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Proteoglycan synthesis in human erythroleukaemia (HEL) cells

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Synthesis of sulphated proteoglycans was compared in human erythroleukaemia (HEL) cells grown under control conditions and under stimulation by dimethyl sulphoxide (DMSO) and phorbol 12-myristate 13-acetate (PMA). Synthesis of [^{35}S]sulphate-labelled proteoglycans by DMSO-treated cells was decreased by about 35% relative to controls, but synthesis of proteoglycans by PMA-treated cells increased 3–4-fold. Control and DMSO-treated cells secreted 65% of the newly synthesized proteoglycans, but PMA-treated cells secreted more than 90%. Sepharose CL-6B chromatography and SDS/PAGE suggested the presence of several proteoglycans in the cells and culture medium. The PMA-treated cells synthesized a low-M_r proteoglycan ($K_{av.}$ 0.3) that was not present in controls and DMSO-treated cultures. The proteoglycans of the cells and medium from control, DMSO-treated and PMA-treated cultures could be separated into three fractions by octyl-Sepharose chromatography. The proteoglycans were resistant to trypsin but were degraded by Pronase and papain to fragments similar in size to the NaOH/$NaBH_4$-generated glycosaminoglycans. The average chain length of the glycosaminoglycans ($K_{av.}$ 0.20 on Sepharose CL-6B for controls) was decreased by DMSO ($K_{av.}$ 0.25) and by PMA ($K_{av.}$ 0.30–0.38). Chondroitin ABC lyase digestion of the proteoglycans from the medium of the control cultures produced two core proteins at M_r 31000 and 36000. The DMSO medium proteoglycans had only the 31000-M_r core protein, and the PMA culture medium proteoglycans had core proteins of M_r 27000, 31000 and 36000. Changes in synthesis of proteoglycans induced by DMSO or PMA may have relevance for the maturation of haematopoietic cells.

INTRODUCTION

A number of normal and malignant haematopoietic and blood cells and cell lines have been shown to contain proteoglycans and to be capable of proteoglycan synthesis [1–21]. Platelets contain proteoglycans [22–24], but the platelet proteoglycans are synthesized during the development of their parent cells, the marrow megakaryocytes [25]. The proteoglycans of the various cell types vary widely in terms of overall size, glycosaminoglycan (GAG) chain length and number of chains, and the constituent disaccharide units of the GAGs, but in general each cell produces a rather homogeneous proteoglycan.

Proteoglycans of some of the haematopoietic cells are stored in secretory granules, and are released along with other granule contents by appropriate stimuli. The secretory granules of mast cells [12,13], natural killer cells [15,26] and large granular lymphocyte tumour cells [7] contain proteolytic enzymes. However, the organelle-associated proteoglycans of platelets are found in the α-granules [23], which are secretory granules that contain many proteins but not proteinases. Other cells, such as monocytes, appear to secrete most of their proteoglycans constitutively rather than store them [1,2,16]. Some haematopoietic cells also have surface-membrane proteoglycans, which may be involved in attachment of cells to substratum during their development [10,27] or in immunological reactions [28].

Several studies *in vitro* have demonstrated changes in proteoglycan synthesis during maturation of haematopoietic cells. These changes involve GAG chain length and the disaccharide composition of the GAGs [3,9,16,27]. A study *in vivo* performed in this laboratory has demonstrated changes in proteoglycan synthesis during development and maturation of megakaryocytes, the precursor cells for platelets, in guinea pigs [25]; we have recently confirmed the results of the study in experiments performed *in vitro* [29]. Agents that induce cell lines to differentiate have been shown to affect proteoglycan synthesis. For example, phorbol 12-myristate 13-acetate (PMA) caused a substantial decrease in GAG synthesis during macrophage-like differentiation of U-937-4 and HL-60 cells [16–18,20].

Human erythroleukaemia (HEL) cells can be induced to differentiate along three cell pathways: haemin induces erythroid differentiation [30]; PMA induces differentiation into macrophage-like cells [31] and also induces production of several proteins that are thought to be specific to platelets and megakaryocytes [32–37]. Dimethyl sulphoxide (DMSO) has also been shown in a morphological study to enhance the production of several platelet-specific proteins [32], and in biochemical studies to enhance expression of platelet factor 4 [38] and platelet-activation-dependent granule external membrane protein (GMP-140) [39].

The current study was undertaken to determine whether HEL cells synthesize proteoglycans that are analogous to those of platelets and megakaryocytes, and to determine whether the same stimuli that enhance production of platelet α-granule proteins could also enhance proteoglycan synthesis.

A preliminary account of this work was presented at the December 1989 meeting of the American Society of Hematology at Atlanta, GA, U.S.A.

MATERIALS AND METHODS

Materials

DEAE-Sephacel, Sepharose CL-6B, Sepharose CL-4B and octyl-Sepharose CL-4B were from Pharmacia (Piscataway, NJ, U.S.A.). Reagents for gel electrophoresis were from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Chondroitin ABC lyase, chondroitin AC-II lyase, *Flavobacterium heparinium* heparitinase, chondroitin 4-sulphate (whale cartilage), chondroitin 6-sulphate (shark cartilage) and ultrapure urea and guanidine were from ICN Biochemicals (Cleveland, OH, U.S.A.). Pronase, papain, trypsin, PMA, CHAPS and DMSO (tissue-culture grade) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). En^{3}Hance

Abbreviations used: HEL cells, human erythroleukaemia cells; DMSO, dimethyl sulphoxide; PMA, phorbol 12-myristate 13-acetate; GAG, glycosaminoglycan.

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was from New England Nuclear (Boston, MA, U.S.A.). $H_2{}^{35}SO_4$ was from ICN Radiochemicals, Irvine, CA, U.S.A. Culture medium (RPMI 1640) and fetal bovine serum were from GIBCO (Grand Island, NY, U.S.A.).

Cells and culture conditions

HEL cells [23] were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). The cells were grown in RPMI 1640 medium supplemented with 10% (v/v) bovine calf serum, 10 mM-glutamine, and penicillin (50 units/ml)/streptomycin (50 μg/ml), and maintained at 37 °C in a humidified incubator in the presence of 5% CO_2 in air.

Cultures were seeded at 2×10^5 cells per ml in 8 ml of culture medium in a 25 ml Nunc plastic culture flask. DMSO at 1.25% (v/v) and PMA at 0.1 μM final concentration were added at the time the cultures were split. [^{35}S]Sulphate (100 μCi/ml) was added at the same time as DMSO or PMA. Incubation times were 24, 48 and 72 h.

Extraction of proteoglycans and isolation of proteoglycans by ion-exchange chromatography

Cells that had remained in suspension during the culture period were harvested by centrifugation at 500 *g* for 5 min, and washed once with Ca^{2+}-free and Mg^{2+}-free Hanks balanced salt solution, pH 7.4. The cells in the pellet were solubilized with 8 M-urea/50 mM-Tris/HCl/0.1 M-NaCl/0.2% Triton X-100, pH 8.0. The adherent cell layer was rinsed once with the Hanks buffer, and then solubilized in the 8 M-urea extraction buffer just described. The culture medium and cell extracts were subjected to DEAE-Sephacel chromatography as described previously [25] in order to isolate the proteoglycans. The proteins were eluted with 0.1 M-NaCl and 0.23 M-NaCl in 8 M-urea/0.5 M-Tris/HCl, pH 8.0; the proteoglycans were eluted with 4 M-guanidinium chloride/50 mM-sodium acetate, pH 8.0, followed by 4 M-guanidinium chloride/50 mM-sodium acetate/2% CHAPS, pH 8.0. The fractions were dialysed against deionized water at 4 °C, and freeze-dried.

Proteoglycan analysis

The proteoglycan fractions obtained from DEAE-Sephacel and their chemical and enzymic digestion products were analysed by Sepharose CL-6B or Sepharose CL-4B chromatography on columns (60 cm × 0.9 cm) eluted with 4 M-guanidinium chloride/50 mM-sodium acetate/0.2% Triton X-100, pH 7.0 [40], with Blue Dextran and Phenol Red as V_0 and V_t markers, and by SDS/PAGE and fluorography using 4–10% acrylamide gradients or 10% acrylamide gels [25,41,42]. In addition, glycosaminoglycans were analysed by PAGE using a Tris/borate/EDTA system described by Min & Cowman [25,43]. Radioactivity profiles from the columns were determined by liquid-scintillation spectrometry, and proteoglycan bands were identified on the gels by fluorography after treatment of the gels with En^{3}Hance followed by exposure to Kodak XAR film. The molecules were characterized by treatment with trypsin, Pronase, papain [40], mild alkaline borohydride [44], chondroitin lyase [45,46] and heparitinase [47]. To determine the disaccharide unit composition of the chondroitin sulphate glycosaminoglycans, the intact ^{35}S-labelled proteoglycans were digested with chondroitin ABC lyase and with chondro-4-sulphatase or chondro-6-sulphatase as described by Saito *et al.* [45] in the presence of either chondroitin 4-sulphate or chondroitin 6-sulphate as carrier. The digestion mixture was chromatographed on a Bio-Gel P-2 column (60 cm high by 0.9 cm wide) and the disaccharides were eluted with 0.25 M-sodium acetate/0.5 M-NaCl, pH 7.0. Each fraction was analysed for uronic acid [48] and radioactivity. To obtain core proteins, the proteoglycans were digested with chondroitin ABC lyase as described by Oike *et al.* [46], and the core proteins were analysed by SDS/PAGE and fluorography.

For octyl-Sepharose CL-4B chromatography the gel and the sample were equilibrated in 4 M-guanidinium chloride/50 mM-sodium acetate, pH 8.0. The column was eluted first with this buffer, and then with the same buffer containing, sequentially, 0.2% Triton X-100 and 2% CHAPS. The columns contained 1–2 ml of packed gel, and 5 times the bed volume of the gel was collected for each solvent fraction.

RESULTS

Cell growth

The control and DMSO-treated cells were in exponential-phase growth and remained almost entirely in suspension during these experiments, with a doubling time of 24 h after the initial lag of several hours. The PMA-treated cells had nearly completely adhered to the culture dishes in a few hours. These cells no longer divide, but instead undergo differentiation [30]. Thus at the end of the 72 h experiment the control and DMSO-treated cultures contained about 6 times as many cells as did the PMA-treated cultures.

Time course of proteoglycan synthesis and distribution of radioactivity between cells and medium

The time course of incorporation of [^{35}S]sulphate into the proteoglycans from the medium and cells of control cultures and

Table 1. Incorporation of [^{35}S]sulphate into proteoglycans of HEL cells and culture medium

Cells were seeded at 2×10^5 cells/ml, and [^{35}S]sulphate and either DMSO or PMA were added to the test cultures. The data represent radioactivity in the 4 M-guanidinium chloride/50 mM-sodium acetate eluate from the DEAE-Sephacel column. Each number is the average of two or three replicate flasks, i.e. cultures taken from the same stock. The variability in radioactivity determinations was no more than 5% for any of the replicate samples. Abbreviation: N.D., not determined. For details of cell number in each growth condition see the text.

	10^{-3} × Incorporation (c.p.m./culture)		
	Medium	Cells	Adherent cells
24 h			
Control	126	66	N.D.
DMSO	67	36	N.D.
48 h			
Control	286	113	N.D.
DMSO	170	78	N.D.
72 h			
Control	346	156	N.D.
DMSO	181	86	N.D.
24 h			
Control	144	53	3
PMA	602	18	35
48 h			
Control	313	106	7
PMA	1375	78	58
72 h			
Control	450	152	9
PMA	2426	163	60

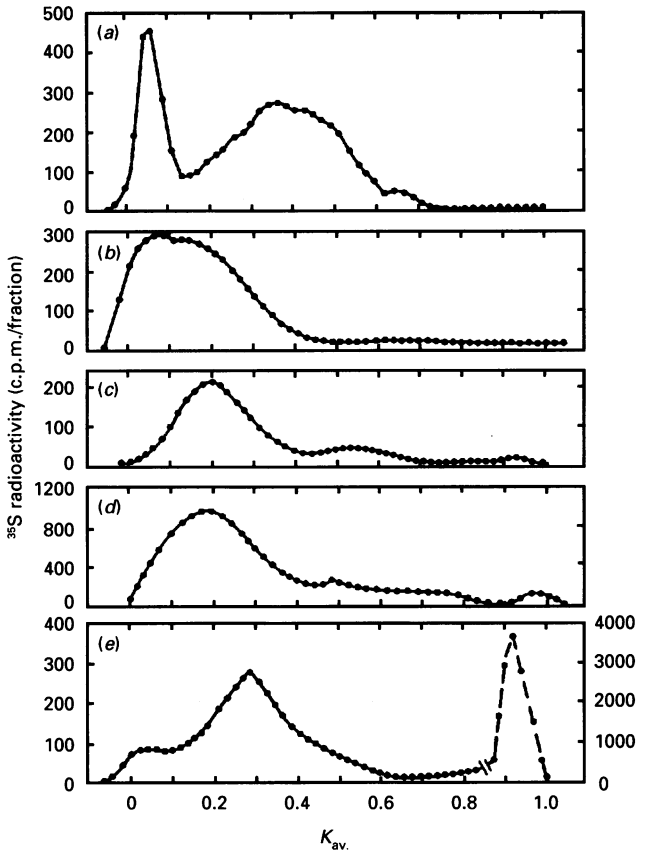

Fig. 1. Proteoglycans from control medium

This Figure represents the 4 M-guanidinium chloride/50 mM-sodium acetate fraction from the DEAE-Sephacel column. (*a*) Sepharose CL-4B chromatography of intact proteoglycans. (*b*) Sepharose CL-6B chromatography of intact proteoglycans. (*c*), (*d*) and (*e*) Mild alkaline borohydride, Pronase and chondroitin ABC lyase digests respectively chromatographed on Sepharose CL-6B.

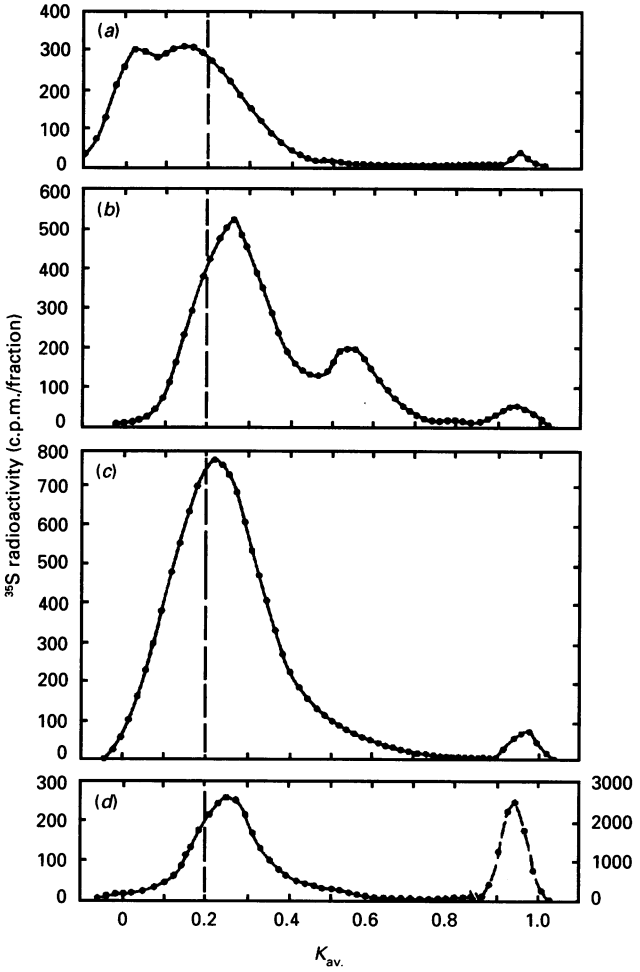

Fig. 2. Proteoglycans from the medium of DMSO-treated cultures

Proteoglycans were from the 4 M-guanidinium chloride/50 mM-sodium acetate eluate from the DEAE-Sephacel column. (*a*) Intact proteoglycans. (*b*), (*c*) and (*d*) Mild alkaline borohydride, Pronase and chondroitin ABC lyase digests respectively chromatographed on Sepharose CL-6B.

of cultures treated with DMSO or PMA over a 72 h incubation period is shown in Table 1. The data represent the 4 M-guanidinium chloride eluate from the DEAE-Sephacel column, which contained 96% of the proteoglycan-associated ^{35}S label. At all time points the DMSO-treated cultures (i.e. cells plus medium) contained only 54–58% as much radiolabelled proteoglycan as controls, and in contrast the PMA-treated cultures contained 3–4 times more radiolabelled proteoglycan than the controls. Control and DMSO-treated cells secreted about 65% and PMA-treated cells secreted 90% of their proteoglycans.

Analysis of proteoglycans

Proteoglycans were isolated from the culture medium and cells by DEAE-Sephacel chromatography. The proteoglycans were characterized by treatment with mild alkaline borohydride, Pronase, papain and chondroitin lyase. The intact proteoglycans and their digestion products were analysed by Sepharose chromatography and SDS/PAGE. The proteoglycans were also subfractionated by octyl-Sepharose chromatography, and the fractions from the octyl-Sepharose column were analysed in the same manner as the total proteoglycans.

Characterization of proteoglycans from culture medium

Control medium. The Sepharose CL-4B and Sepharose CL-6B elution profiles of [^{35}S]sulphate-labelled proteoglycans from control medium after 48 h of labelling are shown in Figs. 1(*a*) and 1(*b*). The profiles were virtually identical at 24 h and 72 h (results not shown). There appear to be at least two distinct populations of proteoglycans, which can be separated on the basis of size.

The GAG chains that were released from the total proteoglycan eluate by alkaline borohydride treatment (Fig. 1*c*) were eluted primarily at $K_{av.}$ 0.20 from Sepharose CL-6B, with a smaller peak at $K_{av.}$ 0.53. When analysed by PAGE as described by Min & Cowman [43], two distinct regions were seen: the major portion of the GAG chains ran just above and into the top fifth of the shark cartilage chondroitin 6-sulphate smear (nominal M_r range 40000–80000) and a minor component was located at the lower-M_r region of this standard (results not shown). In order to determine the distribution of the two types of GAG chains among the proteoglycans, the proteoglycan eluate from the Sepharose CL-4B column was subfractionated into three groups, i.e. the $K_{av.}$ 0.07 peak and the ascending ($K_{av.}$ 0.12–0.4) and descending ($K_{av.}$ 0.4–0.6) halves of the lower-M_r peak, and each subfraction was treated with mild alkaline borohydride. The $K_{av.}$ values of the major GAG peak from these subfractions on Sepharose CL-6B were respectively 0.21, 0.18 and 0.22 (results not shown). The presence of similar-sized GAG chains in the $K_{av.}$ 0.07 peak compared with the $K_{av.}$ 0.1–0.6 peak suggests that

the larger proteoglycan has more GAG chains than the smaller molecules. Only the proteoglycans from the $K_{av.}$ 0.1–0.6 peak contained the $K_{av.}$ 0.53 GAG: this GAG represented 10% of the GAGs of the $K_{av.}$ 0.12–0.4 region and 19% of the $K_{av.}$ 0.4–0.6 region of the proteoglycan peak.

The proteinase-sensitivity of the proteoglycans was assessed. They were not degraded by trypsin (results not shown). However, they were degraded by Pronase (Fig. 1*d*) and papain (results not shown) to fragments similar in size to the GAGs. The ^{35}S-labelled proteoglycans in the control medium were degraded by about 90% by chondroitin ABC lyase (Fig. 1*e*) and chondroitin AC-II lyase (results not shown). The disaccharide unit structures were entirely those of chondroitin 4-sulphate. Approx. 8–10% of the radioactivity appeared at the V_t of the Sepharose CL-6B column after heparitinase digestion (results not shown). Thus the peaks remaining at the V_0 and $K_{av.}$ 0.28 after chondroitin lyase digestion probably represent the heparan sulphate-containing proteoglycans.

Medium from DMSO-treated cells. The proteoglycans from the culture medium of the DMSO-treated cells eluted from Sepharose CL-6B were nearly identical with those of the controls (Fig. 2). Mild alkaline borohydride treatment resulted in release of a major GAG with a peak at $K_{av.}$ 0.25, and the smaller peak at $K_{av.}$ 0.53 that had been seen in controls. On PAGE, the major portion of the GAGs from the DMSO medium ran just below those from the controls, but the lower-M_r minor portion appeared in the same position as in the controls (results not shown). Thus DMSO caused a selective decrease in mean chain length of only the major GAG relative to the control ($K_{av.}$ 0.20). The proteinase-sensitivity (Fig. 2*c*) and chondroitin lyase digestion patterns (Fig. 2*d*) were identical with controls.

Medium from PMA-treated cells. The Sepharose CL-4B and Sepharose CL-6B elution profiles of the culture medium proteoglycans from the PMA-treated cells are shown in Figs. 3(*a*) and 3(*b*). The profile is more complex than that of the controls or DMSO medium, and includes a considerable amount of low-M_r proteoglycan ($K_{av.}$ 0.52 on Sepharose CL-4B), which was not seen in the control and DMSO-treated cultures. The GAG analysis and analysis of Pronase and papain digestions by Sepharose CL-6B are discussed in detail below for proteoglycans subfractionated on octyl-Sepharose. On PAGE, the GAG chains ran at lower M_r values than those from the control and DMSO medium, and the profile was nearly parallel with that of the shark chondroitin 6-sulphate. Chondroitin ABC lyase released 90% of the proteoglycan-associated radioactivity (Fig. 3*c*).

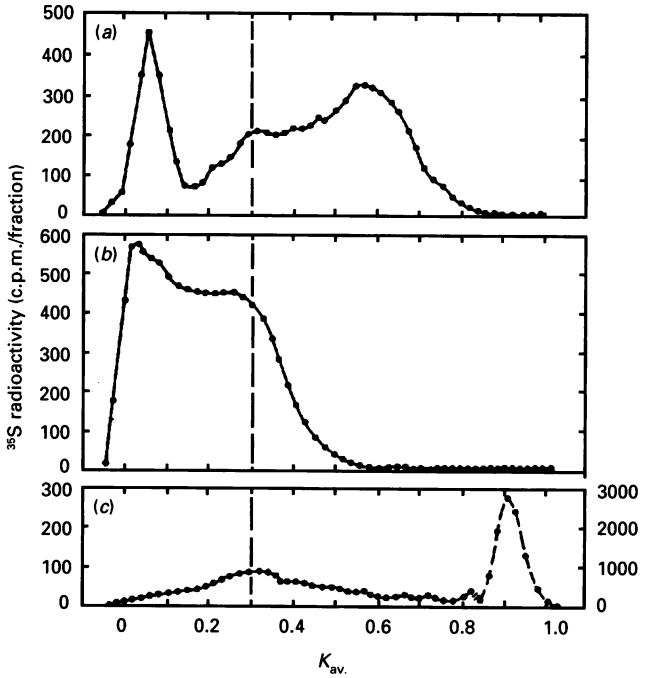

Fig. 3. Proteoglycans from medium of PMA-treated cultures

(*a*) Sepharose CL-4B labelling profile of intact proteoglycans. (*b*) Sepharose CL-6B labelling profile of intact proteoglycans. (*c*) Chondroitin ABC lyase digest chromatographed on Sepharose CL-6B.

Fractionation of proteoglycans by octyl-Sepharose

Proteoglycans from the medium and cells were fractionated on octyl-Sepharose columns. For the medium proteoglycans, the radioactivity was recovered to the extent of 55–65% in the flow-through (4 M-guanidinium chloride eluate), 21–31% in the 0.2% Triton X-100/4 M-guanidinium chloride fraction, and the remainder in the 2% CHAPS/4 M-guanidinium chloride fraction. The fractionation of the cell-associated proteoglycans is described below with the cell proteoglycan analysis.

Fig. 4 shows the SDS/PAGE profile of control and PMA medium proteoglycans obtained from the octyl-Sepharose columns. The DMSO medium proteoglycans were similar to controls. The proteoglycans in all octyl-Sepharose fractions were degraded by Pronase, papain and alkaline borohydride. The digestion products from the proteoglycans from the 4 M-guanidinium chloride/Triton X-100 eluates were of lower M_r than those of the proteoglycans in the 4 M-guanidinium chloride eluate.

Analysis of the PMA medium proteoglycans obtained from octyl-Sepharose fractionation is shown in Fig. 5. The 4 M-guanidinium chloride eluate from octyl-Sepharose (Fig. 5*a*) had an elution profile on Sepharose CL-6B similar to the unfractionated material (see Fig. 3*b*), except that some of the highest-M_r material and some of the lowest-M_r material appeared

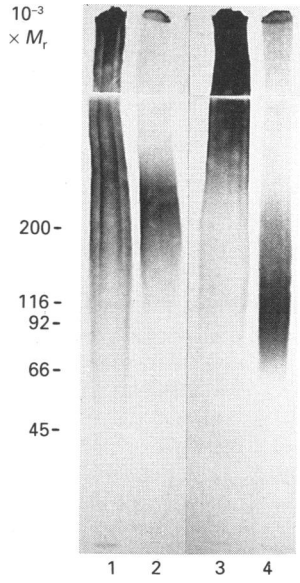

Fig. 4. Octyl-Sepharose separation of proteoglycans from the culture medium

Samples were analysed by SDS/PAGE and fluorography (4–12% gradient gel). Lane 1, Control medium, 4 M-guanidinium chloride fraction (flow-through); lane 2, 4 M-guanidinium chloride/0.2 M-Triton X-100 fraction; lane 3, PMA medium, 4 M-guanidinium chloride fraction; lane 4, 4 M-guanidinium chloride/0.2% Triton X-100 fraction.

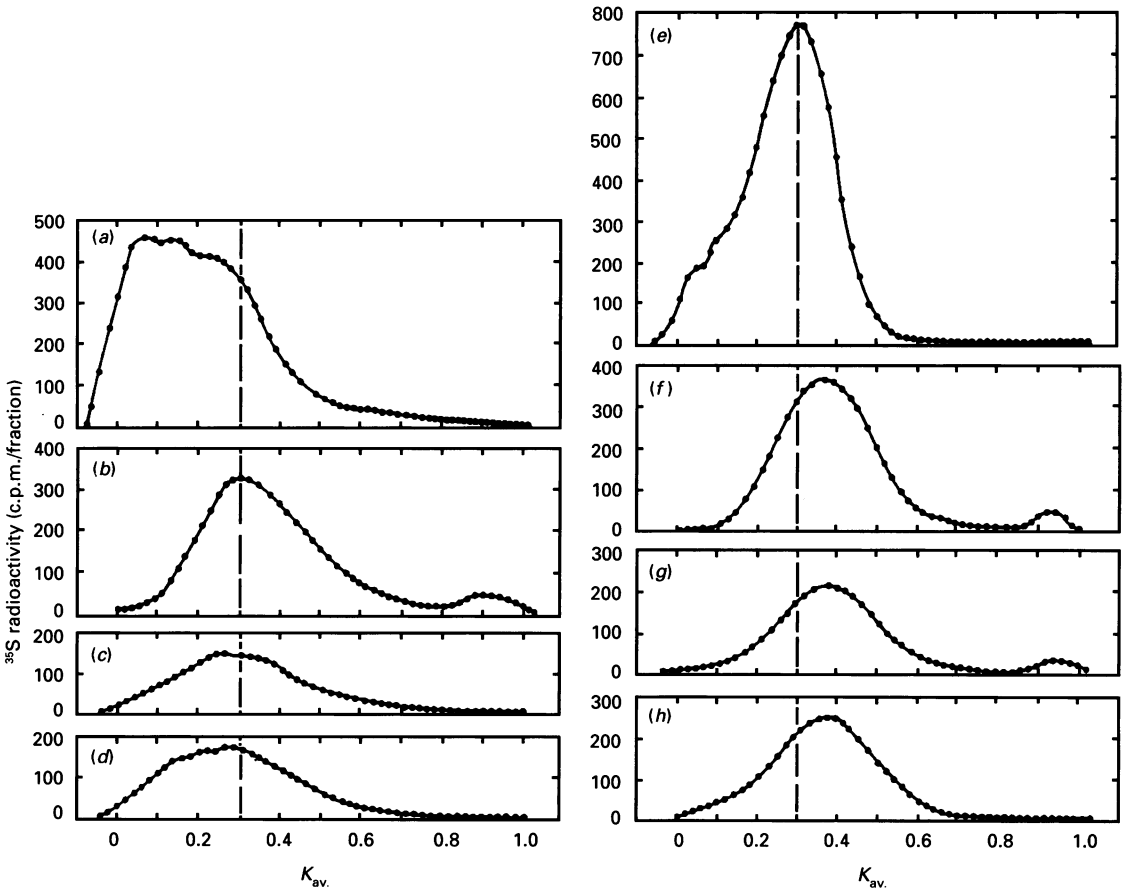

Fig. 5. Analysis of octyl-Sepharose fractions from PMA medium proteoglycans by Sepharose CL-6B chromatography

(*a*)–(*d*) 4 M-Guanidinium chloride eluate; (*e*)–(*h*) 4 M-guanidinium chloride/0.2% Triton X-100 eluate. (*a*) and (*e*) Intact proteoglycan; (*b*) and (*f*) mild alkaline borohydride digest; (*c*) and (*g*) papain digest; (*d*) and (*h*) Pronase digest.

to be lost. These molecules appeared in the 4 M-guanidinium chloride/0.2% Triton X-100 eluate shown in Fig. 5(*e*). All octyl-Sepharose fractions contained proteoglycans that were susceptible to degradation by Pronase and papain, since proteinase-digestion products (Figs. 5*c*, 5*d*, 5*g* and 5*h*) were in the same size range as the GAGs released by the mild alkaline borohydride treatment (Figs. 5*b* and 5*f*). The mean size of the GAG chains synthesized in the presence of PMA was decreased relative to both the control and the DMSO medium proteoglycans; the migration positions were $K_{av.}$ 0.30 for the GAGs in the 4 M-guanidinium chloride fraction (Fig. 5*b*) and 0.38 in the 4 M-guanidinium chloride/0.2% Triton X-100 fraction (Fig. 5*f*). On SDS/PAGE the GAGs ran respectively at M_r 28000–60000 and M_r 25000–44000 (results not shown). In comparison, the control and DMSO medium GAGs ran at M_r 45000–80000 and M_r 35000–70000 (results not shown). The apparent degradation of all proteoglycans by papain, Pronase and alkaline borohydride suggests that there is little or no free GAG in the medium. Pronase and papain digests of proteoglycans from human platelets migrated similarly to those of the PMA-treated HEL cells on SDS/PAGE (not shown).

Core proteins

The core proteins obtained from chondroitin ABC lyase digestion of the culture-medium proteoglycans from the control, DMSO-treated or PMA-treated cells are shown in Fig. 6. The letters a–c refer to core proteins of the size range expected from granule proteoglycans on the basis of the characterization of these molecules from other blood cells, including platelets [25,49]. The control medium proteoglycans had two core proteins, one at M_r 31000 and the other at M_r 36000; the DMSO proteoglycans had only the 31000-M_r core; and the PMA proteoglycans had the 31000-M_r and 36000-M_r cores along with an additional major band at M_r 27000. The three bands were present in similar proportions at 24, 48 and 72 h. In an experiment with PMA-treated cells at 4 h, all three bands were present in the medium, but the heaviest labelling appeared at the 31000-M_r band (results not shown). Two additional bands, marked d and e, at M_r approx. 92000 and 116000, were generated by chondroitin lyase digestion of the proteoglycans from the medium in all three culture conditions. The undigested material most probably represents the heparan sulphate proteoglycans.

Core proteins were also obtained from the proteoglycans from the octyl-Sepharose column fractions (results not shown). In the controls, the 31000-M_r band was associated with the 4 M-guanidinium chloride fraction, and the 36000-M_r band with the Triton X-100 and CHAPS eluates. All three core proteins from the PMA medium proteoglycans were found in the 4 M-guanidinium chloride fraction from octyl-Sepharose, but most of the 36000-M_r band was found in the Triton X-100 and CHAPS fractions. The presumptive core proteins at M_r 96000 and 116000 were present only in the detergent eluates in all samples.

Cell-associated proteoglycans

The cell-associated proteoglycans were analysed by the same procedures described for the medium proteoglycans. The

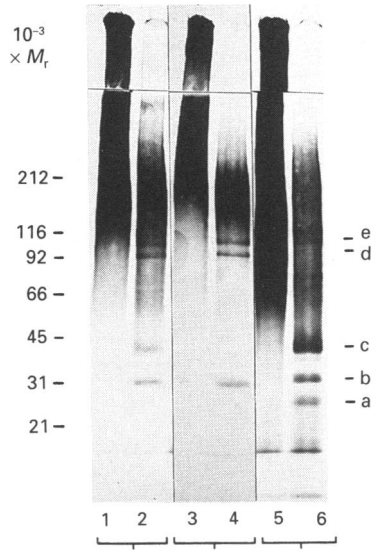

Fig. 6. Core proteins of proteoglycans obtained from the culture media of control (lanes 1 and 2) and DMSO-treated (lanes 3 and 4) and PMA-treated (lanes 5 and 6) cells

The ^{35}S-labelled proteoglycans were isolated by DEAE-Sephacel chromatography, dialysed, freeze-dried and digested with chondroitin ABC lyase as described in the text. The core proteins were analysed by SDS/PAGE and fluorography. The letters a–e represent bands appearing only after chondroitin lyase digestion. Lanes 1, 3 and 5 are undigested material; lanes 2, 4 and 6 are chondroitin lyase digests.

Sepharose CL-6B profiles of the proteoglycans from the control, DMSO-treated and PMA-treated cells and the alkaline borohydride digestion products are shown in Fig. 7. The labelling pattern was essentially the same for each type of culture at 24, 48 and 72 h. No significant differences were observed between the adherent and suspension cells from the same cultures. The cell proteoglycans were eluted over the same general size range as the medium proteoglycans. The patterns of degradation of the proteoglycans by alkaline borohydride and proteinases suggests that there was little or no free GAG present within the cells, in contrast with the presence of free GAGs in other tumour cells [7,50,51]. The papain, Pronase (results not shown) and alkaline borohydride digests of the cell proteoglycans yielded fragments similar to those of the medium proteoglycans. The major GAG was chondroitin 4-sulphate, but about 25% of the radiolabelled material was digested by heparitinase (results not shown). A large peak of sulphated material was seen in all the cell samples at $K_{av.}$ 0.78, and occurred in similar proportions to the total labelling at all time points. This material was not degraded by proteinases, chondroitin lyase or heparitinase, nor was material of this size range generated from the larger molecules by the various digestions. A similar peak was observed by Minguell & Tavassoli [10] in their study of the FDCP stem-cell line. The cell proteoglycans could be subfractionated by octyl-Sepharose chromatography similarly to the medium proteoglycans as described above. About 76% of the radioactivity from control and PMA-treated cells was eluted in the flow-through and the remainder in the detergent fractions, but for the DMSO-treated cells 55% was eluted in the flow-through and 40% with 4 M-guanidinium chloride/0.2% Triton X-100. The SDS/PAGE profiles were consistent with those from the culture-medium proteoglycans (results not shown).

The 4 M-guanidinium chloride/CHAPS DEAE-Sephacel eluate of the HEL-cell extracts did not contain a high-M_r proteoglycan analogous to that seen in guinea-pig platelets [25] and human platelets (B. P. Schick, unpublished work).

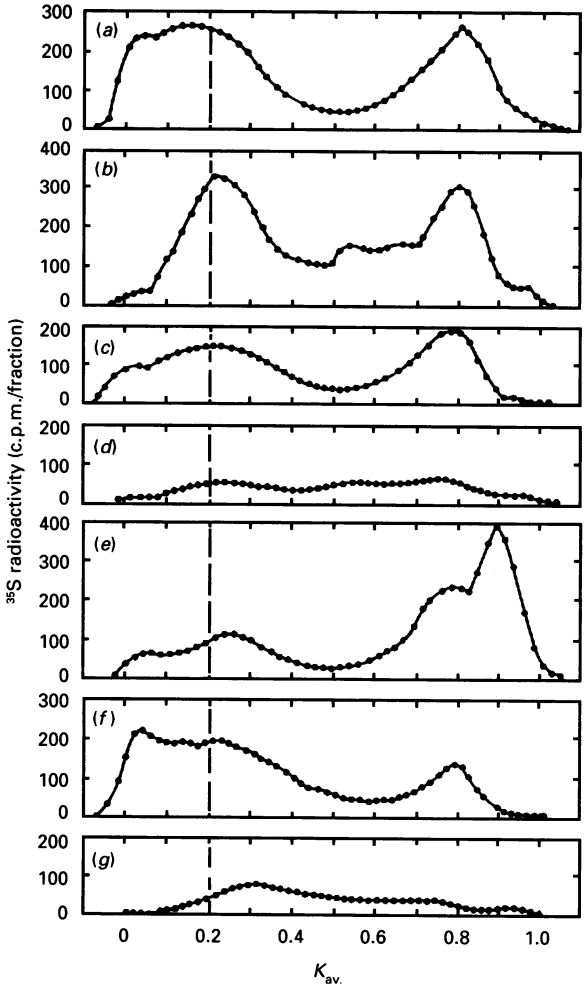

Fig. 7. Proteoglycans obtained from control [(*a*) and (*b*)] and DMSO-treated [(*c*) and (*d*)] and PMA-treated [(*e*) and (*f*)] HEL cells

The proteoglycans were extracted from the cells and isolated by DEAE-Sephacel chromatography as described in the text. All samples were chromatographed on Sepharose CL-6B. The labelling profiles for a given incubation condition were identical at 24, 48 and 72 h. (*a*) Proteoglycans from control cells; (*b*) control proteoglycans digested with alkaline borohydride; (*c*) proteoglycans from DMSO-treated cells; (*d*) DMSO-treated proteoglycans digested with alkaline borohydride; (*e*) DMSO-treated proteoglycans digested with chondroitinase; (*f*) proteoglycans from PMA-treated cells; (*g*) PMA-treated proteoglycans digested with alkaline borohydride.

DISCUSSION

This study has characterized proteoglycan synthesis by HEL cells and the changes induced by PMA and DMSO. We have demonstrated that the HEL cell line, which has the capacity to differentiate along erythrocytic, macrophage and megakaryocytic pathways, synthesizes a heterogeneous population of proteoglycans with a broad range of M_r and GAG chain length. The proteoglycans can be separated into at least two distinct classes by octyl-Sepharose chromatography; thus far no haematopoietic cell proteoglycans have been shown to be retained on octyl-Sepharose columns. The HEL proteoglycans contain primarily chondroitin 4-sulphate chains, as do those of other haematopoietic cells, but are unique, except for mast cells, in also

containing heparan sulphate proteoglycans. Despite their ability to differentiate along the macrophage lineage [31], the HEL cells do not synthesize the disulphated GAGs characteristic of differentiating macrophages [3,52]. Unlike the proteinase-resistant mast-cell, natural killer-cell and eosinophil proteoglycans [6,8,12,15], but similarly to platelet proteoglycans [25], HEL proteoglycans are cleaved by Pronase and papain to fragments similar in size to the alkaline borohydride-generated GAGs. HEL cells may be unique in their response to agents such as PMA and DMSO with regard to proteoglycan synthesis. Both agents decreased the GAG chain length, in contrast with their lack of effects in other cells [16,19]. Of note also are the disparate effects of PMA and DMSO on proteoglycan synthesis: DMSO markedly suppressed and PMA greatly stimulated proteoglycan synthesis. In contrast, in other cells both agents have been found to depress proteoglycan synthesis [19,53–56]. This disparity is also remarkable in that both PMA and DMSO increase the production of several platelet-specific granule and membrane proteins [32–39]. HEL cells are thus far the only haematopoietic cell line shown to contain more than one proteoglycan core protein, and this study is the first, to our knowledge, to demonstrate that PMA and DMSO may alter the synthesis of particular core proteins.

HEL-cell proteoglycans are similar to platelet proteoglycans in several respects. The proteoglycans from control cells are somewhat larger than that of guinea-pig and human platelet proteoglycans [25], probably because of the greater mean chain length of the HEL-cell GAGs. However, the GAG chains synthesized by the PMA-stimulated HEL cells are close in size to those of human platelets (B. P. Schick, unpublished work), ranging from about M_r 35000 to M_r 65000 on PAGE in comparison with known chondroitin sulphate standards. These GAG chains are considerably longer than those of other haematopoietic cells [4,6,16,19,21]. It is interesting to note that HEL cells synthesize a small GAG, $K_{av.}$ 0.53 on Sepharose CL-6B, that is in the same size range as that of monocytes and well below those of platelets [2], and therefore may also synthesize the same proteoglycan found in monocytes.

The ultimate definition of the similarities or differences between the proteoglycans of platelets and HEL cells rests on characterization of the core proteins. Chondroitin lyase digestion of the culture medium proteoglycans generated three core proteins of the general size range associated with granule proteoglycans: the control medium contained 31000-M_r and 36000-M_r proteins, the DMSO medium only the 31000-M_r protein, and the PMA medium both the 31000-M_r and 36000-M_r proteins along with a 27000-M_r protein. The data suggest that DMSO and PMA can influence the synthesis of the core proteins. Alternatively, the 27000-M_r protein could be a proteolysis product of the larger molecules. However, we consider this unlikely because it appears in constant proportion to the other core proteins at all time points. It is interesting that guinea-pig platelets contain at least two core proteins (M_r 31000 and 38000), which are similar in size to the two larger HEL core proteins; these proteins appear to be synthesized at different stages of megakaryocyte maturation [25]. Perin *et al.* [55] have sequenced a chondroitin lyase-generated proteoglycan core protein from human platelets that appears at M_r 27000 on SDS/PAGE. In addition, they have isolated a cDNA from a HEL-cell cDNA library generated from PMA-stimulated cells [49] that corresponds to the sequence of their platelet proteoglycan core protein. This cDNA has significant sequence similarity to the yolk-sac L2-tumour-cell proteoglycan core cDNA characterized as PG-19 [56]. Similar core proteins have been identified in mast cells [57], HL60 cells [58] and rat RBL-2 cells [59]. The extent to which several HEL-cell core proteins are related to the human platelet 27000-M_r core protein remains to be determined.

It is of interest also that higher-M_r core proteins are found at M_r 92000 and 116000. These core proteins are similar in size to those of a proteoglycan that is a transforming-growth-factor-β receptor [60,61]. We find this core protein only amongst the proteoglycans that are retained by octyl-Sepharose. Similarly, Andres *et al.* [62] have found that the extracellular domain of the transforming-growth-factor-β receptor proteoglycan obtained from non-haematopoietic cell lines adheres to octyl-Sepharose. This proteoglycan could conceivably be released from the cell surface to be incorporated into the matrix of the bone marrow *in vivo*.

Our data have shown that the bulk of the proteoglycans are secreted from HEL cells. Constitutive secretion of newly synthesized proteoglycans is characteristic of monocyte/macrophage cells [1,2,16]. In contrast, the proteoglycans synthesized by megakaryocytes *in vivo* appear to be destined for storage in the α-granules or membranes of the platelets [25], and the proteoglycans are only secreted to a minor extent by the megakaryocytes *in vitro* (B. P. Schick, unpublished work). Papayannopoulou *et al.* [34] have reported that most of the platelet-derived growth factor activity of HEL cell cultures is found in the culture medium. No information is available regarding the secretion of other platelet α-granule proteins by HEL cells. Despite the extensive ability of HEL cells to synthesize platelet granule proteins, these proteins may be directed towards a secretory pathway rather than towards the formation of mature α-granules. Since it has been speculated frequently that proteoglycans are necessary to package the granule constituents, perhaps direction of proteoglycans to a secretory pathway in some way disrupts the formation of the granules.

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