Glycoconjugate J (1988) 5: 107-118

The Expression of Proteoglycans in Phorbol Ester Induced U-937 Cells

SVEIN O KOLSET[1]*, IRENE IVHED[2], AUD ØVERVATN[1] and KENNETH NILSSON[2]

[1]*Department of Tumor Biology, Norwegian Cancer Society, Institute of Medical Biology, University of Tromsø, N-9000 Tromsø, Norway*
[2]*Institute of Pathology, University of Uppsala, S-751 85 Uppsala, Sweden*

Received October 10/December 6, 1987.

Key words: differentiation, proteoglycans, monocytes, phorbol esters

U-937 monoblastic cells were differentiated into macrophage-like cells in the presence of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Control cells and differentiated cells were labeled with ^{35}S-sulfate and were both found to produce exclusively chondroitin sulfate proteoglycan. No differences in glycosaminoglycan structure or macromolecular properties of the proteoglycans produced in the two different cell systems could be observed. However, the differentiated cells were found to have a lower capacity for chondroitin sulfate proteoglycan synthesis, both under ordinary experimental conditions, and when exposed to stimulators of glycosaminoglycan biosynthesis such as β-D-xylosides.

Proteoglycans (PG) are highly acidic macromolecules with glycosaminoglycan (GAG) chains covalently attached to a protein core. They have been shown to have structural and organizational functions in tissues [1], and may also be involved in phenomena such as regulation of cell growth and tumor cell killing [2]. The structure of proteoglycans, and the GAG chains in particular, have been studied in cultured cells under various experimental conditions and in different tissues under pathological conditions. Accordingly, the molecular size of GAG chains associated with cartilage proteoglycan was found to decrease with age both *in vivo* [3] and under *in vitro* conditions [4]. Moreover, transformation of cells has been demonstrated to lead to a decrease in the sulfation of GAG [5], a phenomenon also observed in hepatocyte [6] and glomeruli-associated [7] proteoglycans in diabetic rats. Changes in GAG structure have also been demonstrated in conjunction with differentiation of mast cells [8] and monocytes [9]. In the latter case monocytes were found to synthesize chondroitin 4-sulfate, whereas *in*

Abbreviations: SDS, sodium dodecyl sulfate; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PG, proteoglycan; GAG glycoaminoglycan; CS, chondroitin sulfate; CSPG, chondroitin sulfate proteoglycan; NASDAE, naphthol AS-D acetate esterase.

*Author for correspondence.

vitro macrophages synthesized a mixture of chondroitin 4-sulfate and chondroitin 4,6-disulfate [9, 10]. Recently it has also been demonstrated that mature human macrophages obtained from the peritoneal cavity express oversulfated chondroitin sulfate proteoglycan (CSPG) after 24 h incubation *in vitro*, identical in structure to the proteoglycan isolated from *in vitro* macrophages [9, 11]. Changes in the structure of the CSPGs produced by cells in the monocyte-macrophage lineage may therefore be regarded as a parameter of maturation of these cells.

U-937 is a monoblastic cell line [12] which can be induced to differentiate into monocytes (or macrophage-like cells) when exposed to agents such as phorbol esters [13], retinoic acid [14] or 1α,25-dihydroxycholecalciferol [15]. Untreated U-937 cells have been shown to synthesize and secrete CSPG identical in GAG structure to that synthesized by isolated human monocytes *in vitro* [16]. In this particular study it could also be demonstrated that the CSPG produced by U-937 cells was much larger in molecular size than the monocyte species, due to the substitution of larger GAG chains.

This study was initiated in order to investigate further the relationship between differentiation and changes in GAG structure in cells in the monocyte-macrophage lineage. The monoblastic U-937 cells may be regarded as neoplastic monocytes. They are not adherent under *in vitro* conditions, but upon exposure to phorbol esters cell division is completely inhibited and the cells become adherent and acquire many characteristics attributed macrophages. In the present paper the PG and GAG structure have been studied in the U-937 cell line prior to and after differentiation caused by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and the results indicate that the cellular transition induced by this potent agent does not change the PG structure, but rather leads to a decrease in the capacity for PG synthesis.

Materials and Methods

Materials

DNP-alanine, 12-*O*-tetradecanoylphorbol-13-acetate, bacterial chondroitinase AC (E.C. 4.2.2.5), chondroitin sulfate, dextran blue and *p*-nitrophenyl-β-D-xyloside were purchased from Sigma Chemical Co., St. Louis, MO, USA. DEAE-Sephacel, Sephadex G-25, Sephadex G-50, Sepharose CL-2B and Sepharose CL-6B were from Pharmacia Fine Chemicals, Uppsala, Sweden. Heparin from pig intestinal mucosa was a kind gift from Dr. U. Lindahl, Swedish University of Agricultural Sciences, Uppsala, Sweden. Lymphoprep was obtained from Nyegaard & Co., Oslo, Norway. SDS was bought from BDH Chemicals Ltd., Poole, UK and inorganic ^{35}S-sulfate (carrier-free) was from Institutt for Energiteknikk, Kjeller, Norway. ^{3}H-Thymidine was obtained from the Radiochemical Centre, Amersham, UK.

Cells

The U-937 GTB line [12] was maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal calf-serum, 50 μg/ml streptomycin and 100 IU/ml of penicillin (all from Gibco Laboratories, Grand Island Biological Co., Glasgow, Scotland). Differentiation of U-937 cells into macrophage-like cells was accomplished by treating the cells with 1.6×10^{-7}M TPA for 72 h as has previously been described [13]. TPA was dissolved in pure

ethanol. The final concentration in the medium never exceeded 0.1% and had in itself no effect on the differentiation observed in the presence of TPA (data not shown).

Biosynthetic Labeling

Both control and TPA-treated U-937 cells and monocytes were exposed to ^{35}S-sulfate (50 μCi/ml) for 20 h. All cell types were labeled both in the absence and presence of 0.01 and 1 mM *p*-nitrophenyl-β-D-xyloside. After completed labeling cell suspension were harvested and centrifuged at 150 × *g* for 5 min. The media were separated from the pelleted cells and SDS in phosphate-buffered saline (PBS; 0.14 M NaCl, 2 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) was added to each fraction to give a final concentration of 1%. The fractions were boiled for 3 min and frozen.

Proteoglycan Structure

Medium and cell fractions from both control and TPA-induced U-937 cells labeled with ^{35}S-sulfate were subjected to proteolytic digestion and gel chromatography [9] and ^{35}S-labeled macromolecules were subjected to chondroitinase AC digestion as described [9]; 0.5 mg chondroitin sulfate was added to each sample prior to digestion. The products were analyzed by gel chromatography on a Sephadex G-50 column (0.5 × 90 cm) run in 1 M NaCl. Fractions of 1.0 ml were analyzed for content of radioactivity and carbazole-positive material [17]. Heparin and heparan sulfate-related ^{35}S-GAG were depolymerized with HNO_2, pH 1.5 [18], after 0.5 mg heparin had been included in each sample. The reaction products were analyzed by gel chromatography on a Sephadex G-25 column (0.8 × 190 cm) run in 1 M NaCl. Fractions (1.3 ml) were collected and the content of radioactivity and hexuronic acid was determined in each fraction.

In order to separate ^{35}S-labeled macromolecules from free ^{35}S-sulfate, SDS-treated medium and cell samples were subjected to chromatography on Sephadex G-50 columns (0.8 × 25 cm), run in PBS with 0.1% SDS. Markers for void (V_o) and total (V_t) volumes were dextran blue and DNP-alanine, respectively. For determination of ^{35}S-incorporation into macromolecules the V_o-material was collected and counted in a Packard tri-Carb scintillation counter.

The approximate molecular size of medium and cell ^{35}S-GAG chains was determined by Sepharose CL-6B gel chromatography after alkali treatment of the respective fractions [25]. Each sample was treated with NaOH to give a final concentration of 0.5 M, incubated at room temperature for 15 h and thereafter neutralized and subjected to gel chromatography. SDS-treated samples were run in 0.05 M Tris-HCl, pH 8.0 with 0.1% SDS and 0.15 M NaCl. Markers for V_o and V_t were as for the Sephadex G-50 gel chromatography (see above). Fractions of 1.2 ml were collected and analyzed for content of radioactivity. The macromolecular properties of medium and cellular ^{35}S-proteoglycans were analyzed by Sepharose CL-2B gel chromatography (after Sephadex G-50 chromatography as described above). Elution conditions were as for the Sepharose CL-6B gel chromatography.

Cell Counting and Morphology

The incorporation of ^{35}S-sulfate into macromolecules was related to the number of cells. The U-937 cells treated with TPA were removed from the culture plastic with flushing of the medium. Both control and TPA-treated cells were thereafter counted by

Table 1. The effect of TPA on the proliferation and differentiation of U-937 cells.

Cells	Proliferation			Cytochemistry
	Cells/ml[a]	Viability (%)	cpm[b]	NASDAE/+NaF
Control	1.2×10^6	92 ± 4	4479 ± 200	+/+
TPA-treated	0.4×10^6	72 ± 8	334 ± 2	++/(+)

[a] Cells were seeded at 0.5×10^6 cells/ml and counted after three days.
[b] Cell-associated ^{3}H-thymidine after 1 h incubation; after three days incubation in parallel cultures to [a] above.
[c] Naphthol AS-D acetate esterase activity: (+) weak positive reaction; + positive reaction; ++ strong positive reaction. Only the monocyte-specific esterase is inhibited by NaF.

use of Bürker chambers. Viability was determined by trypan blue exclusion. Morphology was determined on Giemsa stained cells after centrifugation on a Shandon cytospin 2 centrifuge (Shandon Southern Products Ltd., Cheshire, UK). Cytospin slides were also stained for activity of naphthol AS-D acetate esterase (NASDAE) with sodium fluoride inhibition (NaF).

Cell Proliferation Assay

Control and TPA-induced cells were incubated with 0.1 μCi ^{3}H-thymidine/ml (specific activity 2 Ci/mmole) under standard incubation conditions for 1 h. The cells were then harvested on Millipore filters (0.45 μm), washed twice with PBS, and treated with 10% trichloroacetic acid and ethanol. The filters were immersed in 5 ml of scintillator and counted in a beta-counter.

Results

Differentiation of U-937 Cells in the Presence of TPA

U-937 cells were cultured in the absence and presence of 1.6×10^{-7} M TPA for three days. Cell proliferation in the two different cell systems was quantitated by measuring the cellular incorporation of ^{3}H-thymidine during a 60 min pulse. As can be seen in Table 1 the incorporation is approximately 13 times higher in untreated cells compared to the TPA-treated counterparts. Furthermore, by cell counting it was possible to establish that TPA-treatment resulted in an almost complete abrogation of cell proliferation, whereas control cells increased from 0.5×10^{-6} cells to 1.2×10^{-6} cells/ml during a three day incubation period (Table 1). The arrest of cell proliferation was furthermore found to correlate to adherence to the plastic culture dish, whereas untreated cells remained in suspension. By cytochemical criteria it was furthermore possible to establish that U-937 cells treated with TPA were more macrophage-like than the corresponding controls (Table 1). In addition, the TPA-treated cells were found to acquire macrophage-like morphology, such as increased cell size, increased ratio cytoplasm/nucleus, increased number of vacuoles, adherence and membrane surface protrusions. By the

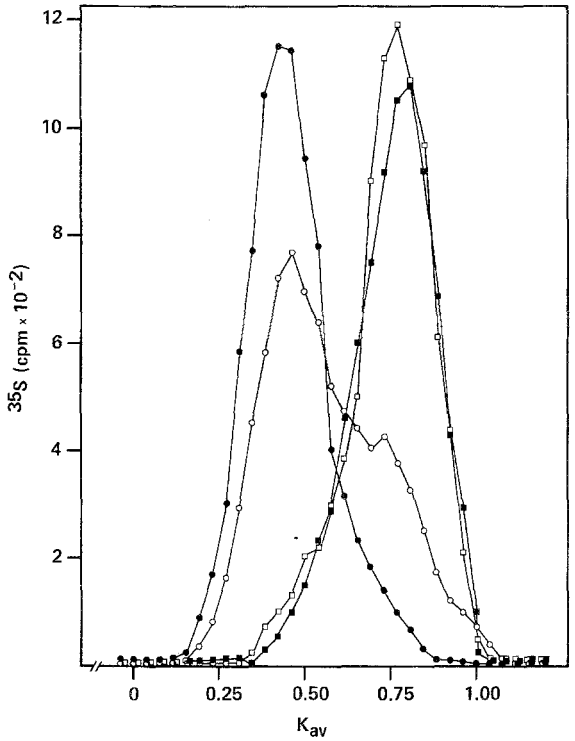

Figure 1. Sepharose CL-2B gel chromatography of ^{35}S-macromolecules from U-937 control and TPA-induced cultures.

SDS-solubilized medium and cell fractions from ^{35}S-labeled cultures were chromatographed on a Sepharose CL-2B column (1 × 90 cm). Control cell (□) and medium fraction (○), and cell (■) and medium fraction (●) from TPA-induced cells.

criteria presented it may be concluded that TPA-treated U-937 cells were more highly differentiated and more macrophage-like than cells kept in culture for three days in the absence of this agent. The studies presented here on proteoglycan biosynthesis were performed on cells exposed to TPA for three days and untreated cells cultured for three days prior to the onset of ^{35}S-labeling.

Proteoglycan Structure and TPA-induced Differentiation

SDS-solubilized medium and cell fractions from both control and TPA-induced cells were analyzed by Sepharose CL-2B gel chromatography. There was no significant difference in elution patterns for the ^{35}S-macromolecules from the respective medium fractions, both displaying a peak K_{av}-value of approximately 0.46 (Fig. 1). The control medium-associated macromolecules were found to contain some minor portion of free GAG chains, as shown by the material eluting as a small peak at K_{av} 0.75. The cell fractions from both control and TPA-induced cells, in contrast, were both found to be much smaller in size (approximate K_{av}-values of 0.77). By Sepharose CL-6B gel chromatography

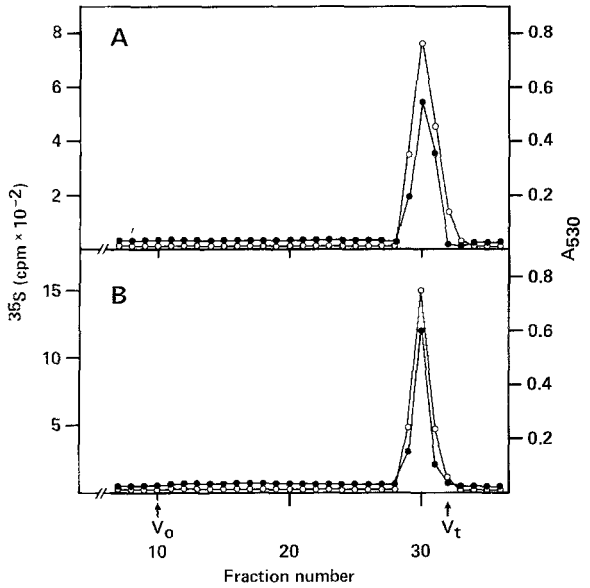

Figure 2. Sephadex G-50 gel chromatography of ^{35}S-glycosaminoglycans from U-937 control and TPA-induced cells.

^{35}S-GAG from media of control and TPA-treated cultures were subjected to chondroitinase AC-digestions followed by Sephadex G-50 gel chromatography. Markers for void (V_o) and total volume (V_t) of the gels were dextran blue and DNP-alanine. Panel A: control medium and panel B: medium from TPA-treated cells. ○, ^{35}S-radioactivity; ●, hexuronic acid.

of both medium and cell fractions prior to and after alkali treatment (resulting in the liberation of free GAG chains), it was possible to establish that only the medium fractions contained ^{35}S-labeled proteoglycans (result not shown), as has also previously been shown for the U-937 control species [16]. The cell fractions from both control and TPA-induced cells contained only free ^{35}S-GAG-chains. An approximate molecular size of the ^{35}S-proteoglycans from both U-937 cell types can be calculated on the basis of previously published data [19, 20] and the presented Sepharose CL-2B gel chromatography (Fig. 1), and Sepharose CL-4B elution profiles (K_{av} of 0.16 for both medium fractions, result not shown) to be approximately 0.75×10^6 - 1.0×10^6. The molecular size of the GAG chains (see below) may have a profound influence on the gel filtration properties, and due to differences in the M_r of the GAG chains in the various PG standards referred to and the U-937 CSPGs, this molecular size can only be regarded as approximate.

The free ^{35}S-GAG chains obtained by alkali-treatment of medium CSPGs were found to elute with almost identical elution patterns after Sepharose CL-6B; peak K_{av}-values of 0.3, corresponding to an average molecular size of 60 000 ([6], see also Fig. 5B for the approximate molecular size of free GAG chains in the cell fraction of control U-937 cells). The differentiation of U-937 monoblasts into macrophage-like cells did not, accordingly, affect the macromolecular properties of the CSPGs produced by the two different cell species.

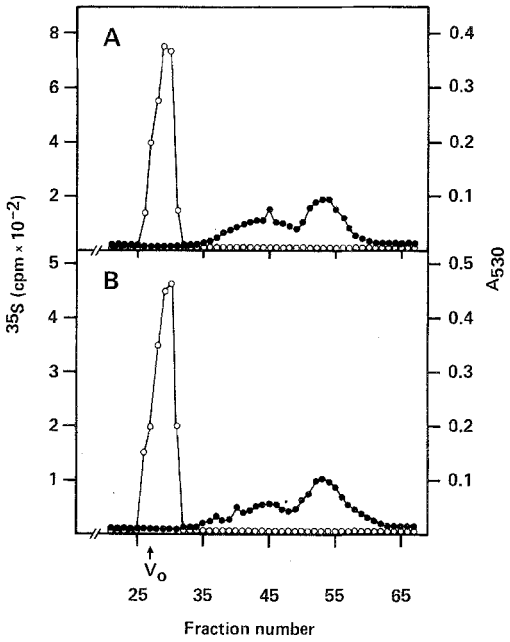

Figure 3. Sephadex G-25 gel chromatography of ^{35}S-glycosaminoglycans from control and TPA-treated U-937 cells.

^{35}S-GAG from media of control and TPA-treated cultures were chromatographed on a Sephadex G-25 column following HNO_2 at pH 1.5. Blue dextran was used as marker for the void volume of the gel (V_o). Panel A: control ^{35}S-GAG and panel B: ^{35}S-GAG from TPA-treated cells. ○, ^{35}S-radioactivity; ●, hexuronic acid.

^{35}S-Labeled macromolecules obtained by proteolytic digestions of both medium and cell fractions were subjected to chondroitinase AC-digestions and gel chromatography. As is evident from Fig. 2 ^{35}S-labeled macromolecules from both media of control and TPA-induced cells were completely depolymerized to disaccharides, as were also the internal chondroitin sulfate standards (Fig. 2A and B, respectively). Identical results were obtained with the respective cell fractions (not shown). The chondroitin sulfate nature of the ^{35}S-labeled macromolecules synthesized by both control and TPA-induced U-937 cells was further corroborated by demonstrating the complete resistance by both medium and cell fractions to HNO_2 degradation (shown only for the medium fractions in Fig. 3A and B). In contrast, the internal standard heparin is depolymerized to oligo-, tetra- and disaccharides as can be seen after Sephadex G-25 gel chromatography (Fig. 3A and B). Hence, both the undifferentiated U-937 cells and their TPA-induced coutnerparts synthesize and release exclusively CSPG.

The possibility that the induced differentiation of this particular cell line might affect the polyanionic properties of the CSPG produced, as has been demonstrated for primary cultures of normal monocytes after differentiation into macrophages *in vitro* [9, 10], was investigated by subjecting medium ^{35}S-GAG fractions to DEAE ion exchange chromatography. No differences in elution patterns between ^{35}S-GAG derived from

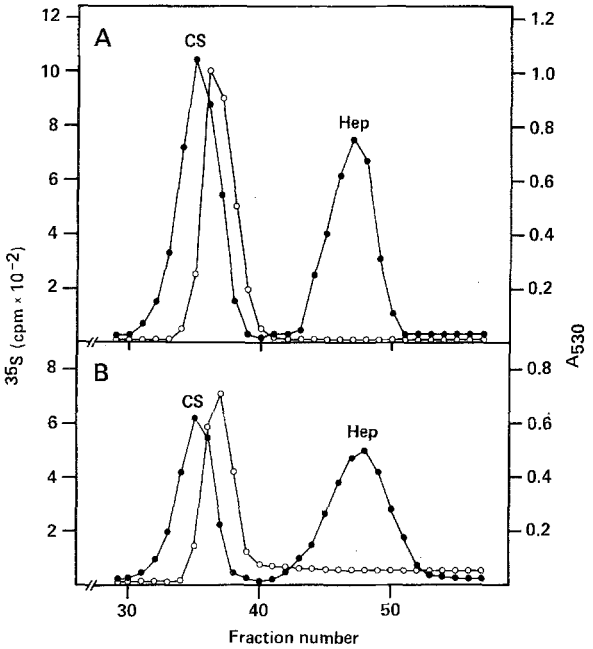

Figure 4. DEAE-Sephacel ion exchange chromatography of ^{35}S-GAG from control and TPA-treated U-937 cultures.

^{35}S-GAG chains from control (panel A) and TPA-treated cultures (panel B) were chromatographed on DEAE-Sephacel columns (1 x 6 cm) together with 1 mg chondroitin sulfate (CS) and 2 mg heparin (Hep). The column was washed with 0.05 M sodium acetate buffer pH 4.0 with 0.05 M LiCl and eluted with a gradient extending from 0.05 M to 1.5 M LiCl in the same buffer as above. Fractions were collected and analyzed for; ○, ^{35}S-radioactivity; ●, hexuronic acid.

control and TPA-induced cell cultures could be observed (Fig. 4A and B), when compared to the elution positions of the chondroitin sulfate and heparin internal standards. The elution profiles for GAG following DEAE ion exchange chromatography have been demonstrated to be molecular size dependent [21]. However, the TPA-induced differentiation did not affect the macromolecular properties of the CSPG synthesized (see Fig. 1 and text above). It may therefore be concluded that TPA-induced differentiation did not affect the polyanionic properties of the CSPG produced.

TPA-Induced Differentiation and the Capacity for PG/GAG Synthesis

Both control and TPA-treated cells incorporate ^{35}S-sulfate exclusively into CSPG. Measurement of ^{35}S-sulfate associated with macromolecules was therefore used to compare the capacity for PG synthesis in the two different cell systems. Furthermore, the incorporation of ^{35}S-sulfate was quantified in the absence and presence of *p*-nitrophenyl-β-D-xyloside. Addition of exogenous β-D-xylosides to cell cultures leads to a competition between xylosylated core protein and the artificial initiator of GAG chain synthesis [22-24]. With increasing concentrations of β-D-xyloside, the GAG synthesis is

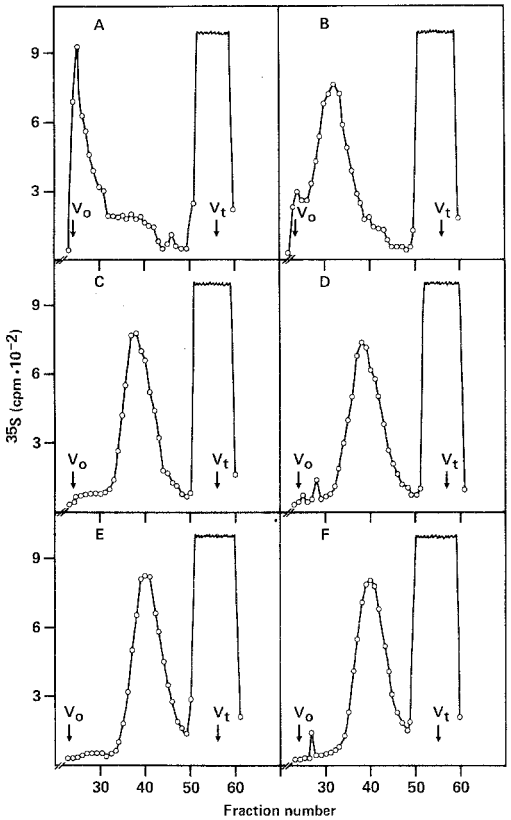

Figure 5. Sepharose CL-6B gel chromatography of U-937 medium and cell fractions.

Medium (A, C, E) and cell (B, D, F) fractions from U-937 cells exposed to ^{35}S-sulfate in the absence (A, B) and presence of 0.01 mM (C, D) and 1 mM (E, F) β-D-xyloside. These fractions were not chromatographed on Sephadex G-50 columns and the large peak in V_t-position in each panel represent unincorporated free ^{35}S-sulfate.

progressively shifted from intact proteoglycans to xylosylated GAG chains, with a concomitant stimulation of GAG-synthesis, as has been shown in cultured human monocytes [25]. When U-937 control cells were exposed to 0.01 and 1.0 mM β-D-xyloside the release of ^{35}S-CSPG (Fig. 5A) was substituted for the release of ^{35}S-macromolecules with more retarded elution patterns as shown in Fig. 5C (0.01 mM xyloside) and 5E (1.0 mM xyloside). Alkali treatment of the two latter medium fractions did not alter the elution pattern displayed in Fig. 5C and E and consequently these two distinct fractions contain only free ^{35}S-GAG chains. The cell fraction from untreated cells was found to contain mostly free GAG chains (Fig. 5B) decreasing in size with increasing xyloside concentrations (0.01 mM xyloside, Fig. 5D; 1.0 mM xyloside, Fig. 5F). The elution profiles for medium and cell fractions from TPA-treated cells were found to be almost identical to those displayed in Fig. 5A-F, and are therefore not shown. At the highest β-D-xyloside concentration medium and cell fractions from both control and TPA-induced cells were found to elute with a peak K_{av}-value of 0.52, corresponding to an approximate molecular

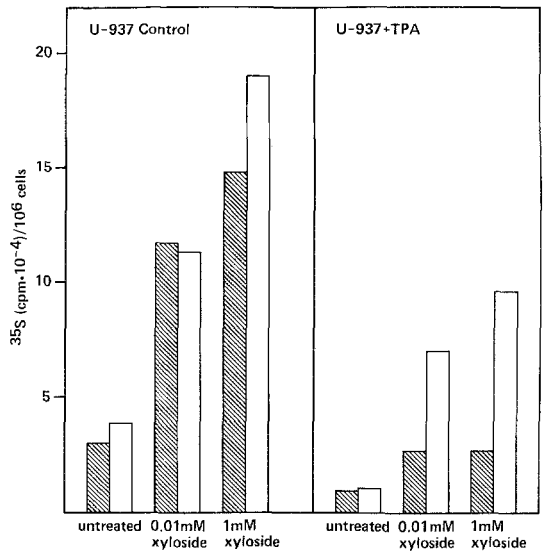

Figure 6. Incorporation of ^{35}S-sulfate into macromolecules in cell and medium fractions in control and TPA-treated U-937 cells.

U-937 control and TPA-treated cells were labeled with ^{35}S-sulfate in the absence and presence of β-D-xyloside. ^{35}S-Macromolecules were isolated by gel chromatography from cell (hatched bars) and medium (open bars) fractions.

size of 18 000 [25], versus 60 000 in the intact medium CSPG (see above), and the cell GAG fraction (Fig. 5B, approximate K_{av} of 0.26 which would indicate a molecular size around 60 000 [25]. When, however, the incorporation of ^{35}S-sulfate into PG/GAG in both cell culture systems was compared it was evident that untreated U-937 control cells synthesized approximately 3.5 times more (cell and medium fractions total) than the TPA-induced cells, as can be seen in Fig. 6. At the highest rate of synthesis, in the presence of 1.0 mM β-D-xyloside, the control cells incorporated 2.7 times more than the TPA-treated cells. The increase in ^{35}S-incorporation in the former cell type was five-fold following xyloside-treatment, whereas the latter displayed a six-fold increment. Thus, TPA-incuded U-937 cells exhibit a lower capacity for PG/GAG synthesis both under normal and experimentally elevated conditions.

Discussion

Changes in GAG synthesis have been related to differentiation and cell division in different cell systems. Accordingly, the formation of macrophage-like cells from TPA-induced HL-60 cells led to a 30-40% reduction in GAG synthesis [26]. Furthermore, HL-60 cells induced to differentiate along the myeloid lineage in the presence of retinoic acid were found to decrease the expression of GAG approximately 50% after maturation [27]. The level of GAG synthesis has also been related to different phases of the cell cycle in chinese hamster ovary cells. GAG synthesis was found to be depressed four-fold during mitosis and stimulated between two- and three-fold in early G1 compared to interphase

cells [28]. In the present study we were not able to demonstrate any differences in the structure of the proteoglycans synthesized by control and TPA-induced U-937 cells. In both cell systems only chondroitin sulfate could be detected by the methods employed. Furthermore, no differences could be observed in the macromolecular properties of the CSPG produced prior to or after differentiation. Finally, no increase in sulfate density of the GAG chains could be demonstrated after TPA-induced differentiation, in contrast to what has been reported for monocytes during differentiation *in vitro* into macrophages [9, 10]. By cytochemical and morphological criteria the TPA-induced cells were found to be more macrophage-like than the corresponding untreated control cells (Table 1). Conceivably, changes in proteoglycan structure in cells in the monocytoid lineage may require normal primary cultures in order to be evident *in vitro*. Although the histocytic lymphoma cell line U-937 was induced to differentiate in the presence of TPA into macrophage-like cells, this may not be sufficient for the distinct changes in PG structure to occur, as has previously been observed in normal monocytes differentiating into macrophages *in vitro* [9, 11]. The possibility also exists that the changes previously observed in monocyte cultures during prolonged cultivation *in vitro* may be related to stimulation of the cells rather than to (*in vitro*) differentiation, as has also been suggested [10].

In the present study we were able to show that TPA-differentiated U-937 cells synthesize PG/GAG at a lower level than untreated control cells, both in the absence and presence of exogenous stimulators. By the use of xylosides in the study of PG/GAG biosynthesis it is possible to circumvent the possible regulation(s) imposed by the protein part of the macromolecule on the rate of synthesis [22-24]. The lower level of GAG synthesis in the presence of β-D-xyloside in TPA-induced cells indicates that differentiation is related to inhibitory effects, possibly on rate-limiting enzymes, in the GAG synthesis machinery. Accordingly, the results presented here suggest that the expression of CS and CSPG decrease following differentiation of cells in the monocytoid lineage, both at normal and stimulated levels of synthesis. Recently, a similar phenomenon has been reported in HL-60 cells differentiated *in vitro* with TPA towards macrophage-like cells [29].

The functional implications of these findings are at present difficult to define. CSPG is a secretory product both from U-937 cells and monocytes [10, 16] and may possibly be involved in the modulation of the extracellular environment of monocytes during inflammatory reactions in the tissues either by interactions with other extracellular components, or as a carrier of molecules destined for release. The lower level of CSPG/CS synthesis in the differentiated cells may at present only be regarded as a phenotypic marker. Further studies with various U-937 clones now available may provide further insight into the implications of PGs, both in neoplastic cells in general, and in relation to the maturation of cells in the monocytoid lineage.

Acknowledgements

This work was supported by grants from the Norwegian Cancer Society and the Swedish Cancer Society.

References

1 Hascall VC, Hascall GK (1981) in Cell Biology of the Extracellular Matrix, Plenum Press, New York, p. 39-63.
2 Hascall VC (1986) Ciba Foundation Symposium 124, John Wiley & Sons, New York.
3 Roughley PJ, White RJ (1980) J Biol Chem 255:217-24.
4 Pacifici M, Fellini SA, Holtzer H, DeLuca S (1981) J Biol Chem 256.1029-37.
5 Underhill CB, Keller JM (1975) Biochem Biophys Res Commun 63:448-54.
6 Kjellén L, Bielefeld D, Höök M (1983) Diabetes 32:337-42.
7 Kanwar YS, Rosenzweig LJ, Linker A, Jakubowski ML (1983) Proc Natl Acad Sci USA 80:2272-75.
8 Levi-Schaffer F, Austen KF, Gravallese PM, Stevens RL (1986) Proc Natl Acad Sci USA 83:6485-88.
9 Kolset SO, Kjellén L, Seljelid R, Lindahl U (1983) Biochem J 210:661-67.
10 Kolset SO, Seljelid R, Lindahl U (1984) Biochem J 219:793-99.
11 Kolset SO (1986) Biochem Biophys Res Commun 139:377-82.
12 Sundström C, Nilsson K (1976) Int J Cancer 17:565-77.
13 Nilsson K, Andersson LC, Gahmberg CG, Forsbeck K (1980) Int Symp Trends Human Immunol Cancer Immunother, Doin, Paris, p. 271-82.
14 Olsson I, Breitman TR (1982) Cancer Res 42:3924-27.
15 Olsson I, Gullberg U, Ivhed I, Nilsson K (1983) Cancer Res 43:5862-67.
16 Kolset SO (1987) Exp Cell Res 168:318-24.
17 Bitter T, Muir HM (1962) Anal Biochem 4:330-34.
18 Shively JE, Conrad HE (1976) Biochemistry 15:3932-42.
19 Silvestri L, Baker JR, Rodén L. Stroud RM (1981) J Biol Chem 256:7383-87.
20 Yurt RW, Wesley Leid, R Jr, Austen KF, Silbert JE (1977) J Biol Chem 252:5818-21.
21 Hallén A (1972) J Chromatogr 71:83-91.
22 Okayama M, Kimata K, Suzuki S (1973) J Biochem (Tokyo) 74:1069-73.
23 Schwartz NB, Galligani L, Ho P-L, Dorfman A (1974) Proc Natl Acad Sci USA 71:4047-51.
24 Robinson HC, Lindahl U (1981) Biochem J 194:575-86.
25 Kolset SO, Ehlorsson J, Kjellén L, Lindahl U (1986) Biochem J 238:209-16.
26 Luikhart SD, Maniglia CA, Sartorelli A-C (1984) Cancer Res 44:2907-12.
27 Reiss M, Maniglia CA, Sartorelli AC (1985) Cancer Res 45:2092-97.
28 Preston SF, Regula CS, Sager PR, Pearson CB, Daniels LS, Brown PA, Berlin RD (1985) J Cell Biol 101:1086-93.
29 Luikhart SD, Sackrison JL (1986) Leuk Res 10:1083-90.