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The Effect of 5-Aminosalicylic Acid, Hydrocortisone and Indomethacin on Gamma Interferon Induced Expression of HLA-DR Antigens on Colonic Epithelium

Wpływ kwasu 5-aminosalicylowego, hydrokortyzonu i indometacyny na indukowaną interferonem gamma ekspresję antygenów HLA-DR na nabłonku okrężnicy

INTRODUCTION

Class II histocompatibility antigens (HLA-DR) play an important role in the development of normal immune response. These glycoprotein cell surface molecules are involved in the process of antigen presentation to lymphocytes T by specialized antigen presenting cells (16). Normally HLA-DR antigens are found on the surface of macrophages, dendritic cells, Langerhans cells in the skin, lymphocytes B, and some endothelial cells, which are antigen presenting cells capable of presenting foreign antigen to lymphocytes and hence induce immunological response by activating T helper cells (8).

Normal colonic epithelium does not express HLA-DR molecules, but expression does occur in the presence of inflammation caused by ulcerative colitis and Crohn's disease (15, 18). The etiology of these two diseases altogether defined as inflammatory bowel diseases (IBD) is unknown, but immunological disturbances seem to play a crucial role in their pathogenesis, especially in perpetuating chronic inflammation in the colon (14). The observation of aberrant HLA-DR expression on colonic epithelium in IBD gave rise to the hypothesis, that colonic epithelial cells may have the potential for acting as antigen presenting cells and this might be a mechanism, whereby following an initial trigger to T-cell activation, the inflammatory response could be perpetuated (15). Experimental studies have provided evidence that epithelial cells can function in this way (13).

As previously reported interferon gamma (IFN_γ) released by lymphocytes of mucosal inflammatory infiltrate is the main factor responsible for induction of colonic epithelial HLA-DR expression (12). Inhibition of this process could be thus an important mechanism in the therapy of IBD.

The mode of action of 5-aminosalicylic acid (5-ASA) as an active moiety of Sulfasalazine and corticosteroids, both of them used in the treatment of IBD remains unclear (17). This prompted us to investigate the effect of 5-ASA and hydrocortisone (HC) on IFNγ induced expression of HLA-DR antigens by colonic epithelial cells. The influence of indomethacin (IND) — prostaglandin synthetase inhibitor was also studied, because prostaglandins were reported to take part in this process (19).

MATERIALS AND METHODS

The human colonic cancer cell line HT-29 (courtesy of Prof. P. Brandtzaeg — Oslo, Norway) was maintained in continuous tissue culture in Leibowitz-15 medium (Gibco—Great Britain) supplemented with 10% fetal calf serum (Flow Laboratories — Great Britain), 2 mM Gluthamine, gentamycin and amphotericin in temp. 37°C in humidified atmosphere with 5% CO_2.

Recombinant interferon gamma — IFNγ (Wellcome Biotechnology, Great Britain) was used in concentration of 10 u/ml, which as reported in our previous work induced maximal HLA-DR expression on HT-29 cells (7).

5-Aminosalicylic acid — 5-ASA (Sigma—Great Britain) was applied in concentrations of 1.5 and 3.0 mg/ml, which were similar to those found in rectal dyalisates in patients taking therapeutic doses of Sulfasalazine (10). Hydrocortisone — HC (Sigma) was studied at a dose of 0.001 mg/ml, which represents the mean serum level after intravenous injection of 100 mg HC (7), and at a dose 0.1 mg/ml. Indomethacin — IND (Sigma) concentrations were 0.001 and 0.005 mg/ml, which approximates to that found in plasma after therapeutic doses (1).

HT-29 cells were seeded at 2×10^5 / well into 96 well plates (Flow Laboratories). After 24 hrs the cells were adherent and entering the logarythmic phase of growth, the medium was discarded and replaced with 150 µl fresh medium and 50 µl of solution of IFNγ with or without studied drug dissolved in medium. The cells were incubated for a further 48 hrs at 37°C before assay. At the end of the culture period the cells were washed 3 times in phosphated buffered saline (PBS) at room temperature and air dried at 37°C for 3 hrs, then fixed using 200 µl of methanol and endogenous peroxidase activity was blocked with 1% hydrogen peroxide. HLA-DR enzyme linked immunoadsorbent assay (ELISA) was performed according to the Baumgarten method (2). Non-specific binding sites in the wells were blocked by incubation with 200 µl 1% gelatin (Sigma) containing 80 µg heat aggregated rabbit immunoglobulin (Dako—Denmark) for 2 hrs at room temperature. The monoclonal antibody to HLA-DR (Monoclonal Mouse Antibody to Human HLA-DR-Dako) was used as a 1:50 dilution. Each well was incubated with 100 µl antibody at 4° for 16 hrs in a humidified chamber. The plates were washed 4 times with 0.1% gelatin in PBS and incubated for 1 hr with peroxidase conjugated rabbit antimouse antiserum (Rabbit Immunoglobulins to Mouse Immunoglobulins HRP-Dako). The plates were washed four times with wash solution and peroxidase content per well assayed using 100 µl ortophylene diamine 1 mg/ml (Dako). The reaction product was terminated after 5 min incubation in the dark at room tempeature by the addition of 100 µl 1 M sulphuric acid. The coloured reaction product was read in a multiscan plate reader (Micro-ELISA reader — Titer Tek — Finland) spectrophotometrically with the use of optical filter 492 nm. The plates were then washed three times with methanol and used for the determination of

cellular protein according to the Bradford technique (3). Briefly 30 µl of Bradford reagent (Biorad Laboratories—Great Britain) was added to each well and plate shaken for 30 min. The reaction product was assayed in a multiscan spectrophotometer (Titer—Tek) with the use of optical filter 595 nm. HLA-DR induction was thus expressed as the ratio of optical densities (OD) measured with both filters OD_{492}/OD_{595}nm. This procedure allowed to eliminate cytotoxic effect of IFNγ and studied drugs to the cells. The cells were also counted in 0.05% trypan blue in a Neubauer chamber before and after exposition to the studied substances. Each experiment was performed 8 times and results were analysed statistically with Student's *t* test.

RESULTS

Nonstimulated HT-29 cells did not exhibit the presence of HLA-DR antigens ($OD_{492\ 59}$ — 0.341 ± 0.04 — which was a background of the assay (Fig. 1). Incubation of the cells with IFNγ at a concentration 10 u/ml caused a strong HLA-DR expression ($OD_{495\ 595}$ — 1305 ± 0.02).

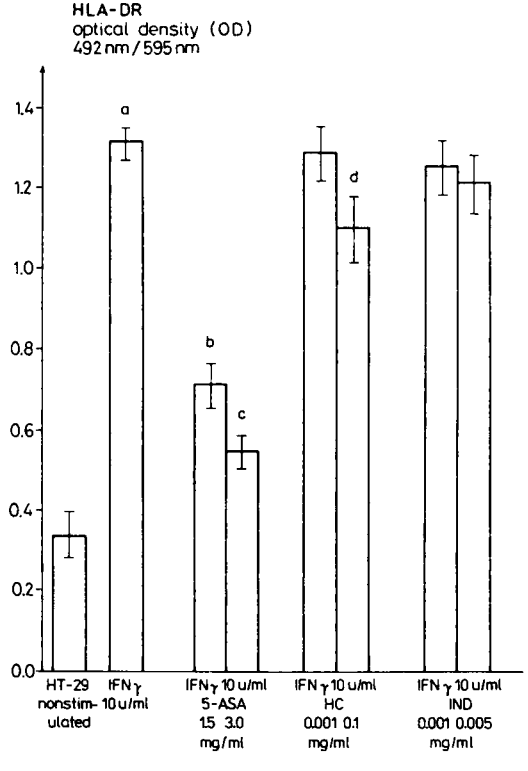

Fig. 1. The effect of 5-aminosalicilic acid (5-ASA), hydrocortisone (HC) and indomethacin (IND) on gamma interferon (IFN_γ) induced expression of HLA-DR antigens on HT-29 cells. Results of ELISA assay expressed as the ratio of optical densities (OD) measured with the use of filters 492 and 595 nm (each bar represents mean value ± standard deviation); a/b — $p<0.001$, a/c — $p<0.001$, a/d — $p<0.01$

HLA-DR expression induced by incubation with IFN was significantly reduced by 5-ASA added to the cells in both concentrations 1.5 mg/ml ($OD_{492\ 595}$ — 0.750 ± 0.04) and 3 mg/ml ($OD_{492\ 595}$ — 0.550 ± 0.05; $p < 0.001$).

HC at concentration of 0.001 mg/ml had no effect ($OD_{492\ 595}$ — 1276 ± 0.04) and at 0.1 mg/ml minimal but significant reduction of HLA-DR expression was observed ($OD_{492\ 595}$ — 1103 ± 0.08; $p < 0.01$). Indomethacin did not inhibit HLA-DR expression at any of the studied doses ($OD_{492\ 595}$ — 1267 ± 0.06, and 1257 ± 0.06, respectively). Cell viability assessed by trypan blue exclusion after 48 hrs incubation with IFNγ and or drug was greater than 90% in all experiments.

DISCUSSION

The aberrant expression of HLA-DR antigens on colonic epithelium may play an important role in the development and chronicity of inflammation in IBD. Continuous presentation of luminal antigens to lymphocytes T by epithelial cells behaving as antigen presenting cells might be responsible for stimulation and perpetuation of immunopathological distrurbances leading to inflammation and tissue injury (20). The ability of intestinal and colonic epithelial cells to act as antigen presenting cells was demonstrated by several authors (4, 13).

The influence of the drugs used in the treatment of IBD on the described phenomenon might be one of the possible therapeutic mechanisms. In our work we observed that 5-ASA in concentrations similar to the ones reported to be found in colonic lumen in patients taking therapeutic doses of Sulfasalazine significantly reduced IFNγ induced HLA-DR expression by colonic epithelial cells. Sulfasalazine is a compound of 5-ASA and sulfapyridine linked by an azo bond. It is poorly absorbed in the small intestine and hence the majority of an orally administered dose is delivered to the colon. The colonic bacteria split the azo bond and thereby release two moieties, from which only 5-ASA is an active one. Sulfapyridine serves only as a carrier molecule (6). The mode of action of 5-ASA is unclear although many possible machanisms have been proposed (9, 17). The inhibition of HLA-DR expression by colonic epithelium may have therapeutic significance, especially in preventing relapses of IBD, where the efficacy of Sulfasalazine is well established (6). Corticosteroids are used mainly in the treatment of acute attacks of IBD, while they appeared to be ineffective as a maintenance therapy (11). In our work HC did not reduce epithelial HLA-DR expression in a concentration corresponding to the plasma levels after administration of therapeutical doses. Minimal though significant inhibition was observed only when a very high concentration was used, normally not found in the therapy. This divergent effects of 5-ASA and HC on HLA-DR expression by colonic epithelium may explain the above mentioned differences in the therapeutical efficacy of the drugs.

The mechanism of reducing the HLA-DR expression by 5-ASA is not completely revealed although there is some evidence that this compound may act by impairing the binding of IFNγ to its specific receptor on the colonic epithelial cell (5).

Indomethacin had no effect on IFNγ induced HLA-DR expression, which suggests that mechanisms other than blocking prostaglandin synthetase are involved in the observed phenomenon.

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STRESZCZENIE

Określono wpływ kwasu 5-aminosalicylowego (5-ASA), hydrokortyzonu (HC) i indometacyny (IND) na indukowaną interferonem gamma (IFN_γ) ekspresję antygenów HLA-DR na komórkach nabłonkowych okrężnicy. Komórki linii HT-29 pochodzącej z ludzkiego raka okrężnicy utrzymywane w ciągłej hodowli tkankowej inkubowane były z IFN_γ (10 j/ml) oraz z badanymi lekami w kilku stężeniach. Ekspresję HLA-DR mierzono przy pomocy metody immunoenzymatycznej (ELISA). 5-ASA w stężeniach 1,5 i 3,0 mg/ml znamiennie hamował ekspresję HLA-DR na komórkach HT-29 indukowaną IFN_γ, podczas gdy HC (0,001 mg/ml) i IND (0,001 i 0,005 mg/ml) efektu takiego nie wywierały. Obserwowane działanie 5-ASA może odgrywać dużą rolę jako mechanizm terapeutyczny w leczeniu nieswoistych zapaleń jelit.