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HLA-D region antigens on isolated human colonic epithelial cells: enhanced expression in inflammatory bowel disease and *in vitro* induction by different stimuli

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SUMMARY

Colonic epithelial cells (CEC) were isolated from actively inflamed mucosa of IBD patients and checked for HLA-DR, HLA-DP, and HLA-DQ. Half of the freshly isolated CEC from IBD tissue expressed DR, and one third were positive for DP and DQ. Normal human CEC were then cultured for 24 h and their expression of these markers in response to different types of *in vitro* stimulation was investigated. A significant increase in the expression of DR, DP and DQ was observed in response to the nonspecific mitogen phytohaemagglutinin (PHA), the lymphokine gamma-interferon (γ-IFN) and the epidermal growth factor (EGF). The enhancement of DR expression was more marked than that of DP and DQ. The effect of γ-IFN was more rapid and significantly more marked than that of either PHA and EGF for all three antigens. EGF appeared to be more potent than PHA in enhancing the expression of DP and DQ. The data from this study indicate that HLA-D region antigens can be induced on human CEC by different types of stimuli and provide further evidence that the expression of these markers in the colonic epithelium is a normal event the magnitude of which can increase under various circumstances. The data also suggest that the increased expression of HLA-D region antigens by IBD CEC occurs as a result of different mechanisms, and that this expression is an indicator of the active participation of the colonic epithelium to the mucosal inflammatory response.

Keywords HLA-D region antigens epidermal growth factor colonic epithelium inflammatory bowel disease gamma interferon

INTRODUCTION

In the gastrointestinal tract of humans HLA-D region antigens are expressed in normal conditions on mature enterocytes in the small intestine and in other areas on the gut epithelium directly adjacent to lymphoid tissue (Scott *et al.*, 1980; Natali *et al.*, 1981; Hirata *et al.*, 1986; Spencer, Finn & Isaacson 1986). The expression of HLA-D region antigens on intestinal epithelium is increased in different inflammatory conditions (Selby *et al.*, 1983; McDonald, Gatter & Jewell 1985; Hirata *et al.*, 1986; Arnaud-Battandier *et al* 1986) and in neoplasia (Daar & Fabre 1983; Ghosh *et al.*, 1986). We have recently reported that in inflammatory bowel disease (IBD) there is an increased expression of HLA-DR as well as of the TFR and the 4F2 antigen on the colonic epithelium of actively inflamed, mucosa, which appeared to be related to an increased proportion of activated lamina propria lymphocytes (LPL) (Fais *et al.*, 1987). These observations suggested that in IBD the epithelial participation in the inflammatory process is associated with a sequence of cell membrane rearrangements and that the expression of HLA-D region molecules is a part of this sequence. In the present study we have further examined this problem investigating *in vitro* the ability of human colonic epithelial cells to express HLA-D region molecules in response to different types of stimulation. The kinetics of the expression of the HLA-D region products DR, DP and DQ was studied on cultured isolated human colonic epithelial cells (CEC) after exposure to different types of stimulus. These included the nonspecific mitogen phytohaemagglutinin (PHA), reported to induce HLA-DR on other epithelial cell types (Pujol-Borrel *et al.*, 1983), the lymphokine gamma-Interferon (γ-IFN), known to be both a modulator and a potent inducer of the MHC-Class II antigens (Rosa & Fellous 1984; Capobianchi *et al.*, 1985) and the Epidermal Growth Factor (EGF) which is essential for the regulation of growth and maturation of the intestinal epithelium (Chopra, Siddiqui & Cooney 1987).

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MATERIALS AND METHODS

Isolated Colonic Epithelial Cells (CEC)

The sources of the CEC used in the study were mucosal samples

Table 1. Expression of HLA-D region antigens and other markers on isolated human colonic epithelial cells (CEC) (mean percentage ± s.d. of cells bearing each marker)

	Freshly isolated CEC		Control human CEC cultured for 24 h		
	Control	IBD	PHA	EGF	γ-IFN
HLA-DR	<2	51±11	44±19	49±7	82±7*
HLA-DP	0	32±11	28±6	38±4†	64±16*
HLA-DQ	<2	36±17	24±12	40±6†	65±14*

*$P < 0.01$ γ-IFN vs PHA and EGF; †$P < 0.05$ EGF vs PHA.

obtained from the involved areas of surgical specimens of eight patients with active inflammatory bowel disease (Crohn's disease of the colon (3) and ulcerative colitis (5)) and from the uninvolved mucosa of 15 controls (colonic cancer (13), diverticular disease (1) and severe chronic constipation (1)). Colonic mucosa was dissected from the surgical specimens within one hour of resection. Strips of the mucosa (2–3 g total weight) were washed in Hank's Balanced Salt Solution free of calcium and magnesium (HBSS-CMF) (Flow Lab, UK) for 10 min. The mucosal strips were then washed in HBSS-CMF containing 1 mM of Dithiothreitol (DTT) (Sigma Chem, USA) and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml and gentamycin 50 μg/ml, fungizone 25 μg/ml) for 15 min at room temperature. The strips of mucosa were then washed in HBSS-CMF three times for 5 min and chopped into approximately 3 × 3 mm pieces. These tiny pieces were incubated 1–2 times in HBSS-CMF containing 0·75 mM ethelenediaminotetraacetic acid (EDTA), 10 mM Hepes buffer and antibiotics for 30 min at 37°C. The supernatants were collected and spun in HBSS-CMF at 300 *g* for 3 min. The pellet was washed again in HBSS-CMF and carefully resuspended in a medium containing 65% Trowell's T8 (Flow Lab, UK), 25% RPMI 1640 + 10 mM Hepes buffer (Flow Lab, UK), 10% Fetal Calf Serum (FCS) (Flow Lab, UK), 22 mM L-Glutamine and antibiotics. The resulting epithelial cells were counted, checked for viability by trypan blue exclusion and resuspended in the medium at the concentration of 1×10^6 CEC/ml. Cell viability was >85% in all experiments. Lymphocyte contamination was assessed by immunofluorescence using OKT3 and OKT8 MoAbs. CD3 and/or CD8 positive cells were consistently <8%. In three experiments the pellet was subjected to density gradient centrifugation (Ficoll–Paque, Pharmacia Swe). The contamination of the resulting purified CEC by OKT3 positive cells was <2%. CEC viability ranged from 20 to 32% before culture, from 15 to 20% after 6 h and was less than 15% after 24 h. For this reason CEC cultures were run for 6 h only.

Expression of HLA-D region molecules on freshly isolated CEC

Freshly isolated CEC obtained as described above from each IBD and control specimen were checked by indirect immunofluorescence for the expression of the three HLA-D region antigens (DR, DP and DQ). The proportion of CEC bearing these markers was assessed using a panel of monoclonal antibodies including anti-HLA-DR (clone L243, Becton-Dickinson, USA), anti-HLA-DP (clone B7/21, Becton-Dickinson, USA), anti-HLA-DQ (clone SK10, Becton-Dickinson, USA). The OKT6, OKT11 (Ortho-mune) were used as non-relevant first layer antibodies.

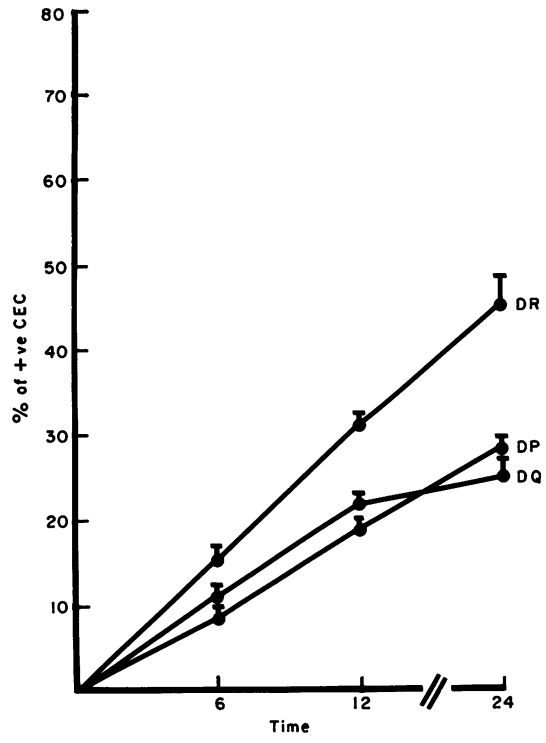

Fig. 1. Kinetics of HLA-D region antigen expression by cultured normal colonic epithelial cells (CEC) after *in vitro* exposure to PHA. Points in the curve are the mean percentages (±s.d. vertical bars) of CEC bearing each of the HLA-D region antigens. Counts at 12 h are significantly higher ($P < 0.01$) than those at 6 h for each antigen. Counts at 24 h are higher ($P < 0.05$) than those at 12 h for DR and DP.

In vitro *induction of HLA-D region molecules on isolated normal human CEC*

Portions of freshly isolated CEC from each of the control specimens were resuspended in the medium described above at a concentration of 1×10^6 and placed in flat-bottomed, 24-well culture plates (Falcon Plastic, UK). CEC were cultured in CO_2 humid atmosphere at 37°C with and without the addition, on hour 0, of each of the following: 1 μg/ml phytohaemagglutinin (PHA) (PHA HA15, Wellcome, UK), 5 ng/ml epidermal growth factor (EGF) (Collaborative Research, Mass. USA) and 50 Units *Escherichia coli*-derived human recombinant gamma-interferon (γ-IFN) (Genentech, Inc., USA). After 6, 12 and 24 h the cells were harvested, washed and checked by immunofluorescence with the panel of monoclonal antibodies indicated above. Cell viability was 85±5% at 6 h, 80±8% at 12 h and 70±10% at 24 h. Each experiment was performed in duplicate.

Blocking experiments with an anti-γ-IFN antibody

In four experiments 50 Units of γ-IFN were incubated at 37°C for 30 min with 200 neutralizing units (4-fold excess) of a monoclonal anti γ-IFN antibody (supplied by Roche Pharmaceuticals, Switzerland) (titre 10,000 neutralizing units/ml). After incubation the mixture was added to isolated CEC. CEC cultures were run as indicated above and at 6, 12 and 24 h the

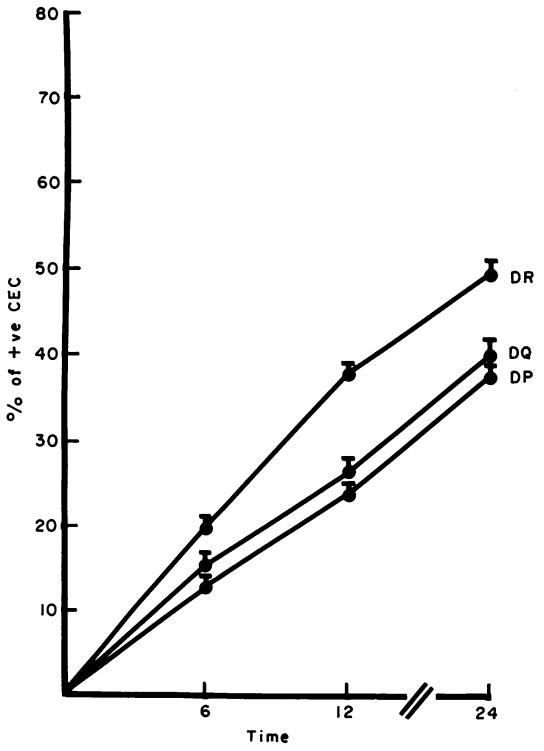

Fig. 2. Kinetics of HLA-D region antigen expression on cultured normal colonic epithelial cells (CEC) after *in vitro* exposure to EGF. Points in the curve are the mean percentages (±s.d. vertical bars) of CEC bearing each of the HLA-D region antigens. Counts at 12 h are significantly higher ($P<0{\cdot}01$) than those at 6 h and significantly lower ($P<0{\cdot}01$) than those at 24 h.

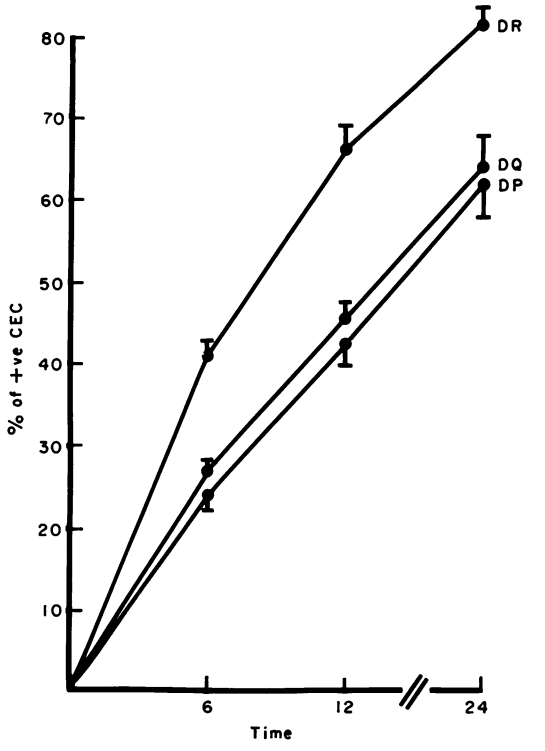

Fig. 3. Kinetics of HLA-D region antigen expression by cultured normal colonic epithelial cells (CEC) after *in vitro* exposure to γ-IFN. Points in the curve are the mean percentages (±s.d. vertical bars) of CEC bearing each of the HLA-D region antigens. Counts at 12 h are significantly higher ($P<0{\cdot}001$) than those at 6 h and significantly lower ($P<0{\cdot}01$) than those at 24 h.

CEC were checked by immunofluorescence. Portions of CEC from the same tissue samples were also cultured in the presence of PHA (1 μg/ml) with and without the addition of 200 neutralizing units of the anti-γ-IFN monoclonal antibody.

Statistical analysis

Student's *t*-test for unpaired data and the Wilcoxon rank-sum test were used where appropriate for the statistical analysis of the data.

RESULTS

We first assessed the proportion of freshly isolated CEC bearing HLA-DR, HLA-DP and HLA-DQ antigens. As shown in Table 1, 51±11% of the isolated CEC from the actively inflamed mucosa of patients with IBD showed the DR antigen on their surface, while 32±11% expressed the DP and 36±17% the DQ. The proportion of cells bearing each antigen was consistently <3% when CEC suspensions from uninvolved mucosa of IBD patients and macroscopically normal mucosa from control subjects were examined. A granular staining was observed with the anti-DR, anti-DP and anti-DQ mainly at the basolateral region of the cell membrane but the apical surface also appeared to react. Staining of the supranuclear region of the cytoplasm was also observed in some experiments.

Normal human isolated CEC were cultured for 24 hours in the presence of the non-specific mitogen PHA, EGF and γ-IFN. The addition of PHA to CEC cultures evoked a significant increase of the HLA-D region antigen-bearing cells (DR, DP and DQ) after 6 h of culture (16±4%, 9±5% and 10±4% respectively), significantly increasing ($P<0{\cdot}01$) after 12 h (30±4%, 19±6% and 20±4%) (Fig. 1). After 24 h there was a significant further increase of DR and DP (44±19%, 28±6% respectively, $P<0{\cdot}05$) while the counts of DQ-bearing cells did not differ from those at 12 h (Fig. 1 and Table 1).

The exposure of CEC to EGF appeared to markedly enhance the HLA-D region antigens (Fig. 2). The kinetics of the EGF-induced DR, DP and DQ expression showed a progressive increase in the proportion of CEC bearing each of the three markers throughout the culture period. The expression after 6 h of EGF was 20±2%, 13±3% and 16±4% respectively and it was 39±2%, 24±6% and 26±5% after 12 h, further increasing ($P<0{\cdot}01$) after 24 h (49±7%, 39±4% and 39±6%) (Fig. 1). The counts of DP- and DQ-bearing cells after 24 h were significantly higher with EGF than with PHA (38±4 and 40±6) ($P<0{\cdot}05$ and $P<0{\cdot}01$, respectively) (Table 1). The expression of DR, DP and DQ promptly increased 6 h after CEC exposure to γ-IFN (Fig. 3) (40±11%, 24±8% and 25±5%), further increasing after 12 h ($P<0{\cdot}01$) (66±19%, 42±9% and 45±10%) and reaching a peak after 24 h (82±7%, 64±16% and 65±14%). The DR, DP and DQ bearing CEC counts after γ-IFN at 6, 12 and 24 h were significantly higher ($P<0{\cdot}01$) than those after PHA and EGF at each time interval (Fig. 3 and Table 1).

In three experiments CEC suspensions were depleted of contaminating lymphocytes. After 6 h culture of PHA DR, DP and DQ expression was $10 \pm 1\%$, $5 \pm 1\%$ and $7 \pm 1\%$ respectively. In the EGF stimulated cultures DR expression was $10 \pm 2\%$, DP was $8 \pm 1\%$ and DQ $9 \pm 1\%$. After stimulation with γ-IFN DR+CEC were $29 \pm 3\%$, DP+$18 \pm 2\%$ and DQ+$20 \pm 2\%$. The expression of HLA-D region antigens on cultured CEC in response to γ-IFN was markedly inhibited ($> 90\%$ inhibition) ($P < 0{\cdot}001$) by the addition to cultures of an anti-γ-IFN monoclonal antibody. The inhibition of the PHA-induced HLA-D antigens was less pronounced (50% inhibition).

DISCUSSION

Several observations arise from the results of this study. A pattern in the expression of MHC Class II antigens appeared to occur on isolated CEC from actively inflamed IBD tissue, characterized by an increased expression of HLA-DR, DP and DQ. It also appeared that after the *in vitro* exposure of fresh normal human CEC to either PHA or EGF, a pattern in the expression of HLA-D region antigens evolved which was qualitatively and quantitatively similar to that observed with freshly isolated CEC from actively inflamed mucosa of IBD patients. These observations suggest that different mechanisms may be operating *in vivo* in enhancing HLA-D region antigen on the colon epithelium in IBD. These may include the production of γ-IFN by either activated lamina propria lymphocytes (Pallone *et al.*, 1987) or intraepithelial lymphocytes (Cerf-Bensussan *et al.*, 1984) as well as increased epithelial proliferation (Shorter, Spencer & Hallenbek 1966). It is also likely that luminal factors play a role. Studies in experimental animals have shown that Ia is inducible on the gut epithelium of pathogen-free rats by infection with *Trichinella spiralis* (Barclay & Mason 1982) and that grafts of gut epithelium in the kidney capsule failed to express Ia (Mayrhofer, Pugh & Barclay 1983). Furthermore, it has been demonstrated that the intestinal epithelium of both rat fetuses and newborn rats (Cerf-Bensussan *et al.*, 1984) does not express Ia while that of adult conventional rat does. All these observations lend support to the concept that luminal factors are important in inducing and modulating Ia expression on intestinal epithelial cells.

This study shows that an increased MHC Class II antigen expression can be induced *in vitro* on human colonic epithelial cells by different types of stimulus. As with other cell types (Wong *et al.*, 1982; Rosa & Fellous 1984. Houghton *et al.*, 1984) human CEC were promptly and potently induced to express HLA-D region antigens by exposure to γ-IFN. The effect of γ-IFN appeared to be slightly more potent for DR than for either DP or DQ in contrast to that observed for other cell types (Capobianchi *et al.*, 1985). Our kinetic experiments showing a progressive increase of DR expression up to $> 80\%$ over 24 h may indirectly confirm the assumption that γ-IFN acts as both a modulator and an inducer.

The mechanism by which PHA was capable of enhancing HLA-D region antigens on CEC is not clear. As with other human epithelial cells (Pujol-Borrel *et al.*, 1983) this mechanism may not be related to the known mitogenic effect of PHA. This is further supported by the observation that ^{3}H-Thymidine uptake was not increased in our PHA-stimulated CEC cultures (data not shown). The data from this study showed that EGF also is capable of markedly increasing MHC Class II molecules on human CEC. EGF is essential in promoting cell growth but also has other functions (Carpenter, 1985). There is evidence that EGF plays a role in regulating the synthesis and secretion of cellular macromolecules and in maintaining morphological differentiation of the colonic epithelial cells in culture (Chopra *et al.*, 1987). Whether the Class II induction by EGF is related to its mitogenic effect or to these other functions remains to be established. However, this is to our knowledge the first demonstration that EGF has the property of inducing Class II antigens on human intestinal epithelial cells in culture.

The increased HLA-D region antigen expression induced *in vitro* by both PHA and EGF might have occurred as a result of these substances stimulating contaminating lymphocytes to produce γ-IFN (or γ-IFN-like substances), which in turn might have induced DR, DP and DQ on the CEC. This seems to be unlikely since the expression patterns with PHA and EGF differed both quantitatively and qualitatively from that observed after γ-IFN exposure. Moreover, our results indicate that either PHA or EGF are capable of inducing HLA-D region antigens on CEC cell suspensions depleted of lymphocytes. Finally, the addition of the anti-γ-IFN monoclonal antibody only partially inhibited the PHA-induced expression of HLA-D region molecules. On the other hand, evidence indicates that lymphocytes and particularly T cells are not obligatory for the expression of HLA-D region products on the gut epithelium (Mayrhofer *et al.*, 1983).

Several lines of investigation have shown that normal intestinal epithelial cells are capable of synthesizing and expressing MHC Class II molecules (Barclay & Mason, 1982; Mayrhofer *et al.*, 1983; Cerf-Bensussan *et al.*, 1984; Bland & Warren, 1986a; Spencer *et al.*, 1986). Our findings of an *in vitro* enhancement of HLA-D region antigens on CEC by different types of stimulation provide further evidence that MHC Class II antigen expression on the colonic epithelium is a normal phenomenon, the magnitude of which can increase under various circumstances. There is now increasing evidence that intestinal epithelial cells may function as antigen presenting cells *in vitro* (Bland & Warren, 1986a; Bland & Warren, 1986b; Mayer & Shlien, 1987). Although the *in vitro* induction of HLA-D region molecules does not necessarily imply that the cell is committed to function as an antigen presenting cell (Geppert & Lipsky, 1985), the data from the present investigation lend support to the concept that CEC play a role in the mucosal immune reactions and suggest that the increase in HLA-D region antigens on CEC in IBD is an indicator of the active participation of the epithelium in the mucosal inflammatory response.

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