treated explants showed similar levels of E-cadherin expression up to 60 hours of culture. By 96 hours, however, loss of epithelial integrity was apparent in some pokeweed mitogen stimulated explants. In the explants where epithelium was disrupted E-cadherin expression was low in the remaining epithelium, possibly reflecting a regenerative process similar to that found in UACL.

Unlike the intact intestinal epithelium, E-cadherin expression in the HT29 cell line was low. This could be upregulated by conditioned medium from PHA stimulated tonsil cells. Human recombinant interferon- γ did not affect E-cadherin expression in this cell line (data not shown). Enhanced E-cadherin expression after treatment with conditioned medium could be an intrinsic property of this cell line, similar to the induction of intercellular adhesion molecule-1 expression,⁹ which can be induced in HT29 cells by cytokines, but is seldom seen during inflammation in intestinal epithelium *in vivo*.^{5,10}

In summary, we have shown that E-cadherin is strongly and evenly expressed by epithelium in normal and inflamed intestine and suggest that this expression is reduced in the epithelium adjacent to ulcers to assist regeneration.

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