Increased expression of laminin/collagen receptor (VLA-1) on epithelium of inflamed human intestine

T T MacDonald, M A Horton, M Y Choy, P I Richman

Abstract

Immunohistochemical techniques were used to investigate the epithelial expression of VLA-1 in inflammatory bowel disease in six patients with Crohn's disease, in four patients with ulcerative colitis, and in one patient with indeterminate colitis, and compared with that in the small intestine and colons of 10 normal controls. In normal small bowel VLA-1 was expressed on crypt epithelial cells and only weakly or not at all on surface epithelium. VLA-1 was again expressed weakly in normal colon, except in one case, a 1 year old child with diarrhoea but no histological abnormalities. In small and large intestine affected with Crohn's disease, ulcerative colitis, or indeterminate colitis, there was increased expression of VLA-1 on the basolateral aspects of crypt cells and de novo expression on surface epithelium. It is suggested that this is an adaptive response to prevent epithelial cell loss as a result of inflammation in the underlying lamina propria.

Cell surface adhesion molecules of various classes have been described and shown to be involved in cellular adhesion to proteins in the interstitium and in intercellular interactions.¹⁻³ They almost certainly have an important role in the human intestine. They may be concerned with adherence of epithelial cells to the basement membrane.⁴ Migration of epithelial cells from the crypts of Lieberkuhn to the tips of the villi presumably may also involve such molecules in the formation and disruption of cell-cell and cell-stromal adhesive interactions.

We have previously shown that epithelial cells express VLA-1,2,3 and 6 molecules in normal human small intestine.⁴ The VLA cell surface heterodimers are members of the integrin superfamily and function as receptors for extra-cellular matrix proteins such as laminin, collagen, and fibronectin.^{1-3,5,6} The expression of VLA-1, a cell surface heterodimer originally identified on the surface of activated T cells,⁷⁻⁹ was particularly interesting. It was weak or absent at the bases of the crypts, was expressed on the middle to upper crypt, and was then absent or only weakly present on surface epithelium.⁴ This distribution is suggestive of a possible role for VLA proteins in normal bowel organogenesis.

In this paper we report on the expression of VLA-1 in the intestines of patients with

inflammatory bowel disease and show that there is a dramatic increase in VLA-1 expression in Crohn's disease, ulcerative colitis, and indeterminate colitis.

Methods

Colonic biopsy specimens were obtained from four children undergoing investigation for suspected inflammatory bowel disease but in whom no intestinal disease was found. Jejunal biopsy specimens were obtained from four patients (three adults and a child) suspected of having coeliac disease but who were healthy. Resected ileum from an adult with serosal adhesions was also studied.

One colonic biopsy specimen and four pieces of resected colon from children with active ulcerative colitis were studied. A single biopsy specimen from a child with indeterminate colitis and one with Crohn's disease were also obtained. Resected ileum was obtained from five children undergoing surgery for active Crohn's disease unresponsive to medical management.

IMMUNOHISTOCHEMISTRY

Tissues were snap frozen in liquid nitrogen and stored at -70°C . Cryostat sections ($8\text{ }\mu\text{m}$) of intestine were cut and stained immunohistochemically with monoclonal anti-VLA-1 antibody (T Cell Sciences, Cambridge, Massachusetts) using the indirect alkaline phosphatase anti-alkaline phosphatase (APAAP) technique.¹⁰ Fast red was used to localise antibody binding: sections were counterstained with Mayer's haematoxylin.

Staining of epithelial cells was graded semiquantitatively on a four point scale ($\pm$, $+$, $++$, or $+++$). The range of staining was from very weak cytoplasmic and basolateral membrane staining ($\pm$) to intense cytoplasmic and membrane staining ($+++$). For statistical analysis the grades were converted to numbers ($\pm = 0.5$, $+$ = 1, $++ = 2$, and $+++ = 3$), and the groups were compared using the Kolmogorov-Smirnov 2 group test.

Results

In normal small bowel VLA-1 was expressed on crypt epithelial cells, and was expressed only weakly or was absent from surface epithelium (table). In normal colon VLA-1 was again expressed weakly, except in one patient who had moderate staining on both crypt and surface epithelium. This was a 1 year old child with diarrhoea but in whom no histological

St Bartholomew's
Hospital, London
Department of
Paediatric
Gastroenterology
T T MacDonald
M Y Choy

ICRF Haemopoiesis
Research Group
M A Horton

Department of
Histopathology
P I Richman

Correspondence to:
Dr T T MacDonald,
Department of Paediatric
Gastroenterology,
St Bartholomew's Hospital,
London EC1A 7BE

Accepted for publication
12 October 1989

Expression of VLA-1 in normal human small intestinal epithelium (a) and in epithelium from a patient with active Crohn's disease (b). In the section of normal intestine there is brush border staining (BB), a VLA-1 positive intraepithelial lymphocyte is present (large arrow), subepithelial smooth muscle cells are also VLA-1 positive (small arrows), as are endothelial cells (e) in the lamina propria. The epithelial cells themselves are negative. In Crohn's disease epithelium there is dense VLA-1 staining on the basolateral aspects of the epithelial cells (arrows). The endothelial cells of the subepithelial capillary network are also strongly VLA-1 positive. (Immunalkaline phosphatase.)

abnormality was detected. In contrast, there was increased VLA-1 expression on both surface and crypt epithelium in intestine affected with inflammatory bowel disease (table, figure).

Discussion

The idea that surface expression of cell adhesion molecules can be upregulated in inflammatory sites is not new. It is well established that the expression of ICAM-1, the ligand for the leucocyte integrin LFA-1,¹¹ is

Expression of VLA-1 on normal and diseased human intestine

	Epithelium	
	Crypt	Surface
Normal small intestine:		
1	+	-
2	+	-
3	+	+
4	+	+
5	+	±
	p = 0.006*	p = 0.04†
Crohn's small intestine:		
1	+++	++
2	+++	+++
3	++	++
4	++	++
5	+++	+
Normal colon:		
1	++	++
2	±	±
3	±	±
4	±	-
	p = 0.023‡	p = 0.067§
Ulcerative colitis colon:		
1	+++	++
2	+++	++
3	+++	+++
4	++	++
5	+++	++
Crohn's disease:		
1	+++	+++
Indeterminate colitis:		
1	+++	Surface epithelium not identifiable in section of biopsy

Key
- No staining
± Very weak cytoplasmic staining
+ Weak cytoplasmic staining, densest basolaterally
++ Moderate cytoplasmic staining, densest basolaterally
+++ Strong cytoplasmic staining, densest basolaterally
*Comparison of crypts in normal small intestine with crypts in Crohn's small intestine
†Comparison of villus epithelium in normal small intestine with villus epithelium in Crohn's small intestine
‡Comparison of crypt epithelium in normal colon with crypt epithelium in ulcerative colitis, Crohn's disease, and indeterminate colitis combined.
§Comparison of surface epithelium in normal colon with surface epithelium in ulcerative colitis, Crohn's disease, and indeterminate colitis combined.

increased on accessory cells and endothelium in inflammatory sites.¹² This adaptive response is mediated by inflammatory cytokines.¹² Our results further emphasise the dynamic changes in molecules important in the maintenance of normal epithelial integrity which can occur following local inflammation in disease. The observation that VLA-1, an integrin receptor involved in cell adhesion to basement membranes, is upregulated in inflamed intestinal mucosa, is important for our understanding of the consequences of host responses at mucosal surfaces. Moreover, we would predict that increased VLA-1 expression on epithelium would not be confined to inflammatory bowel disease, but will be found in other conditions such as food sensitive enteropathy and parasitic infection where inflammatory cell responses into the mucosa are well recognised. This is well established in the case of HLA-DR expression in the bowel epithelium, which is increased non-specifically at any inflammatory site in the gut.¹³⁻¹⁵ The function, however, of HLA-DR on epithelium is poorly understood. In contrast, at least one of the functions of VLA-1 is known—that is, it is a cellular receptor for laminin and collagen. These two proteins are important constituents of basement membrane,¹⁶ and it is therefore not surprising that in normal bowel VLA-1 is

predominantly expressed on the basolateral aspect of the enterocyte where such an interaction would be expected to occur. In the specimens of inflamed intestine examined here, it was also on the basolateral aspects of the cells that VLA-1 expression was most noticeably increased (figure). Epithelial cells themselves can secrete extracellular matrix proteins,¹⁷ which may be the reason for the expression of VLA-1 on their lateral aspects. Increased expression of extracellular matrix adhesion molecules by epithelial cells during inflammation in the underlying lamina propria is probably an adaptive response to prevent extensive epithelial cell sloughing by inflammatory mediators. It will be important to analyse the regulation by inflammatory cytokines of integrin receptor expression and extracellular matrix secretion in intestinal epithelium.

Loss of epithelial integrity results in leakage of plasma proteins and fluid directly into the lumen, with severe deleterious effects on the host. It would make teleologic sense to have a mechanism in the bowel to prevent denudation of the mucosa during protective intestinal immune responses, such as parasite expulsion.

T T MacDonald is a Wellcome senior lecturer. M A Horton is supported by the Imperial Cancer Research Fund. M-Y Choy is in receipt of an award from the National Foundation for Ileitis and Colitis, New York. This work was also supported by Crohn's in Childhood Research Appeal.

- 1 Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. *Science* 1987;238:491-7.
- 2 Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987;48:549-54.
- 3 Hemler ME. Adhesive protein receptors on haematopoietic

- cells. *Immunology Today* 1988;9:109-13.
- 4 Choy M-Y, Richman PI, Horton MA, MacDonald TT. Expression of the VLA family of integrins in human intestine. *J Pathol* (in press).
- 5 Hemler ME, Huang C, Schwarz L. The VLA protein family. Characterisation of five distinct cell surface heterodimers each with a common 130,000 molecular weight β subunit. *J Biol Chem* 1987;262:3300-9.
- 6 Kishimoto TK, O'Connor K, Lee A, Roberts TM, Springer TA. Cloning of the β subunit of the leucocyte adhesion proteins: homology to an extracellular matrix receptor defines a novel supergene family. *Cell* 1987;48:681-90.
- 7 Hemler ME, Ware CF, Strominger JL. Characterization of a novel differentiation antigen complex recognised by a monoclonal antibody (A-1A5): unique activation specific molecule on stimulated T cells. *J Immunol* 1983;131:334-40.
- 8 Hemler ME, Jacobson JG. Cell matrix adhesion related proteins VLA-1 and VLA-2: regulation of expression on T cells. *J Immunol* 1987;138:2941-8.
- 9 Hemler ME, Jacobson JG, Strominger JL. Biochemical characterisation of VLA1 and VLA2 cell surface heterodimers on activated T cells. *J Biol Chem* 1985;260:15246-52.
- 10 Isaacs M, Wright DH. Immunocytochemistry of lymphoreticular tumours. In: Polak J, van Noorden S, eds. *Immunocytochemistry. Practical applications in pathology and biology*. Bristol: John Wright and Sons, 1986:249.
- 11 Marlin SD, Springer TA. Purified intracellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function associated antigen (LFA-1). *Cell* 1987;51:813-9.
- 12 Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA. Induction by IL-1, tissue distribution, biochemistry and function of a natural adherence molecule (ICAM-1). *J Immunol* 1986;137:245-54.
- 13 Selby WS, Janossy G, Mason DY, Jewell DP. Expression of HLA-DR antigens by colonic epithelium in inflammatory bowel disease. *Clin Exp Immunol* 1983;53:614-8.
- 14 Arato A, Savilahti E, Taenio V-M, Verkasalo M, Klemola T. HLA-DR expression, natural killer cells and IgE containing cells in the jejunal mucosa of coeliac children. *Gut* 1987;28:988-94.
- 15 Spencer JM, Pugh S, Isaacson PG. HLA-D region antigen expression on stomach epithelium in absence of auto-antibodies. *Lancet* 1986;ii:983.
- 16 Madri JA, Pratt BM, Yurcheno PD, Furthmayr H. The ultrastructural organisation and architecture of basement membranes. In: *Basement membranes and cell movement*. London: Pitman, 1984:6-24.
- 17 Bissel MJ, Hall HG, Parry G. How does the extracellular matrix direct gene expression. *J Theoret Biol* 1982;99:31-68.