Prostaglandin E_2 Down-Regulates the Expression of HLA-DR Antigen in Human Colon Adenocarcinoma Cell Lines†

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ABSTRACT: Prostaglandins (PG) have been implicated in the pathogenesis of cancer and play an important role in immune regulation. Colon cancer is associated with elevated levels of PGE_2, while aspirin, the prototypical inhibitor of PG synthesis, appears to reduce the incidence of colon cancer by 50%. We have observed that in human colon cancer the expression of HLA class I and II antigens is reduced or lost; loss of HLA antigens is suspected to be a mechanism by which the malignant cell escapes the immune surveillance. We investigated the effect of these eicosanoids on the expression of HLA antigens in human colon adenocarcinoma cell lines. PGE_2 down-regulated the expression of the class II antigen HLA-DR in SW1116 cells (65% reduction at 2.8×10^{-8} M). This effect was dose- and time-dependent, reversible, and specific ($PGF_{2\alpha}$ and LTB_4 had no effect; the expression of carcinoembryonic antigen and class I genes were not affected). Aspirin induced the expression of HLA-DR in HT29 cells, a cell line not expressing constitutively HLA-DR. The reduction of HLA-DR by PGE_2 was accompanied by reduced messenger RNA (mRNA) levels of HLA-DRα and reduced transcription of the corresponding gene. In contrast to HLA-DR, none of these three eicosanoids affected the expression of HLA class I genes, as assessed via determination of protein expression by fluorescence flow cytometric analysis and evaluation of the corresponding class I mRNA levels. We conclude that PGE_2 specifically down-regulates the expression of HLA-DR, while it does not affect the expression of class I antigens. These findings may be relevant to the general problem of immune surveillance of cancer and the mode of action of aspirin in protecting from colon cancer.

Eicosanoids play an important role in immune regulation and have complex interactions with various cytokines (Goodwin, 1985; Lewis, 1990). During the last two decades they have been implicated in the pathogenesis of cancer, although their role is still unclear (Levine, 1988). For example, prostaglandins (PGs) have been considered to participate in tumor promotion and tumor metastasis (Fischer et al., 1985; Honn et al., 1983; Karmali et al., 1983). Several lines of evidence suggest that eicosanoids may be involved in colon carcinogenesis. First, work in animal models of colon carcinogenesis demonstrated that nonsteroidal anti-inflammatory drugs (NSAIDs), such as piroxicam, indomethacin, and sulindac, all of which inhibit PG synthesis, significantly reduce the number of tumor bearing animals and the number of tumors per animal (Pollard & Luckert, 1981, 1983; Moorghen et al., 1988; Reddy et al., 1990). Second, most (e.g., Waddell et al., 1989; Thun et al., 1991; Giovannucci et al., 1994) but not all (Paganini-Hill et al., 1989; Gann et al., 1993) epidemiologic studies have demonstrated that aspirin halves the risk of colon cancer in long-term users of aspirin and other NSAIDs. Finally, sulindac causes regression of large bowel polyps in patients with familial polyposis (Giardiello et al., 1993).

Colon cancer is associated with elevated levels of PGE_2. For example, we recently determined the levels of PGE_2, $PGF_{2\alpha}$, PGI_2, thromboxane (TX) A_2, and leukotriene (LT) B_4 in pairs of human colon cancer and histologically normal mucosa distant from the tumor. Compared to normal mucosa, in colon cancer the levels of PGE_2 were elevated, while those of PGI_2 were decreased; the levels of $PGF_{2\alpha}$, TXB_2, and LTB_4 were not significantly different (Rigas et al., 1993). Elevated levels of PGE_2 have also been reported in blood from the draining vein of colon cancer (Kubota et al., 1992).

The expression of HLA class I and II antigens is very frequently reduced in human colon cancer. We observed that, in 96% of colon cancers, the expression of HLA class I antigens was either undetectable or diminished and that, in 92% of colon cancers, at least one class II antigen was undetectable (McDougall et al., 1990). Reduced expression of HLA antigens was also noted in colonic adenomas, which are the premalignant lesions of the colon. In addition, HLA antigens are reduced not only in adenomas but also in histologically normal mucosa distant from the adenomas (Tsioulias et al., 1992, 1993). These findings indicate that reduced expression of HLA antigens is very common in colon cancer, preceding the onset of histological changes toward neoplasia.

Loss of HLA antigens is suspected to be the mechanism by which the malignant cell escapes the immune surveillance of the organism in which it develops. For example, loss of HLA class I molecules, which are required for the presentation of tumor antigens to cytotoxic T lymphocytes, may lead

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to the escape of these tumors from immune surveillance (Bodmer, 1991). The important role of class II antigens in the immunology of cancer is currently being elucidated. Class II antigens present endogenous proteins, such as *ras* oncoproteins (Peace et al., 1991), to $CD4^+CD8^-$ lymphocytes bearing the appropriate T cell receptor to generate antigen specific Th (T helper) cells. Since an immune response depends heavily on Th cells, it has been postulated that impaired generation of tumor-specific Th cells may explain, at least partially, the inability of an organism to mount an effective antitumor immune response (Ostrand-Rosenberg et al., 1991).

In view of the profound changes of HLA antigens in colon cancer and the changes in eicosanoid levels in human colon cancer, we examined the possibility that either PGE_2 or other eicosanoids may affect HLA class I antigen expression. Also examined was the role of aspirin in these events. This paper describes the findings from our study of the role of PGE_2, $PGF_{2\alpha}$, and LTB_4 in the regulation of HLA gene expression in cultured human colon adenocarcinoma cells.

EXPERIMENTAL PROCEDURES

Cell Lines. The human colon adenocarcinoma cell lines SW1116, HT29, SKC01, HCT15, and LoVo were obtained from American Tissue Type Culture (ATTC, Rockville, MD) and grown as monolayers, according to the instructions of ATTC. Culture medium consisted of RPMI1640 for SW1116, LoVo, and HCT15, and McCoy 5A for HT29 cells. Both media were supplemented with 10% fetal calf serum, nonessential amino acids, streptomycin (10 000 units/mL) and penicillin (10 000 units/mL). Cells were incubated at 37 °C, in 5% CO_2 and 90% relative humidity. Cell morphology was recorded daily.

PGE_2 and $PGF_{2\alpha}$ (Sigma Chemical, St. Louis, MO) were dissolved in dimethyl sulfoxide (DMSO), while LTB_4 (Cayman Chemical Co., Ann Arbor, MI) was dissolved in ethanol. To avoid coprecipitation of LTB_4 with denatured proteins of the culture medium, the ethanolic solution (typically 10−20 μL) was first dissolved in 250 μL of PBS and then added to 10 mL of culture medium. Eicosanoids (or PBS for controls) were added 12 h after plating the cells, which were harvested at either 24 h, for the study of HLA-DR, or at 48 h for the study of HLA class I antigens. Acetylsalicylic acid (Sigma Chemical, St. Louis, MO) was dissolved in culture medium, whose pH was adjusted appropriately.

Immunofluorescence Analysis. Immunofluorescence was performed by incubating cells with the appropriate monoclonal antibody (mAb). Single cell suspensions, obtained by incubating the monolayers with enzyme-free Cell Dissociation Solution (Specialty Media, Inc, Lavalette, NJ), were washed with PBS/1% bovine serum albumin (BSA) (Sigma Chemical, St. Louis, MO). HLA-DR, -DP, -DQ, and carcinoembryonic antigen (CEA) were determined by indirect immunofluorescence, using anti-HLA-DR, -DP, and -DQ mAbs (Sera-Lab, Westbury, NY) or an anti-CEA polyclonal antibody (Dako, Carpinteria, CA), respectively, or isotypic controls. Cells were reacted with the primary antibody for 30 min at 4 °C in the presence of 0.5% Tween 20, washed twice with PBS/1% BSA, incubated with FITC-conjugated secondary antibody (goat anti-mouse for the HLA class II antigens, and goat anti-rabbit for CEA) for 30 min at 4 °C in the presence of 0.5% Tween 20, and then subjected to flow cytometric analysis with an EPICS 752 flow cytometer (Coulter Cytometry Systems, Hialeah, FL). HLA class I antigens were determined by direct immunofluorescence, using the FITC-conjugated monoclonal antibody W6/32 (IgG2a), which recognizes shared determinants of HLA-A, -B, and -C or isotypic control (Sera-Labs). Background immunofluorescence was determined by using only the FITC-conjugated antibody. For each sample, 10 000 events were analyzed at flow rate of 150 events/s. Cell viability was assessed by the trypan blue exclusion method. In these experiments binding by isotypic control mAb was <5% and was subtracted.

Northern Blot Analysis. Following washing of the cells with PBS, total cellular RNA was extracted using RNAzol (Cinna/Biotecx Labs, Friendswood, TX), following the instructions of the manufacturer. Ten micrograms of RNA was size fractionated on formaldehyde/agarose gels, transferred to nitrocellulose membranes (Nitrin UV cross-link membrane, Scheider & Scheider), fixed on the membrane with UV cross-linking in the Cross Linker (Stratalinker, Stratagene, La Jolla, CA) at 2000 J, and hybridized to nick-translated DP001, a general HLA class I probe (Sood et al., 1981). The hybridized probe was then stripped from the filter by washing it for 2 h at 65 °C in (0.5% sodium pyrophosphate/0.1× Denhardt's/5 mM Tris, pH 8.0/0.2 mM EDTA), following the instructions of Stratagene. Rehybridization of the filter to a β-actin DNA probe (Kost et al., 1983) showed that practically the same amounts of RNA were immobilized on it (data not shown). Bands were quantitated using the PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Results were expressed as percent of control, which was assigned an arbitrary value of 100.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Equal quantities of mRNA from control and test samples were reverse-transcribed and amplified using the RT-PCR kit (Perkin-Elmer Cetus, Norwalk, CT) and following the instructions of the manufacturer. Preliminary experiments indicated that 500 ng of mRNA per RT-PCR reaction were the appropriate amount to use as template to synthesize its complementary DNA through the action of reverse transcriptase. The resulting double-stranded DNA was heat denatured at 94 °C for 5 min and cooled in ice before use in the polymerase chain reaction. The oligonucleotide primers used were 5′ AGA GGT AAC TGT GCT CAC GAA 3′ and 5′ CCC AAG GCA CAC ACC ACG TTC 3′ [complementary to bases 1150−1171 and 1762−1741, respectively, of the HLA-DRα gene (Bodmer, 1991)]. For the purposes of the present study, PCR was performed for 30 cycles, at a denaturing temperature of 94 °C for 1 min and an annealing temperature of 72 °C for 1 min. Control experiments revealed that at these PCR settings the amplification of DNA was in the linear range. As a control, actin mRNA was also reverse-transcribed and amplified, using the following oligonucleotide primers: 5′ GTT TGA GAC CTT CAA CAC CCC 3′ and 5′ GTG GCC ATC TCC TGC TCG AAG TC 3′ (Peace et al., 1991). Following amplification, an aliquot of the reaction mixture was fractionated on a 2% agarose gel, transferred to nitrocellulose membranes (Nitrin UV cross-link membrane), fixed on the membrane with UV cross-linking in the Cross Linker at 2000 J, and hybridized to the nick-translated HLA-DRα or actin (Kost et al., 1983) DNA probes, and autoradiographed. Bands were quantitated using the PhosphorImager. Results were expressed as

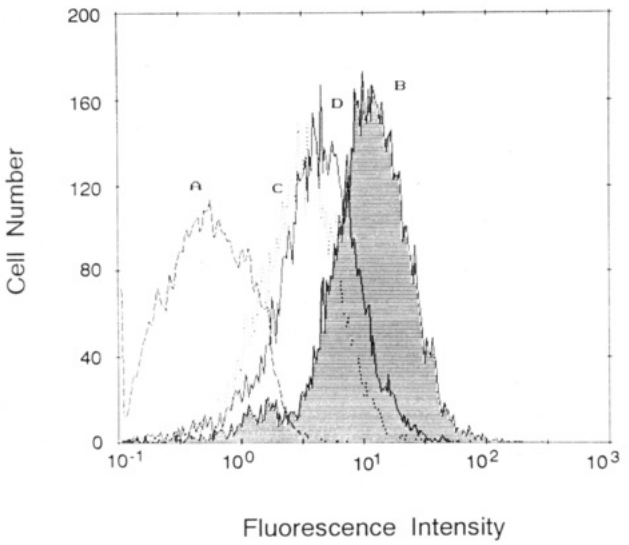

FIGURE 1: Expression of HLA-DR in SW1116 cells treated with PGE_2. The expression of the HLA-DR antigen by SW1116 cells treated with various concentrations of PGE_2 for 24 h was determined by flow cytometry, as described under Experimental Procedures. In these flow cytograms A = isotypic control; B = control; C = PGE_2 2.8×10^{-8} M; D = PGE_2 1.4×10^{-8} M.

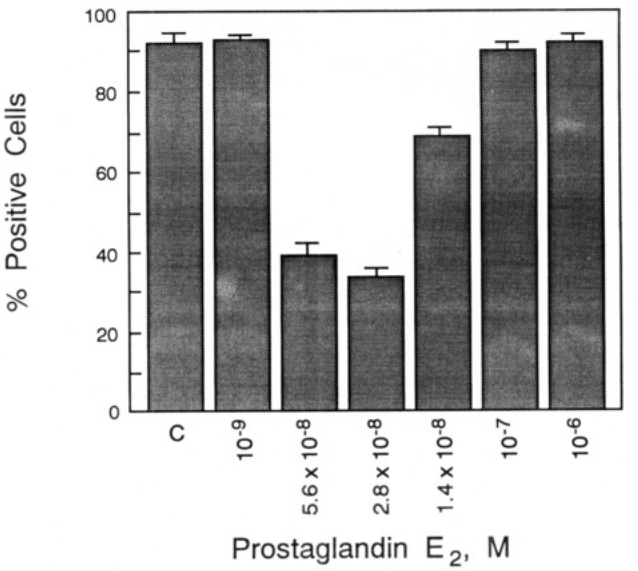

FIGURE 2: Effect of PGE_2 on HLA-DR expression: dose−response. The percentage of cells expressing the HLA-DR antigen, when treated with various concentrations of PGE_2 for 24 h, was quantitated using flow cytometry, as described under Experimental Procedures. Data represent mean ± SD of at least two triplicate experiments.

percent of control, which was assigned an arbitrary value of 100.

Nuclear Transcriptional Analysis. The ability of PGE_2 to affect transcriptional gene expression was monitored by nuclear run-on analysis. Nuclei were isolated from cultured cells (Minakuchi et al., 1990) treated with PGE_2 10^{-8} M. Cells were harvested by gentle scraping following washing with PBS. Cells were pelleted and lysed by incubating with NP-40 lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40) for 5 min on ice; this was repeated once. Nuclei were counted using a hemocytometer. Aliquots of the nuclear pellet were stored in liquid nitrogen until further use. For the nuclear run-on transcription assay (Groudine et al., 1981), frozen nuclei were thawed, and equal numbers (typically 10^8 nuclei) were incubated in a polypropylene tube with ATP, CTP, and GTP, each at 1 mM, and [α-^{32}P]UTP at 0.1 mM, in a reaction buffer containing 5 mM Tris HCl pH 8, 150 mM KCl, 2.5 mM $MgCl_2$, and 2.5 mM DTT, at 30 °C for 30 min with shaking. This was followed by successive treatments with DNase I and proteinase K for 5 min at 30 °C and 30 min at 42 °C, respectively. Nucleic acids were extracted with phenol/chloroform/isoamyl alcohol, and precipitated with 10% trichloroacetic acid and 60 mM sodium pyrophosphate. The precipitate was filtered onto a GF/C filter paper (Whatman, Maidstone, U.K.) and, following washes, treated again with DNAse I. RNA was eluted following heating in the presence of 1% SDS, 10 mM Tris-HCl, pH 7.5, and 5 mM EDTA at 65 °C for 10 min. RNA was then extracted and hybridized with target DNA immobilized on a nitrocellulose filter. DNA species used include the β-actin gene; the HLA-DRα gene; DP001, a general class I probe; and M13 (negative control). Filters were autoradiographed and bands quantitated using the PhosphorImager.

RESULTS

PGE_2 Reduced the Expression of HLA-DR in SW1116 Colon Adenocarcinoma Cells. Of the colon adenocarcinoma cell lines that we examined, only SW1116 expresses HLA class II antigens. HT29, HCT15, SKC01, and LoVo cells do not express appreciable amounts of any of the three class II antigens (HLA-DR, -DP, -DQ). While SW1116 cells express HLA-DR strongly, HLA-DP and HLA-DQ are barely detectable.

We examined the effect of PGE_2, $PGF_{2\alpha}$, and LTB_4 on the expression of HLA-DR antigen in the SW1116 cells. Cells were exposed for 24 h to each of these three eicosanoids, and HLA-DR antigen expression was determined. The percentage of cells expressing this antigen and the mean fluorescence intensity of positive cells (an expression of relative antigen density) were quantitated and results were compared to those of untreated control cultures.

PGE_2 induced a significant reduction in the expression of HLA-DR antigens in SW1116 cells. Figures 1 and 2 depict the effect of various concentrations of PGE_2 on the expression of HLA-DR in the SW1116 cells. The expression of HLA-DR is reduced only at PGE_2 concentrations in the 10^{-8} M range: it is 61%, 65%, and 31% lower compared to control at 5.6×10^{-8}, 2.8×10^{-8}, and 1.4×10^{-8} M, respectively ($p < 0.01$ for all). There is no statistically significant change at PGE_2 concentrations higher or lower than 10^{-8} M (i.e., 10^{-9}, 10^{-7}, and 10^{-6} M). Similar results were obtained for the mean fluorescence intensity of positive cells. Therefore, it is apparent that the response is bell-shaped, in accordance with previous experience with PG effects (Horrobin, 1978). In contrast, $PGF_{2\alpha}$ and LTB_4, tested at the same concentrations, had no effect on the expression of HLA-DR; parallel PGE_2 controls did manifest the expected reduction in the expression of HLA-DR. Neither of the two solvents (DMSO and ethanol) used to dissolve the eicosanoids affected the expression of the HLA-DR antigen. No eicosanoid affected the morphology of the cells or their viability. PGE_2 had no effect on the expression of CEA, a cell surface protein (Shiveley & Beatty, 1985) that is expressed strongly in these cells (data not shown), thus suggesting that the effect of PGE_2 on HLA-DR expression was not part of a generalized suppression of gene expression.

The kinetics of the response to PGE_2 were evaluated at three concentrations (2.8×10^{-8}, 2.8×10^{-7}, and $2.8 \times$

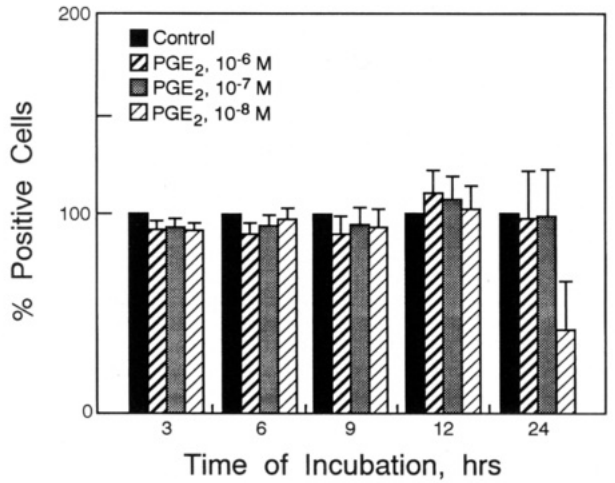

FIGURE 3: Kinetics of HLA-DR in response to PGE_2. HLA-DR expression by SW1116 cells treated with three different concentrations of PGE_2 was evaluated as described under Experimental Procedures, over a 24 h period. Since the expression of HLA-DR in these cells follows a circadian variation, results are expressed as percent of the corresponding control cells. Data represent mean ± SD of two triplicate experiments.

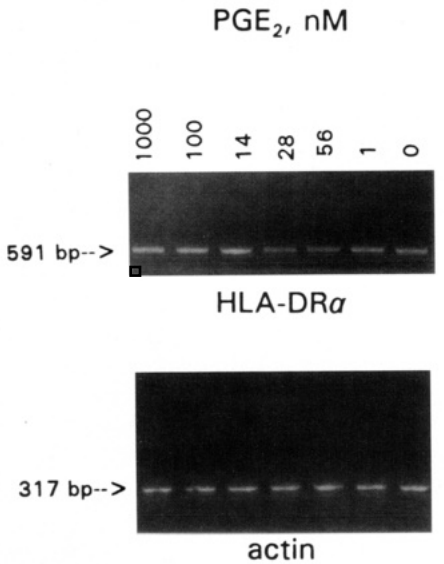

FIGURE 4: Effect of PGE_2 on the steady-state mRNA level of HLA-DRα in SW1116 cells. Total cellular RNA, extracted from cells treated with various concentrations of PGE_2, was amplified by RT-PCR using oligolnucleotide primers for either HLA-DRα (top) or actin (bottom), as described under Experimental Procedures. The size of the PCR products is indicated by the arrows.

10^{-6} M). The effect of PGE_2 on HLA-DR expression required 24 h, not being apparent during the first 12 h (Figure 3). Of note is that the expression of HLA-DR in SW1116 cells follows a circadian variation (Rigas et al., 1994); results are, therefore, expressed as percent of control cells.

The effect of PGE_2 on the expression of HLA-DR was reversible. This was demonstrated by treating cells with PGE_2 (5.6×10^{-8} M) for 24 h, at which time the cells were washed twice and incubated for an additional 24 h with medium without PGE_2. Twenty-four hours following the removal of PGE_2 from the culture medium, both the percentage of cells positive for HLA-DR expression and the mean fluorescence intensity of positive cells were virtually identical between PGE_2 and control cells. Parallel controls confirmed the reduction of HLA-DR expression by PGE_2 at 24 h.

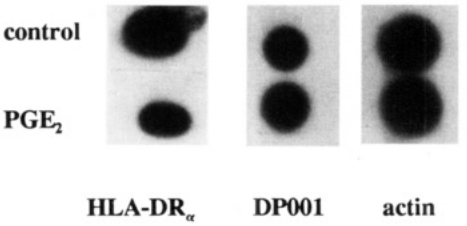

FIGURE 5: Effect of PGE_2 on the transcription of HLA-DRα in SW1116 cells. Nuclear run-on was performed using nuclei of SW1116 cells treated with PGE_2 (5.6×10^{-8} M) as described under Experimental Procedures. The resultant radiolabeled mRNA was hybridized to DNA fixed on a nitrocellulose membrane (the amounts of the plasmids containing the HLA-DRα, actin and DP001 (general class I) sequences were 22, 34, and 42 pmol, respectively). Quantitation of bands by Phosphorimager revealed that the HLA-DR transcription rate was reduced by 64% while that of actin and DPOO1 remained unaffected. A repeat duplicate experiment gave similar results (within 10%).

PGE_2 Reduced the Steady-State HLA-DRα mRNA Levels in SW1116 Cells. To elucidate the mode of PGE_2 action, we evaluated by RT-PCR the steady-state mRNA levels of HLA-DRα in both PGE_2-treated and control cells; the HLA-DRα gene encodes one of the two polypeptide chains that constitute the heterodimeric HLA-DR protein (Klein, 1986). As shown in Figure 4, PGE_2 reduced the steady-state mRNA level of HLA-DRα at the three concentrations in the range of 10^{-8} (14−56 nM), while it had no effect on it at 10^{-9}, 10^{-7}, and 10^{-6} M (indicated, for convenience in the figure as 1, 100, and 1000 nM, respectively). In contrast, the levels of actin mRNA were not changed in PGE_2-treated cells. When these bands were quantitated, by hybridizing to the appropriate radiolabeled probes as described under Experimental Procedures, it was found that, compared to controls, the HLA-DRα mRNA levels were reduced by 53% at PGE_2 concentrations of 2.8×10^{-8} and 5.6×10^{-8} M, and by 48% at 1.4×10^{-8} M, with no change at the three remaining PGE_2 concentrations. Similarly, there was no significant quantitative change in the actin mRNA levels between control and PGE_2-treated cells.

PGE_2 Down-Regulated the Transcription of HLA-DRα in SW1116 Cells. Nuclear run-on assays were performed to evaluate the mechanism by which the steady-state levels of HLA-DRα were reduced in response to PGE_2. As shown in Figure 5, PGE_2 at 2.8×10^{-8} M reduced the transcription of the HLA-DRα gene by 64%, while it did not affect that of actin or the HLA class I genes; the latter were assayed collectively by using a general class I sequence (DP001) (Sood et al., 1981).

Aspirin Induced HLA-DR Expression in HT29 Colon Adenocarcinoma Cells. We also evaluated whether PGE_2 may indeed participate in the regulation of HLA-DR expression. To this end, we assessed the effect of aspirin, a known inhibitor of prostaglandin synthesis, on the expression of HLA-DR in a colon cell line which does not express this antigen constitutively. HT29 cells (5×10^5 per 100 mm dish) were plated, and aspirin (0, 100, or 200 μM) was added to the culture medium at that time. Cells were harvested 24 h later and evaluated for the expression of HLA-DR and carcinoembryonic antigen (control surface antigen). As shown in Figure 6, <10% of control cells were positive for HLA-DR, whereas up to 87% of the aspirin-treated cells were positive. The expression of CEA remained unchanged. Similarly, indomethacin and sulindac, two aspirin-like drugs, increased the expression of HLA-DR in these cells (to be

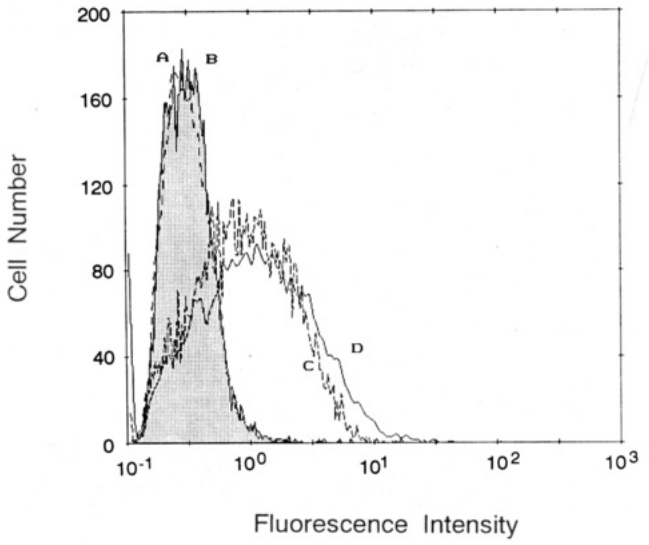

FIGURE 6: Effect of aspirin on HLA class II antigen expression. Aspirin increases the expression of HLA-DR in HT29 cells. A = isotypic control; B = untreated cells; C = aspirin 100 μM, D = aspirin 200 μM. This protocol was repeated twice giving similar results.

published elsewhere). These data demonstrate that treatment of HT29 cells, which do not express HLA-DR, with aspirin and aspirin-like drugs, leads to expression of this antigen.

Eicosanoids Did Not Affect the Expression of HLA Class I Antigens in Human Colon Adenocarcinoma Cell Lines. We evaluated the effect of PGE_2, $PGF_{2\alpha}$, and LTB_4 on the expression of HLA class I antigens in cultured human colon adenocarcinoma cells. A total of 2.5×10^5 SW1116 cells were plated per 100 mm dish and exposed, 12 h later, to each of the three eicosanoids at concentrations ranging between 3×10^{-10} and 3×10^{-6} M for a 48 h period. Table 1 shows that SW116 cells treated with PGE_2, $PGF_{2\alpha}$, and LTB_4 showed no statistically significant change either in the percentage of cells displaying immunofluorescence, when reacted with FITC-labeled anti-class I monoclonal antibody, or the mean intensity of fluorescence, compared to controls.

To further evaluate this finding, we tested the effect of PGE_2, the eicosanoid whose levels are elevated in colon cancer tissues, on the expression of HLA class I surface antigens in the HT29 colon adenocarcinoma cell line; HT29 cells express constitutively HLA class I antigens. PGE_2, at concentrations of 3×10^{-8}, 3×10^{-7}, and 3×10^{-6} M, had no effect on the expression of HLA class I antigens compared to controls. We also examined whether adding PGE_2 to the culture medium more frequently (every 24 h instead of once in a 48 h period) had any effect on HLA class I antigen expression. Again, we observed no effect of PGE_2 on HLA class I antigen expression in either SW1116 or HT29 cells.

The steady-state mRNA levels of HLA class I genes were assessed by Northern blotting in both SW116 and HT29 cells treated with PGE_2. No change was noted between PGE_2-treated and untreated cells; rehybridization of the filters with actin also showed no difference between any of these samples (data not shown). Of note, in the nuclear run-on experiment assessing the effect of PGE_2 on HLA-DRα transcription rate, the transcription of class I genes was assessed collectively as a control and was not altered, further supporting this observation.

DISCUSSION

Our data demonstrate that PGE_2 decreases the expression of the HLA class II antigen HLA-DR in a human colon adenocarcinoma cell line. This effect is brought about, at least partially, by decreasing the transcription rate of the HLA-DRα gene. The effect of PGE_2 on the transcription of HLA-DRα is reflected in the reduction of the corresponding steady-state mRNA levels.

Table 1: Effect of PGE_2, $PGF_{2\alpha}$, and LTB_4 on HLA Class I Antigen Expression in SW116 Colon Adenocarcinoma Cells[a]

	PGE_2		$PGF_{2\alpha}$		LTB_4	
concentration (M)	% (+) cells	mean intensity	% (+) cells	mean intensity	% (+) cells	mean intensity
0	85.2	83.1	83.3	80.3	81.3	79.8
3×10^{-10}	82.7	81.5	79.5	77.4	80.0	77.6
3×10^{-9}	80.8	79.6	83.6	81.4	84.7	83.1
3×10^{-8}	84.6	80.3	82.4	80.1	83.7	81.6
3×10^{-7}	89.0	82.9	86.9	82.5	82.6	80.0
3×10^{-6}	83.3	81.7	82.2	80.9	84.8	83.6

[a] Results are the average of duplicate samples. This protocol was repeated once giving similar results.

The reduction of HLA-DR antigens in SW1116 cells was both time- and concentration-dependent. It is evident at 24 h but does not occur during the first 12 h and only at PGE_2 concentrations between 1.4×10^{-8} and 5.6×10^{-8} M. The concentration-response curve of the effect of PGE_2 on HLA-DR antigen expression is bell-shaped. Such a response, in contrast to plateau-type curves often encountered in biology, is characteristic of a prostaglandin effect (Horrobin, 1978). That this unusual response is not an artifact is indicated by (a) the fact that a range of concentrations at 10^{-8} M is manifesting this result, (b) the concordance of the mRNA and protein data, and (c) the reproducibility of the findings.

The effect of PGE_2 is specific in terms of its structure and chemical class, and also the target gene. This is evidenced by two findings: (a) $PGF_{2\alpha}$, which is a structurally closely related product of the cyclooxygenase pathway, and LTB_4, a product of the lipoxygenase pathway, do not affect the expression of HLA-DR, and (b) the expression of CEA and HLA class I genes is not affected by PGE_2.

The steady-state mRNA levels of HLA-DRα, one of the two genes encoding the heterodimeric HLA-DR protein, are reduced in response to PGE_2. Again, the bell-shaped response is evident. Our data suggest that PGE_2 reduces the expression of the HLA-DR antigen by a transcriptional effect. Although we have demonstrated an effect on only HLA-DRα (HLA-DRβ was not evaluated), down-regulation of only one of the genes is sufficient to reduce the expression of the antigen, since both chains are required for its insertion into the membrane of the cell. A similar effect of PGE_2 on HLA-DRβ is also possible.

None of the three eicosanoids tested (PGE_2, $PGF_{2\alpha}$, and LTB_4) affected the regulation of the expression of HLA class I antigens. First, no such effect was demonstrated in two colon adenocarcinoma cell lines, one of which manifested the effect of PGE_2 on HLA-DR (the other did not express constitutively HLA-DR). Second, HLA class I mRNA levels were not affected by PGE_2. Also unaffected by PGE_2 was the transcription of class I genes assessed in the nuclear run-on study. The induction of HLA-DR, following treatment with aspirin and other NSAIDs in HLA-DR negative colon adenocarcinoma cells suggests that endogenously produced PGE_2 may maintain the HLA-DR gene(s) in a state of down-regulation. This is, however, a tentative conclusion, as our data leave open the possibilities of (a) a direct effect by aspirin and other NSAIDs on these genes, (b) an effect

through one of the complex eicosanoid loops, independent of PGE_2, or (c) an effect totally independent of prostaglandins or any other eicosanoids. Nevertheless, these findings suggest a potential mechanism for aspirin's effect in colon cancer that may be worth exploring.

Prostaglandins have pleiotropic effects on immune regulation. The effect of prostaglandins on MHC class II antigens has been demonstrated in immune cells. Snyder et al. (1982) first showed in murine macrophages that PGE_2 suppressed the expression of Ia; both endogenous and synthetic PGE_2 (and other PGs) suppressed Ia expression (Tripp et al., 1986). PGE_2 at 10^{-5} M reduced HLA-DR expression in circulating monocytes (Hassan et al., 1989), while PGE_1 methyl ester analogs suppressed HLA-DR expression in transformed lymphocytes (Pollak et al., 1990). Mechanistic insights into the regulation of class II expression by cAMP, the level of which is modulated by PGE_2, have been obtained (Mayer & Shlien, 1987). That PGE_2 suppresses HLA-DR expression in colonic epithelial cells is in keeping with the ability of these cells to participate in immune phenomena (Ivashkiv & Glimcher, 1992).

The mechanism by which these changes are brought about by PGE_2 is not clear. PGE_2 is known to modulate the expression of other genes, such as those for tumor necrosis factor-a (Kunkle et al., 1988; Ferreri et al., 1992) and IL-2 and IL-2Ra (Anastassiou et al., 1992). Intracellular signal transduction resulting in PGE_2-induced suppression may be mediated by the second messenger cAMP (Minakuchi et al., 1990; Rincon et al., 1988). Whether such a mechanism is operative in the system examined in our work is uncertain.

In conclusion, our data demonstrate that PGE_2 suppresses, in a specific manner, the expression of HLA-DR in human colon adenocarcinoma cells by an effect on gene transcription, while it does not modulate the expression of class I genes. $PGF_{2\alpha}$ and LTB_4, two eicosanoids derived by the cyclooxygenase and 5-lipoxygenase pathways, respectively, do not affect the regulation of HLA genes. Aspirin and other NSAIDs upregulate the expression of HLA-DR in colon cancer cells that do not express it constitutively. These findings may be relevant to the general problem of immune surveillance of cancer and the mode of action of aspirin in protecting from colon cancer.

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