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Expression of HLA-DR antigens by colonic epithelium in inflammatory bowel disease

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SUMMARY

The expression of HLA-DR and HLA-A,B,C antigens by human colonic epithelium has been examined in tissue sections of patients with inflammatory bowel disease using an immunohistological technique. Colonic epithelial cells from all 21 control subjects with histologically normal colonic mucosa were HLA-DR$^-$. In contrast, in nine of 13 patients with active ulcerative colitis and 11 of 12 with active Crohn's disease the epithelium of involved colonic mucosa was HLA-DR$^+$. HLA-DR antigens were found on the epithelium of only one of six patients with ulcerative colitis in remission and one of three with inactive Crohn's disease. Moreover, these antigens were not present on the epithelium of non-inflamed colonic mucosa in two patients with Crohn's disease in whom adjacent involved mucosa showed strong epithelial reactivity. This difference between patients with active and those with inactive disease is highly significant ($P < 0{\cdot}005$). These findings provide further evidence of the importance of cell-mediated immune mechanisms in the pathogenesis of inflammatory bowel disease.

Keywords ulcerative colitis Crohn's disease HLA-DR Ia antigens epithelium

INTRODUCTION

Cell-mediated immune mechanisms appear to be important in the pathogenesis of inflammatory bowel disease (ulcerative colitis and Crohn's disease) (Kraft, 1979). Recent studies have demonstrated that, following cell-mediated immune reactions such as graft versus host disease and contact hypersensitivity, keratinocytes in the inflamed epidermis show expression of Ia or HLA-DR antigens (Lampert, Suitters & Chisholm, 1981; Lampert *et al.*, 1982; Mason, Dallman & Barclay, 1981; Suitters & Lampert, 1982; Poulter *et al.*, 1983). Although normal colonic epithelial cells, in contrast to those in the small intestine, do not express these antigens (Scott *et al.*, 1980; Selby *et al.*, 1981) it was noticed while examining mucosal cell populations in patients with these disorders that in several with active disease the colonic epithelium showed strong expression of HLA-DR antigens. The present study examines more closely the distribution of histocompatibility antigens in the colonic mucosa of patients with inflammatory bowel disease, with particular regard to disease activity.

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MATERIALS AND METHODS

Patients. Four groups of patients were studied. (1) Twenty-one patients with non-inflammatory gastrointestinal disease, in whom macroscopically and histologically normal colonic mucosa was examined (10 with colonic carcinoma, three with previous colonic polyps or carcinoma, five with irritable colon syndrome, two with haemorrhoids and one with lymphoid hyperplasia). (2) Nineteen patients with ulcerative colitis, clinically and histologically active in 13 and inactive in six. Seven patients were receiving topical or systemic corticosteroids, 13 sulphasalazine and two (both with active disease) were on no therapy. (3) Fifteen patients with Crohn's disease, active in 12 and inactive in three. In two patients, involved and non-involved mucosa were studied separately. Five of the patients were receiving corticosteroids, five sulphasalazine and five no treatment. (4) One patient with acute colitis due to *Salmonella enteritidis*, and one with radiation colitis following radiotherapy for a uterine cervical neoplasm.

Tissues. Rectal or colonic biopsies were obtained endoscopically or at the time of colectomy. The specimens were orientated on cork, mounted in OCT compound (Ames Co) and frozen in isopentane suspended over liquid nitrogen.

Five micron frozen sections were cut on a cryostat, air dried, fixed in absolute alcohol at 4°C for 10 min and then washed in phosphate-buffered saline (PBS). Indirect immunofluorescence was performed as described previously (Selby *et al.*, 1981). The sections were incubated with first layer antibodies for 30 min, washed in PBS for the same period and then the procedure repeated with second layer antisera. After washing, slides were mounted in 50% glycerol in PBS.

Antibodies. HLA-DR antigens were detected using a specific antiserum raised in chickens against p28,33 antigens (C anti-Ia). Details of this antiserum have been published previously (Janossy *et al.*, 1979; Selby *et al.*, 1981). In addition, a monoclonal antibody, C3R/43, which reacts with the β-chain of human HLA-DR antigens, was also used (Gatter *et al.*, 1982). HLA-A,B,C antigens were detected with the monoclonal antibody W6/32 (Seralab) which reacts with a common component of these antigens (Barnstable *et al.*, 1978). Culture supernatants of both monoclonal antibodies were applied undiluted. W6/32 was used in combination with C anti-Ia. Controls were performed using normal chicken serum and NA1/34, a monoclonal antibody to human thymocyte antigen which is unreactive with human intestinal tissue (Selby *et al.*, 1981).

Monoclonal antibodies were used with fluorescein conjugated goat anti-mouse Ig as second layer reagent (GAM FITC; Nordic), and C anti-Ia with rhodamine labelled sheep anti-chicken Ig (SAC TRITC; Royal Free). Sections were examined under a Zeiss fluorescence microscope with selective filters for FITC and TRITC. Assessment of the expression of histocompatibility antigens was determined without knowledge of the clinical history of histopathological findings.

RESULTS

Identical results were obtained using either chicken antiserum or monoclonal antibody to HLA-DR antigens. The epithelium of histologically normal colon showed no detectable expression of HLA-DR antigens (Fig. 1a). However, within the lamina propria of the same sections numerous histiocytic cells were seen, which showed strong expression of these antigens. HLA-A,B,C antigens were expressed on epithelial cells, intraepithelial lymphocytes and on cells in the lamina propria. These findings are in agreement with those reported previously (Selby *et al.*, 1981).

In nine of 13 patients with active ulcerative colitis, and in 11 of 12 with active Crohn's disease, the epithelium of involved colonic mucosa demonstrated expression of HLA-DR antigens (Table 1 & Fig. 1b). This expression was strong and, in general, was more marked on epithelial cells lining the colonic glands than on those of the surface epithelium. The expression appeared to be membrane associated rather than cytoplasmic, and there were no HLA-DR$^+$ granules in the apices of the cells. Although the entire cell surface was HLA-DR$^+$, in several cases the apical half of the cell membrane appeared to react more strongly than the basal half. There were no distinguishing clinical features in these patients with active disease between those in whom the epithelial cells were HLA-DR$^+$ and

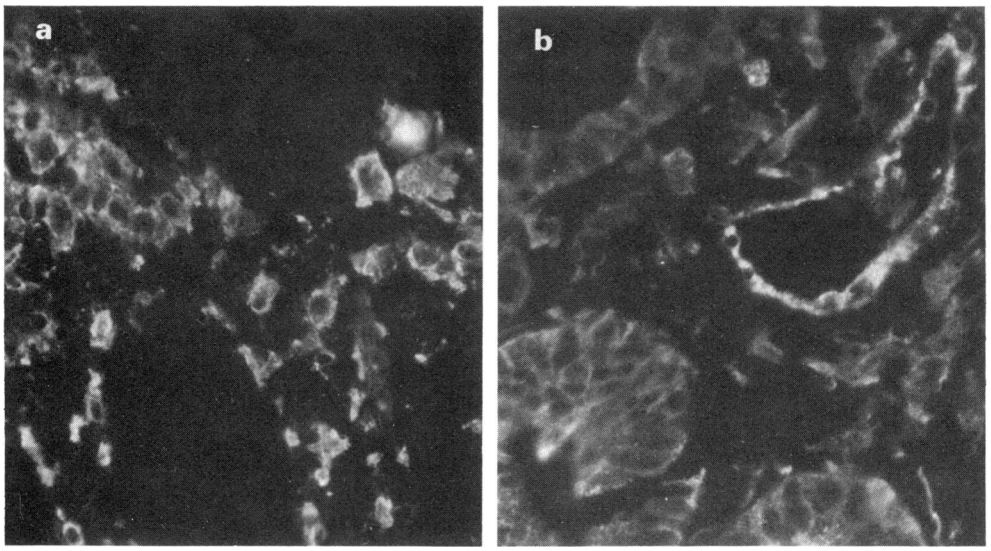

Fig. 1. (a) Section of normal colonic mucosa: although numerous HLA-DR$^+$ histiocytic cells can be seen in the lamina propria, the epithelial cells are HLA-DR$^-$. (b) Colonic biopsy from a patient with active Crohn's colitis. The epithelial cells show strong expression of HLA-DR antigens.

Table 1. Expression of HLA-DR antigens by colonic epithelium in patients with ulcerative colitis or Crohn's disease

	Active	Inactive or non-involved	Total
HLA-DR$^+$	20	2	22
HLA-DR$^-$	5	9	14
Total	25	11	36

$\chi^2 = 9{\cdot}82$; $P < 0{\cdot}005$.

those in whom they were HLA-DR$^-$. Moreover, there was no correlation between epithelial expression of HLA-DR antigens and form of treatment being received, if any.

In contrast to the patients with active disease, HLA-DR antigens were found on the epithelium of only one of the six patients with inactive ulcerative colitis, and one of the three with inactive Crohn's disease. In addition, these antigens were not present on the epthelium of non-inflamed colonic mucosa in the two patients with Crohn's disease in whom adjacent involved mucosa showed strong epithelial reactivity. The difference in the occurrence of HLA-DR antigens on epithelial cells between patients with active and those with inactive disease, and between involved and non-involved, mucosa was highly significant ($P < 0{\cdot}005$). In the patients with salmonella colitis (1) or radiation colitis (1) the epithelium was HLA-DR$^-$.

In patients with inflammatory bowel disease, either active or inactive, the pattern of expression of HLA-A,B,C antigens was identical to that seen in normal colonic mucosa.

Studies using control antibodies resulted in no staining of epithelium or lamina propria cells.

DISCUSSION

In the epidermis of normal skin, HLA-DR antigens are found only on Langerhans cells (Klareskog *et al.*, 1977). However, following experimental graft versus host disease in the rat, strong Ia antigen

expression develops on keratinocytes (Mason *et al.*, 1981; Lampert *et al.*, 1981; Barclay & Mason, 1982). Expression of HLA-DR antigens on epidermal cells is seen also in human graft versus host disease following bone marrow transplantation (Lampert *et al.*, 1982). Suitters & Lampert (1982) showed that Ia antigen expression can be induced in rat epidermis by contact hypersensitivity reactions, but not by either mechanical or chemical trauma. The epithelium of the colon and small intestine also expresses Ia antigens in graft versus host disease in rats (Mason *et al.*, 1981; Barclay & Mason, 1982). In addition, the immune response following experimental infection with Trichinella spiralis results in expression of Ia antigens by rat intestinal epithelial cells (Barclay & Mason, 1982). By analogy, this suggests that the expression of HLA-DR antigens by inflamed colonic epithelium in patients with ulcerative colitis or Crohn's disease is the result of a cell-mediated immune reaction within the mucosa, perhaps directed against the epithelium. Epithelial cell damage *per se* is unlikely to result in expression of these antigens, since such damage can be seen in otherwise non-involved mucosa in Crohn's disease, and affects surface, but not glandular, epithelium (Dourmashkin, Davies & Wells, 1981). In the two patients studied, epithelial damage due to irradiation or to bacterial invasion also failed to result in epithelial expression of HLA-DR antigens.

The expression of HLA-DR antigens by human colonic epithelium is not restricted to patients with inflammatory bowel disease, as it has also been described in variable proportions of carcinoma cells in about 50% of patients with colonic adenocarcinoma. It is also seen on crypt cells adjacent to the tumour, but not on those at a distance from it (Darr *et al.*, 1982).

Whether the HLA-DR antigens are synthesized by the epithelial cells themselves or whether they are passively adsorbed onto the cell surface is unknown. The distribution of these antigens appears to be predominantly membrane associated. In inflammatory bowel disease there is an increase in the number of macrophage like cells in the affected mucosa, and these cells have strong expression of HLA-DR antigens (Selby *et al.*, 1983). Thus, these cells may be the source of the epithelial HLA-DR antigens. However, in graft versus host disease, the epithelial Ia antigens are of host origin, and are not derived from the Ia^+, bone marrow derived cells in the lamina propria, strongly suggesting that the Ia antigens are synthesized by the epithelial cells themselves (Barclay & Mason, 1982). The stimulus for the HLA-DR antigen expression by colonic epithelial cells is also unknown. It may be a lymphokine produced by locally stimulated lymphocytes (Lampert *et al.*, 1981). In particular, a lymphokine with properties of interferon-γ has been shown to be capable of stimulating Ia antigen expression by macrophages (Steeg *et al.*, 1982). The population of T cells within the gut epithelium, the intraepithelial lymphocytes (IEL), may be important in the production of such a lymphokine.

Acinar cells in the lactating breast of the guinea-pig synthesize and express Ia antigens, and this is under hormonal control (Klareskog, Forsum & Peterson, 1981). It was proposed that this expression may be a homing stimulus for the IgA plasma cells which migrate to the breast during lactation. However, a similar role for colonic epithelial HLA-DR antigens in inflammatory bowel disease seems unlikely. The normal colon contains large numbers of IgA plasma cells in the lamina propria (Brandtzaeg & Baklien, 1976) yet the epithelium is $HLA\text{-}DR^-$. Moreover, neither plasma cells nor B lymphocytes are found in the colonic epithelium of normal subjects or of patients with ulcerative colitis or Crohn's disease. If HLA-DR antigens do have homing properties, then they are much more likely to be associated with the strongly $HLA\text{-}DR^+$ cells present in large numbers in the lamina propria (Selby *et al.*, 1981; 1983).

The influx of lymphocytes into the skin before the appearance of Ia antigens on keratinocytes in contact hypersensitivity reactions in the rat (Suitters & Lampert, 1982) as well as the infiltration of the mucosa with lymphocytes and plasma cells seen in the present study in some patients without detectable epithelial HLA-DR reactivity, and vice versa, also argues against a priming role for epithelial cell HLA-DR antigens in this cellular infiltration.

The role of HLA-DR antigens on colonic epithelium in inflammatory bowel disease is unknown. This expression is not associated with an alteration in the proportions of helper/type ($OKT4^+$) or suppressor/cytotoxic type ($OKT8^+$) T cells either in the epithelium or in the lamina propria. HLA-DR antigens on macrophages and B cells are involved in the presentation of antigens to T cells (McDevitt, 1981). A similar role has been shown for HLA-DR antigens on non-immune cells such as vascular endothelial cells (Hirschberg, Bergh & Thorsby, 1980). If such a function exists for

these antigens on the colonic epithelium of patients with inflammatory bowel disease, then this may lead to enhancement of the immune response to any invading antigen. Whatever the exact role these antigens play on colonic epithelial cells, the results of this study provide further evidence of the importance of cell-mediated immune mechanisms in the pathogenesis of inflammatory bowel disease.

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