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Biochemical and Morphological Differentiation of the Human Colonic Epithelial Cell Line SW620 in the Presence of Dimethylsulfoxide

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Abstract In vitro models of intestinal cell differentiation provide an important adjunct for studying normal and abnormal intestinal epithelial cell differentiation. The studies reported herein describe morphologic and biochemical changes in the colonic epithelial cell line SW620 following dimethylsulfoxide (DMSO) incubation. Cells cultured in the presence of DMSO showed striking changes in morphology characterized by enlargement, elongation, and formation of process-like structures by light microscopy and a propensity to form microvillus-like structures by electron microscopy. These changes were accompanied by significant differences in the expression of the cell surface markers CD4 (HIV gp120 receptor), CD44 (hyaluronate receptor), and KS1 (adenocarcinoma/epithelial specific antigen). There was a marked decrease in CD4 expression (38% to 2%), an increase in CD44 expression (4% to 50%) and a decrease in KS1 expression (98% to 66%) as detected by flow cytometry following incubation of SW620 cells in DMSO. Parallel changes in the expression of these markers were seen by metabolic and surface labeling studies. Although SW620 cells were infected by HIV-1, DMSO-treated SW620 cells could not be infected. DMSO-induced changes in surface expression of CD4, CD44, and KS-1 were reversible over time upon removal of DMSO from the culture medium. Secretory component, sucrase, neuron-specific enolase, chromogranin-A, and mucin were not detectable in SW620 cells with or without DMSO treatment. SW620 cells provide a useful model for studying specific biochemical and molecular events involved in intestinal epithelial cell differentiation and function.

Key words: SW620 cells, CD4, CD44, KS1/4, dimethylsulfoxide

Present methodology for isolating and growing human intestinal epithelial cells is limited by difficulties in attaining adequate viability, the propensity of epithelial cell autolysis, and limitation of culture duration [1,2]. Therefore, the development and use of in vitro models of differentiation have provided particularly useful systems for studying epithelial cell growth, differentiation, and function [3–7]. Examples include HT29 cells grown in the absence of sugar or replacement of glucose by galactose which, after confluency, differentiate along an enterocytic pathway as determined by the formation of tight junctions, an apical brush border, and new expression of sucrase isomaltase [5,6]. In addition, a differentiating clone of HT29 cells, HT29-18, was further subcloned to isolate cells that can differentiate to absorptive or goblet-like cells [7].

Dimethylsulfoxide (DMSO) is commonly used for inducing differentiation in hematopoietic [8,9] and epithelial cell lines [3,4]. Following incubation of the colonic epithelial cell line SW620 with 2% DMSO for eight days, cells underwent morphological changes, as determined by light microscopy, and alterations in their whole membrane protein profile [3]. Recently, we reported that SW620 cells, among a panel of colonic epithelial cell lines tested, uniquely express the HIV gp120 receptor (CD4) on the cell surface [10]. Further, we showed that SW620 cells can be infected with HIV and hence serve as a useful model for studying HIV infection and expression in colonic epithelial cells.

Epithelial tissues express a number of tissue-specific antigens that likely play important functional roles. For some antigens, such as CD44, a

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function is known, while for other antigens, such as KS-1, their function remains unknown. CD44 is an integral membrane glycoprotein that is the receptor for hyaluronate [11]. A new variant of this glycoprotein, which encodes an additional 162 amino acids in the extra cellular domain, was recently identified and confers a malignant potential to rat carcinoma cells [12]. The function that this additional domain imparts and whether a similar variant is present in humans remain to be determined. KS-1 is an adenocarcinoma/epithelial specific antigen whose gene has been cloned [13–15], yet its function remains unknown despite detailed structural characterization. Due to its epithelial specificity and oncofetal association [16], KS-1 antigen has been used as a target for the treatment of epithelial malignancies using vinca-alkaloids conjugated to anti-KS-1 monoclonal antibodies (MAb) [17]. In this report, we describe changes in the morphology and expression of CD4, CD44, and KS-1 on DMSO-treated SW620 cells.

MATERIALS AND METHODS

Cell Culture

SW620 cells, originally established as colonic epithelial cells from a lymph node metastasis of a colonic carcinoma [18], were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). For culture in the presence of DMSO, cells were plated in 10 or 15 ml ($0.5–1 \times 10^5$ cells/ml) in 100-mm tissue culture dishes. Media was changed three times weekly.

Antibodies

The MAb used were: Leu3a (anti-CD4, Becton Dickinson, Mountain View, CA); I4D4 (anti-KS-1/4 antigen [10]); L3D1 (anti-CD44 as determined by immunodepletion experiments using an antibody that recognizes CD44 [19]); A3/10 (anti-HLA class I [20]); L2A1 (anti-cytokeratin polypeptides 8 and 18 [21]); MU126-UC (anti-chromogranin; Biogenex Laboratories, San Ramon, CA); anti-sucrase isomaltase (generously provided by Dr. A. Quaroni, Cornell University).

Immunological Techniques

SW620 cells were detached from tissue culture dishes by incubating in 1 mM ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline (PBS), pH 7.4 for 10 min at 37°C, then washed twice with PBS (4°C). For indirect immunofluorescence, washed cells ($5–10 \times 10^5$ in 100 μl) were incubated with 100 μl of antibody supernatant for 45 min (4°C) followed by washing. Fluorescein isothiocyanate (FITC) coupled rabbit anti-mouse $F(ab')_2$ fragments (Zymed, South San Francisco, CA) were added (100 μl of 25 μg/ml) after which cells were incubated for 30 min at 4°C and then washed. Fluorescence activated cell sorter (FACS) analysis used an Ortho Systems #50H cytofluorograph (Ortho Pharmaceutical, Ravitan, NJ). For background staining, the first antibody was omitted or a nonspecific isotype matched antibody was used. Immunoprecipitation was done as described before [21].

Light Microscopy

SW620 cells were cultured on coverslips in the presence or absence of 3% DMSO. Cells were fixed in 3% formaldehyde in PBS, pH 7.4 for 1 h, then the coverslips were mounted on microscope slides using 90% glycerol. A Nikon Microphot-FX light microscope was used and images were obtained using Kodak Tri-X pan film, ASA 400.

Transmission Electron Microscopy

Cells were fixed on the culture dish for 1 h (22°C) in 0.1 M Pipes (pH 7.4) containing 3% formaldehyde and 2% glutaraldehyde. Cells were then harvested into a pellet or left as a monolayer on the culture dish, post-fixed for 1 h in 1% OsO_4 in 0.1 M Pipes (pH 7.4, 4°C), dehydrated through a graded ethanol series, and embedded in epon. Sections were obtained using a Reichert OMU2 ultramicrotome then double-stained in 2% uranyl acetate and lead citrate. A Philips EM 410 was used at 60 kV and images were obtained using Kodak EM 4489 film.

Radiolabeling

Cell surface labeling was carried out using lactoperoxidase catalyzed iodination and Na ^{125}I (carrier-free, New England Nuclear) as described before [19]. Metabolic labeling using [3,4,5-^{3}H]-leucine (150 Ci/mmol, Research Products International Corp.) was in leucine-free RPMI 1640 medium supplemented with 10% dialyzed fetal calf serum (2×10^6 cells/ml, 12-h labeling). After labeling with ^{125}I or [^{3}H]-leucine,

cells were washed in PBS, then detergent solubilized and processed for immunoprecipitation [21].

Proliferation and Protein Synthesis Assays

Cells were labeled with [^{3}H]-thymidine (84 Ci/mmol, New England Nuclear) in standard culture media or with [^{3}H]-leucine in leucine-free medium. Quadruplicate samples were labeled for each time point and each isotope. For radiolabeling, cells were removed from the original incubating dish then replated in a 96-well plate (2.5×10^4 cells in 0.2 ml/well) after which 1 μCi of [^{3}H]-thymidine or 2 μCi of [^{3}H]-leucine were added. After 12 h of labeling, 20 μl of 0.5 M EDTA was added (30 min, 37°C) followed by harvesting onto GF/C filters (Whatman) using a cell harvester (Skatron Inc., Sterling, VA). Filters were then counted in 10 ml of scintillation fluid and counts were normalized to the amount of cellular protein present. Protein concentrations were determined using the Bradford method [22].

HIV-1 Infections

SW620 cells cultured with or without a 12-day treatment with 3% DMSO were exposed to a cell-free preparation of HIV-1 (strain LAV_{BRU}) exactly as described [10]. Cells were then washed three times to remove excess virus inoculum. Culture medium was harvested on day 0 (after the washes) and on day 7 to measure HIV-1 p24 antigen levels. An enzyme-linked immunosorbent assay was used to measure p24 antigen levels (ELISA; Abbott Laboratories, North Chicago, IL).

RESULTS

Morphologic Changes in SW620 Cells Treated With DMSO

In preliminary experiments, we tested several DMSO concentrations (0.5%–5% in 0.25% increments) and incubation periods (4–16 days) to determine the conditions that result in the most dramatic morphologic change in SW620 cell shape without effecting cell viability. Cells grown in 3% DMSO for 12–16 days underwent the most striking changes in cell shape without exhibiting decreased cell viability (data not shown). As shown in Figure 1, DMSO concentrations greater than 3% resulted in significant cell death. There was also a progressive blunting in the increase in cell number as the DMSO concentration increased (Fig. 1).

DMSO treatment of SW620 cells resulted in an enlarged appearance, elongation, and formation of process-like structures as visualized by light microscopy (Fig. 2). Similar but less pronounced changes were described by Kim et al. [3] when SW620 cells were incubated in the

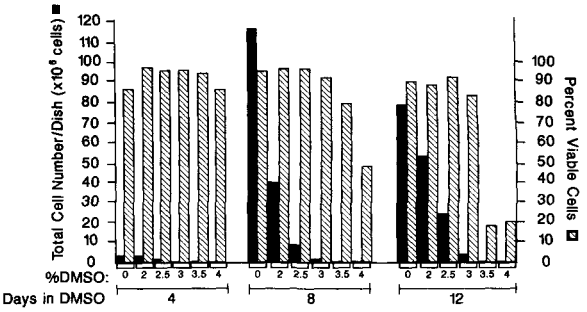

Fig. 1. Effect of several DMSO concentrations on SW620 cell number and viability. SW620 cells (5×10^5 cells/dish, 10 ml of medium/dish) were incubated with 0, 2%, 2.5%, 3%, 3.5% and 4% DMSO for 4, 8, or 12 days. On indicated days, cells were collected using 0.5% trysin, 1 mM EDTA in RPMI 1640 medium, then counted. Cell viability was determined by trypan blue exclusion.

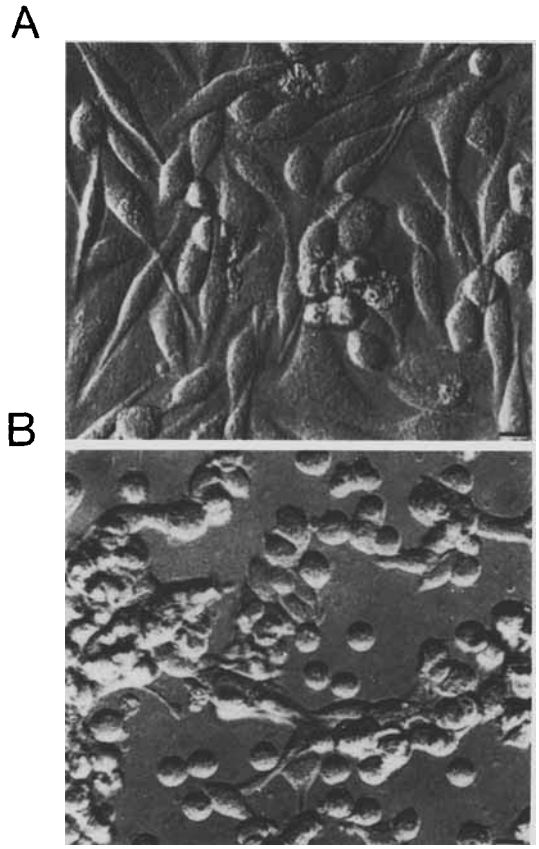

Fig. 2. Effect of DMSO on SW620 cells as visualized by light microscopy. SW620 cells were grown in the presence of 3% DMSO for 12 days (**A**) or without DMSO (**B**) as described in Methods. Bar indicates 20 μm. Magnification was 400×.

presence of 2% DMSO for eight days. Transmission electron microscopy (EM) revealed that DMSO-treated cells developed microvillus-like structures that appeared somewhat irregular and of varied length (Fig. 3). Whereas some cells were essentially covered with the microvillus-like structures, other cells showed only marginal formation of these structures. There was no evidence of tight junction formation, and DMSO-treated cells did not form a polarized monolayer when grown on Transwell filters (data not shown). The observed enlargement of cells seen by light microscopy may be due to cell flattening rather than an increase in cell volume. The flattened nature of the cells is seen in the EM vertical sections (Fig. 3, panels B and D) whereas EM sections of a cell suspension (Fig. 3, panels A and C) shows that the DMSO-treated cells have a similar diameter to the non-DMSO-treated cells.

DMSO Treatment of SW620 Cells Modulates the Expression of CD44, CD4, and KS-1 Cell Surface Markers

Kim et al. [3] reported that treatment of SW620 cells with DMSO resulted in changes in the overall profile of membrane glycoproteins, although individual glycoproteins were not investigated. The present study examined specific cell surface marker changes that accompany the above described morphological changes.

Using FACS analysis, we found marked changes in the expression of CD44, KS-1, and CD4 glycoproteins after SW620 cells were treated with DMSO (Fig. 4). CD44 levels increased from minimally detectable levels to 50% of cells staining above background. In contrast, KS-1 and CD4 levels decreased dramatically, with CD4 levels becoming barely detectable and KS-1 levels decreasing by more than 15-fold (Fig. 4). HLA class I antigen expression was essentially unchanged by DMSO treatment. Another differentiating agent, sodium butyrate (up to 2 mM), which was shown to induce differentiation in colonic tissue culture cell lines [3,4], had no effect on KS-1, CD4, and CD44 antigen expression in our cells (not shown).

Immunofluorescent binding studies were confirmed by immunoprecipitation of CD44, KS-1, and CD4 from ^{125}I-cell surface-labeled or [^{3}H]-leucine metabolically labeled untreated or

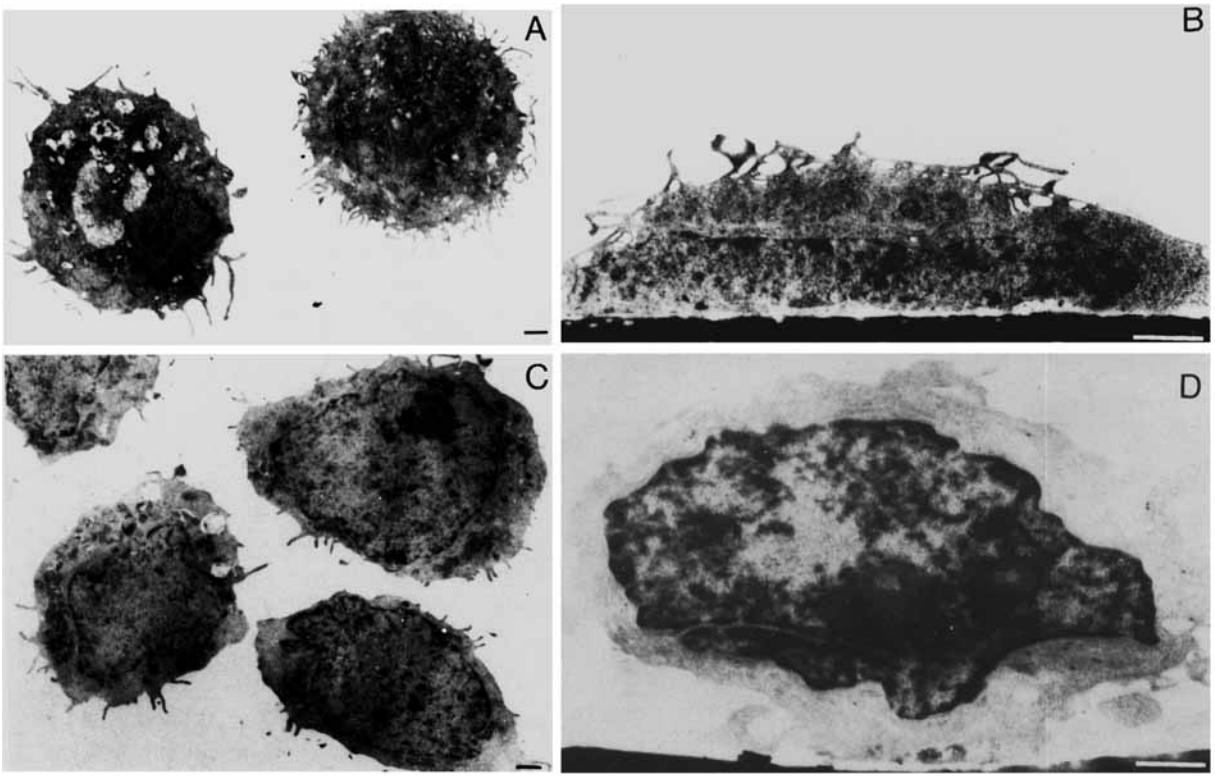

Fig. 3. Effect of DMSO on SW620 cells as visualized by transmission electron microscopy. SW620 cells were grown in the presence of 3% DMSO for 12 days (**A, B**) or without DMSO (**C, D**). Transmission EM was performed on a cross-section of the cell pellet (A, C) or on vertical sections obtained directly from cells on the tissue culture dish (B, D). Bars correspond to 1 μ.

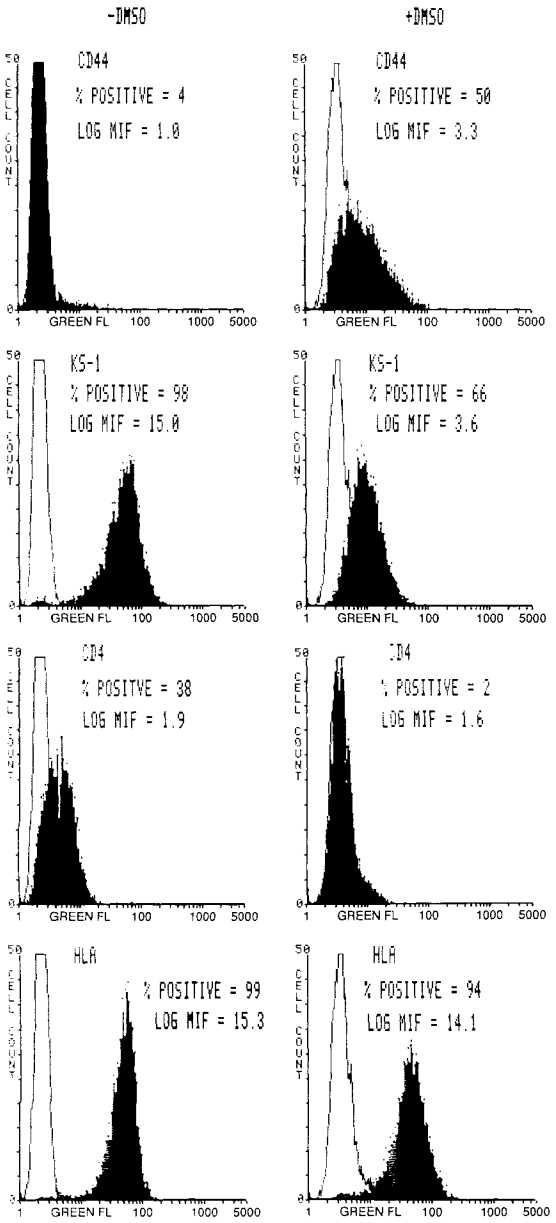

Fig. 4. Detection of CD44, KS-1, CD4, and HLA glycoproteins using FACS analysis. SW620 cells were incubated in the absence of DMSO (left column of histograms) or in the presence of 3% DMSO for 12 days (right column of histograms). Immunofluorescence was carried out as described in Methods. Background antibody staining is shown in clear tracings and specific antibody staining is shown in shaded tracings. Each tracing was generated by counting 50,000 cells. Histograms show percent positive cell staining above background and log of the mean intensity of fluorescence (MIF).

DMSO-treated cells (Fig. 5). As shown in Figure 5, DMSO treatment of SW620 cells resulted in the expression of the 90-kD CD44 glycoprotein with a concomitant decrease in the expression of CD4 (M_r 60 kD) and KS-1 glycoproteins. The glycoprotein KS-1 migrated as three bands with M_r 35–42 kD as reported by Perez and Walker [15].

We previously showed that SW620 cells could be infected with HIV-1 and that anti-CD4 antibody blocked this infection, suggesting that CD4 was important for HIV-1 infection in these cells [10]. The importance of CD4 was confirmed by our inability to infect SW620 cells that were treated with DMSO for 12 days. Hence, inoculation of SW620 cells with HIV-1 resulted in more than an eightfold increase above background in p24 antigen levels (303 pg/ml at day 0 compared with 2,452 pg/ml after seven days) as was shown previously [10], whereas DMSO-treated cells showed near background p24 levels (420 pg/ml after seven days).

We also examined the presence of several markers that characterize epithelial cell subtypes in DMSO-treated and untreated SW620 cells (Table I). All of the markers tested were absent except for cytokeratin polypeptides 8 and 18, which are markers for "simple" single layer epithelial cells such as the intestine [23]. Cytokeratins 8 and 18 were present in amounts that allowed easy visualization by Coomassie staining (not shown).

Time Course of the DMSO-Induced Change in Antigen Expression, Protein and DNA Synthesis in SW620 Cells

The expression level of KS-1, CD44, and CD4 was examined in SW620 cells after cells were cultured in the presence of DMSO for 0, 4, 8, 12, and 16 days. As shown in Figure 6, CD4 levels decreased to near baseline by day 4, whereas KS-1 levels decreased more gradually. In contrast, CD44 levels increased over time as cells were grown in the presence of DMSO. The effect of DMSO on cell growth was such that after 16 days, minimal [^{3}H]-thymidine incorporation was noted (Fig. 6, inset) and this was paralleled by an arrest of cell growth as determined by cell counts (not shown; however, see Fig. 1). In contrast, protein synthesis levels decreased to 50% of their starting rate after 12 days in the presence of DMSO as assessed by the incorporation of [^{3}H]-leucine into protein (Fig. 6, inset).

Expression Levels of KS-1, CD4, and CD44 Glycoproteins After Removal of DMSO From Culture

We tested whether the effect of DMSO on KS-1, CD4, and CD44 was reversible upon re-

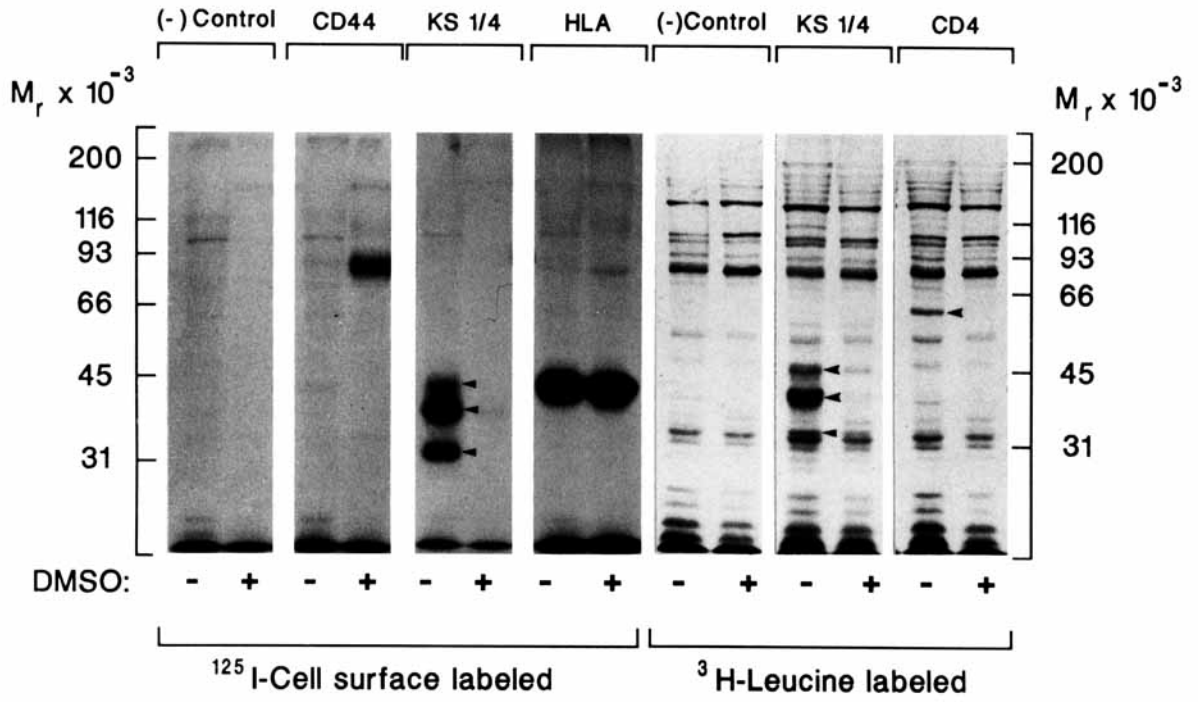

Fig. 5. Detection of CD44, KS-1, CD4, and HLA glycoproteins using immunoprecipitation. SW620 cells (−, untreated; +, DMSO-treated) were labeled on the cell surface with ^{125}I using lactoperoxidase catalyzed iodination or labeled metabolically with [^{3}H]-leucine as described in Methods. Glycoproteins were immunoprecipitated using their corresponding antibodies followed by SDS-PAGE (8% gel) and autoradiography.

TABLE I. Marker Profile of Untreated or DMSO-Treated SW620 Cells

Marker	Percent of cells positive[a] −DMSO	+DMSO
Alkaline phosphatase[b]	none	none
Sucrose isomaltase[c]	1	2
Secretory component[c]	2	2
Cytokeratins 8/18[d]	98	99
Chromogranin A	1	2
Mucin[e]	none	none
Carcinoembryonic antigen[c]	1	1

[a]Percent of cells positive above background as determined by flow cytometry in a single representative experiment. Similar results were obtained in at least three repeat experiments.
[b]Determined using a colorimetric assay.
[c]Also confirmed by immunoprecipitation of radiolabeled cells.
[d]Also confirmed by immunoprecipitation from unlabeled cells followed by SDS-PAGE and Coomassie blue staining.
[e]Determined using mucicarmine staining.

moval of DMSO. As shown in Figure 7, KS-1 showed rapid recovery to levels seen in untreated cells. In contrast, CD4 and CD44 showed a gradual return to baseline levels with the return of CD44 levels to baseline requiring 6–8 weeks (not shown). Similarly, SW620 cells that were thawed after liquid nitrogen freezing in the presence of 5% DMSO also required 6–8 weeks of culture in the absence of DMSO for the complete disappearance of CD44 (not shown).

DISCUSSION

The primary observation in our study is that DMSO induces significant changes in the expression levels of CD4, CD44, and KS-1 glycoproteins in the colonic epithelial cell line SW620. The observed changes should allow the use of this cell line to carry out functional studies related to these molecules as well as studies related to their regulation.

Most DMSO effects on tissue cultured cells reported to date have shown differentiation and loss of tumorigenicity of these cells [8,24]. However, DMSO has also been shown to induce a highly metastatic phenotype in several epithelial cell lines [25,26]. The type of differentiation induced by DMSO in SW620 cells appears to be incomplete. Although the formation of villus-like structures suggests progression along an enterocyte pathway, we did not detect CEA, secretory component (well differentiated enterocyte markers); mucin (goblet cell marker); and

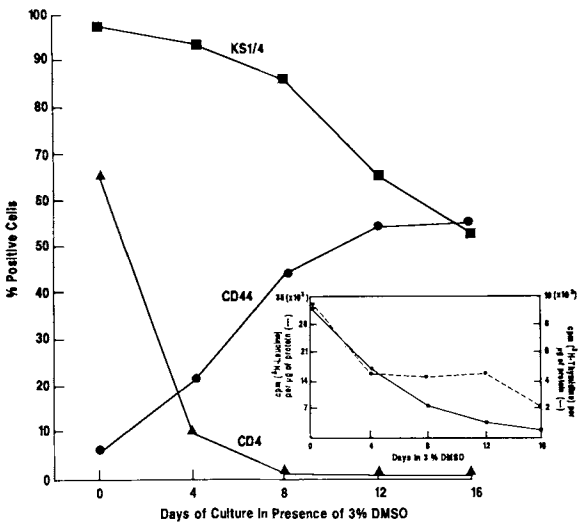

Fig. 6. Effect of DMSO on protein and DNA synthesis and on the kinetics of expression of KS-1, CD44, and CD4 glycoproteins in SW620 cells. SW620 cells were grown in medium containing 3% DMSO for the indicated days after which they were analyzed for [^{3}H]-leucine and [^{3}H]-thymidine incorporation (inset) or for KS-1, CD44, and CD4 expression using FACS analysis. The percent of cells staining above background is shown for the indicated antigens. Labeling with [^{3}H]-leucine or [^{3}H]-thymidine was carried out as described in Methods. Findings were reproducible in two additional experiments.

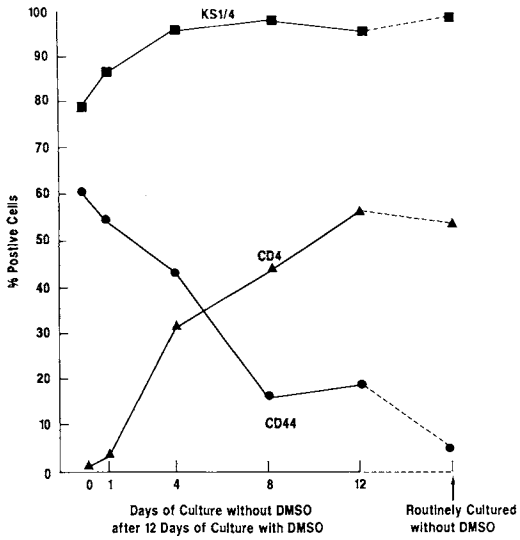

Fig. 7. Reappearance of KS-1, CD4, and CD44 after removal of DMSO. SW620 cells were first cultured in the presence of 3% DMSO for 12 days then switched to media without DMSO (day 0). On days 0, 1, 4, 8, and 12 after switching to normal media, cells were analyzed for the expression of KS-1, CD4, and CD44 using FACS analysis.

chromogranin or neuron-specific enolase (neuroendocrine markers). However, cloning of SW620 cells did result in a clone with a similar phenotype to the parental cells upon DMSO stimulation. This clone expressed secretory component after DMSO treatment (data not shown).

The presence of CD4 in SW620 cells has been described previously and shown to be involved in HIV infection of these cells [10]. CD4 was not detectable by immunoprecipitation and FACS analysis after treatment of SW620 cells with DMSO. Interestingly, we find that DMSO-treated SW620 cells are no longer infectable with HIV. Therefore, SW620 cells, with and without DMSO treatment, can provide a useful model for studying HIV infection of colonic epithelial cells.

DMSO treatment of SW620 cells did not completely abolish KS-1 expression, although levels of KS-1 were decreased by more than 15-fold. Although the amino acid and nucleotide sequence of the coding region of KS-1 shows no direct homology to any protein of known sequence, it does contain multiple cysteine residues which are usually conserved and implicated in functions including DNA-binding domains and receptor sites [15]. The marked change in the expression of KS-1 after DMSO treatment should provide a useful model for studying potential functions for KS-1.

In marked contrast to KS-1, CD44 levels were strikingly increased in DMSO-treated cells. CD44, also called Pgp-1, extracellular matrix receptor III, or Hermes antigen, is the hyaluronate receptor [11]. SW620 cells lacked any detectable CD44; however, treatment with DMSO resulted in clearly detectable levels of this glycoprotein. Although the functional significance for the expression of CD44 in DMSO-treated cells remains to be determined, several possibilities can be considered. For example, expression of CD44 can allow for better attachment of the cells to the substratum which may account for the observed flattening of the cells.

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