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Identification of cells responsible for synthesis of sulphated glycosaminoglycans in schistosome-induced hepatic granulomas

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Summary. Sulphated glycosaminoglycans were isolated from schistosome-induced hepatic granuloma and from the pericellular, intracellular and extracellular compartments of two murine cell lines derived from granulomas: the primary cell line GR, and the permanent cell line GRX, established spontaneously from GR. The glycosaminoglycans composition in the whole granuloma was similar to that observed in the intracellular and extracellular compartments of GR cells. This result suggests that GR cells may be the major cell population involved in the synthesis and accumulation of glycosaminoglycans in the granulomas, and play an important role in the process of hepatic fibrosis.

The conversion of the primary cell line GR into the established GRX cells did not modify the ratios that prevail among different glycosaminoglycans of the cell surface. However, it decreased the synthesis and secretion of glycosaminoglycans, reduced the proportion of iduronic acid units in the chondroitin sulphate*, and increased the proportion of heparan sulphate in intracellular and extracellular pools. These characteristics of the GRX cells are similar to those observed in long-term cultures of smooth-muscle cells. In agreement with the general phenomenon of progressive de-differentiation during in-vitro culture of primary cell lines, these data indicate that the connective tissue cells of liver may belong to the myofibroblastic cell lineage.

Keywords: glycosaminoglycans, schistosomiasis, granuloma, chondroitin sulphate, heparan sulphate

Schistosomiasis is one of the major infectious diseases of humans and one of the most widespread causes of hepatic fibrosis (Warren 1978). The pathogenesis of fibrosis is related, at least in part, to formation of granulomas around schistosome eggs, which are trapped in the hepatic portal microvasculature. Periovular granulomas are complex structures composed of various cell types and an extensive extracellular matrix (Warren 1982), where large amounts of collagen, glycosaminoglycans,

* Chondroitin sulphate, designating copolymers of chondroitin 4- and 6-sulphate and dermatan sulphate, is used according to Höök *et al.* (1984).

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fibronectin and other glycoproteins may be found (Al Adnani 1985; Junqueira *et al.* 1986; Grimaud *et al.* 1987). A previous study suggested that glycosaminoglycan deposition in granulomas occurs early, maximum amounts being deposited in the acute phase of the disease and maintained throughout the evolution of the disease to its chronic phase (Silva *et al.* 1989). In contrast, collagen accumulation has been shown to be progressive, and to increase considerably in the late chronic stage of the disease. This inversion in the patterns of glycosaminoglycan and collagen deposition may be related to the reversibility of granuloma evolution in the acute phase and its irreversibility in the chronic phase of the disease (Warren & Klein 1969; Melhorn *et al.* 1982).

Information on the type of cell involved in the synthesis of glycosaminoglycan in the extracellular matrix of the granuloma may be important for understanding the role of this fibrogranulomatous reaction in the establishment of hepatic fibrosis in schistosomiasis. Granulomas are composed of macrophages, lymphocytes, granulocytes and connective-tissue-like cells (Moore *et al.* 1977). The extent to which each of these cell types contributes to glycosaminoglycan synthesis is not known. Comparison between glycosaminoglycan synthesis in cultures of connective-tissue cells isolated from granulomas and synthesis in whole granulomas may provide a clue to the type of cell that is involved in glycosaminoglycan synthesis and accumulation in granulomas. In addition, the use of cell cultures to study these processes makes it possible to distinguish among types of glycans in different cell compartments (Kraemer 1971; Mourão *et al.* 1983).

The main purpose of our study was to compare glycosaminoglycans isolated from the whole granulomas with those obtained from the pericellular, intracellular and extracellular compartments of two murine cell lines derived from schistosome-induced granulomas. We have studied the primary cell line GR and the permanent cell line GRX. Both cell lines have been shown to belong to the smooth-muscle cell lineage; by biological, morphological and biochemical criteria they correspond to myofibroblasts and they may be induced to differentiate *in vitro* into lipocytes (Borojevic *et al.* 1985a, 1990b; Margis & Borojevic 1989). Similar to most murine primary cell lines, after several months of culture *in vitro*, GR cells give rise to the permanent cell line GRX. However, GRX cells continue to depend upon serum (maximal growth rate is observed with 20–30% FBS), and the cells do not grow in soft agar, showing that this line is not transformed. The reported morphological and biochemical characteristics of these cells led us to consider them to be representative of murine liver connective-tissue cell lines, shown to be involved in repair and regenerative processes in liver and in secretion of extracellular matrix in hepatic fibrosis (Borojevic *et al.* 1985b; Mak *et al.* 1984; Friedman *et al.* 1985; Gressner & Haarmann, 1988).

Materials and methods

Formation and isolation of schistosome-induced granulomas

C3H/HeN mice of both sexes, 1 month old, were infected by transcutaneous penetration of 30 cercariae of *Schistosoma mansoni* (BH strain, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil). Mice were sacrificed by ether overdose after 90 days of infection. This period corresponds to the beginning of the chronic phase of the disease, as characterized in previous work (Borojevic *et al.* 1984). Granulomas were isolated from liver tissues following the method described by Pellegrino and Brener (1956). Briefly, livers of infected mice were homogenized in an Omni-Mixer (Sorvall, Norwalk, CT, USA), in phosphate-buffered saline (PBS) (pH 7.4), until all the soft hepatic tissue was dispersed. Granulomas were separated by repeated sedimentation at 1 *g* in ice-cold PBS, washed, and used in further studies.

Culture procedures

Isolated granulomas were washed with Hanks' balanced salt solution and incubated overnight in 0.3 mg/ml collagenase (Type IA, Sigma Chemical Co., St Louis, MO, USA) in Dulbecco's modified minimum essential medium (MEM) supplemented with 3 g/l HEPES buffer (both from Sigma), pH 4, for 20 min at 37°C. Granulomas were then washed for 20 min in calcium and magnesium-free balanced salt solution, and incubated for 1 h in 0.125% trypsin and 0.05% EDTA (both from Sigma) in the same balanced salt solution (pH 7.8). Cells were harvested in Dulbecco's modified MEM supplemented with 10% foetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil), and 3 g/l HEPES buffer (standard medium). They were plated in 25-cm^2 tissue culture flasks (Nunclon, Roskilde, Denmark) and incubated overnight. Non-adherent cells were washed off and adherent cells were cultured in the standard medium. After two subsequent trypsinizations, a homogeneous cell population of fibroblastoid cells was obtained, designated here as the primary GR cell line.

The GRX permanent cell line was originally obtained by spontaneous migration of cells from plated granulomas. It was characterized as myofibroblastic and described in detail in previous publications (Borojevic *et al.* 1985a; Margis & Borojevic 1989).

Labelling of sulphated glycosaminoglycans in isolated granulomas

Approximately 40 mg (wet weight) of freshly isolated granulomas were incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. The medium contained 40 μCi carrier-free $H_2{}^{35}SO_4$ (Instituto de Pesquisas Energéticas e Nucleares, São Paulo, SP, Brazil) dissolved in 8.0 ml sulphate-free Eagle's MEM (Eurobio, Paris, France) supplemented with 10% FBS. At the end of the labelling period, the medium was decanted and granulomas were washed twice with PBS. Isolated granulomas were immersed immediately in 10 volumes of acetone, where they were kept for 24 h at 4°C and subsequently vacuum-dried.

Labelling of sulphated glycosaminoglycans from the cell cultures.

Thirty μCi of $H_2{}^{35}SO_4$ in fresh nutrient Eagle's MEM without sulphate, containing 10% FBS, were added to confluent monolayer cultures of GR cells, at the third to fourth passage, and to GRX cells. The cultures were incubated in this medium for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. At the end of the labelling period, the media were decanted, the cells were washed twice with PBS (pH 7.4), and the ^{35}S-sulphated glycosaminoglycans from the pericellular, intracellular and extracellular pools were prepared as described below.

Isolation of the glycosaminoglycans from schistosome-induced granulomas

Glycosaminoglycans were isolated from the granulomas following the method previously described for other tissue by Toledo and Mourão (1979). Briefly, dried granulomas (400 mg) were suspended in 2.0 ml of 0.1 M sodium acetate buffer (pH 5.5), containing 40 mg papain (E. Merck, Darmstadt, Germany), 5 mM EDTA and 5 mM cysteine, and incubated at 60°C for 24 h. The suspension was centrifuged at 2000 *g* for 10 min at room temperature, and the supernatant was precipitated with 2 volumes of 95% ethanol and maintained at −10°C for 24 h. Precipitates were collected by centrifugation (2000 *g* for 10 min at room temperature) and vacuum dried. The residue was re-dissolved in 1.0 ml PBS and approximately 0.5 mg deoxyribonuclease I from bovine pancreas (Sigma) was added, followed by incubation for 12 h at 37°C. The digestion was interrupted by immersing the tubes in boiling water for 1 min. The supernatants obtained after centrifugation (2000 *g* for 10 min at room temperature) were precipitated with 2 volumes of 95% ethanol, collected by centrifugation and dissolved in 0.2 ml distilled water.

Isolation of the glycosaminoglycans from the cell cultures

At the completion of the labelling period, media were decanted and the cells were rinsed with PBS (pH 7.4). Cells were detached by trypsinization with 2 ml of 0.05% trypsin in 0.05% EDTA for 10 min at room temperature. Centrifugation of the trypsinate (2000 *g* for 10 min at room temperature) separated the supernatant and cell pellet, which contained the *pericellular* and *intracellular* glycosaminoglycans, respectively. Both fractions were incubated for 12 h at 37°C with 2 ml of PBS (pH 7.4), containing 0.05% trypsin and 0.05% EDTA. These solutions were centrifuged (2000 *g* for 10 min) and the glycosaminoglycans in the supernatants were precipitated with 4 volumes of 95% ethanol and maintained at −10°C for 24 h. The precipitates were collected by centrifugation, washed once with 80% ethanol, vacuum dried, and dissolved in 0.2 ml distilled water.

For isolation of *extracellular* glycosaminoglycans, the culture medium (2 ml) was incubated with 10 mg of papain in the presence of 5 mM EDTA and 5 mM cysteine at 60°C for 24 h, then dialysed against distilled water and finally lyophilized. This preparation was dissolved in 2 ml of distilled water and applied to a DEAE-cellulose column (3.5 × 2.0 cm), equilibrated with 0.05 M sodium acetate buffer (pH 5.0). The column was washed with 100 ml of the same buffer and then eluted step-wise with 25 ml of 0.1 M and 1.0 M NaCl in the same acetate buffer. The ^{35}S-glycosaminoglycans eluted from the column with 1.0 M NaCl were exhaustively dialysed against distilled water, lyophilized, and dissolved in 0.2 ml of distilled water.

Identification of glycosaminoglycans

Glycosaminoglycans were identified according to two criteria: (a) by their electrophoretic mobilities on agarose gels and (b) by the products of their enzymatic degradation with chondroitinases AC or ABC, and by deaminative cleavage with nitrous acid.

Agarose gel electrophoresis was carried out as previously described (Dietrich & Dietrich 1976). Approximately 20 μg glycosaminoglycans (dry weight) were applied to 0.5% agarose gels in 0.05 M 1,3-diaminopropane/acetate buffer (pH 9.0). After electrophoresis, glycosaminoglycans were fixed in the gel with 0.1% Cetavlon (*N*-cetyl-*N*, *N*, *N*-trimethylammonium bromide) in water, and stained with 0.1% toluidine blue in acetic acid:ethanol:water (0.1:5:5, v/v). Non-radioactive glycosaminoglycans were quantified in the gel by densitometry using a Quick Scan densitometer (Helena Laboratories, Beaumont, TX, USA). The ^{35}S-labelled glycosaminoglycans were visualized by autoradiography of the stained gels. The radioactive bands having identical electrophoretic migration with standard glycosaminoglycans were scraped into 10 ml of 0.5% PPO/toluene solution and counted in a liquid scintillation counter.

Degradation with chondroitinases AC and ABC was carried out according to Saito *et al.* (1968). Approximately 100 μg of non-labelled glycosaminoglycans or 10000 c.p.m. of ^{35}S-labelled glycosaminoglycans were incubated with 0.01 units of chondroitinase AC-II from *Arthrobacter aurescens* or 0.01 units of chondroitinase ABC from *Proteus vulgaris* for 8 h at 37°C in 0.05 M ethylenediamine:acetate buffer (pH 8.0) in a final volume of 50 μl. After incubation, 10 μl of each mixture was analysed by agarose gel electrophoresis, as described above. The remaining 40 μl was applied to Whatman No. 1 chromatography paper and developed in isobutyric acid:1 M NH_4OH (5:3, v/v) for 24 h. The products were detected by silver nitrate staining, and the compounds showing chromatographic migration identical to that of standard disaccharides (ΔGlcUA-GalNAc4S and ΔGlcUA-GalNAc6S) were quantified by densitometry. The ^{35}S-labelled degradation products were detected by autoradiography of the chromatograms. The radioactive bands having identical chrom-

atographic migration to standard disaccharides were cut out and counted in 10 ml of PPO/toluene.

Deamination by nitrous acid at pH 4.0 was performed as described by Shively and Conrad (1976). Briefly, approximately 10000 c.p.m. of ^{35}S-labelled glycosaminoglycans were incubated with 6 μl of 5% $NaNO_2$ and 2 μl glacial acetic acid at 30°C for 90 min. About 10 μl 2% ammonium sulphamate was added to the incubation mixture to destroy the nitrous acid.

Results

Glycosaminoglycans from schistosome-induced granulomas

Glycosaminoglycans extracted from chronic granulomas were identified by agarose gel electrophoresis and degradation with specific mucopolysaccharidases (Fig. 1A). The major electrophoretic band had the same mobility as the dermatan sulphate standard; it decreased slightly in intensity after incubation with chondroitinase AC and disappeared totally after chondroitinase ABC treatment. The less intense band had the same mobility as the heparan sulphate standard and resisted chondroitinase ABC (Fig. 1A), but disappeared totally from the gel after deamination by nitrous acid (not shown).

The products of chondroitinase AC and ABC digestion were also analysed by paper chromatography. After incubation with chondroitinase ABC only unsaturated 4-sulphated disaccharides were formed. No unsaturated non-sulphated disaccharides (ΔGlcUA-GalNAc or ΔGlcUA-GlcNAc) or 6-sulphated disaccharide (ΔGlcUA-GalNAc6S) appeared after treatment with either chondroitinase (not shown).

When granulomas were incubated with radiolabelled sulphate during 24 h, the migration of the ^{35}S-labelled glycosaminoglycans on agarose gels and the pattern of chondroitinase degradation (Fig. 1B) were the same as for non-labelled glycans, except for slight increases in the relative amount of heparan sulphate in the ^{35}S-labelled extracted.

Overall, these results indicate that the main glycosaminoglycan in schistosome-induced granuloma is dermatan sulphate.

Distribution of sulphated glycosaminoglycans in different compartments of confluent cultures of GR and GRX cells

Table 1 shows the total and relative amounts of ^{35}S-labelled glycosaminoglycans in the intracellular, pericellular and extracellular pools of GR and GRX cell lines after a 24-h labelling period. For both cell lines, distribution of the ^{35}S-labelled glycosaminoglycans among the three compartments was similar. However, the total amount of ^{35}S-labelled glycosaminoglycans in the GR cell line was about twice that in the GRX, which suggests that GR cells have a higher rate of glycosaminoglycan synthesis than GRX cells.

Characterization of ^{35}S-sulphated glycosaminoglycans from various compartments of GR and GRX cell lines

On agarose gels, the ^{35}S-sulphated glycosaminoglycans from all three compartments of both cell lines had one component that migrated at the same rate as the standard heparan sulphate and a second component, hereby denominated chondroitin sulphate, that migrated between the dermatan sulphate and chondroitin 4/6-sulphate standards (Fig. 2a). The identification of this component as ^{35}S-chondroitin sulphate was based on the results of digestion with chondroitinase AC and ABC, as follows.

Chondroitinase AC degraded most of the ^{35}S-chondroitin sulphate from GRX cells, while most of the ^{35}S-chondroitin sulphate from GR cells were still detectable in the agarose gel after chondroitinase AC digestion (Fig. 2b). Chondroitinase ABC totally degraded the ^{35}S-chondroitin sulphate from both cell lines (Fig. 2c). Overall, these results suggested that ^{35}S-chondroitin sulphate from GR cells possesses higher proportions of

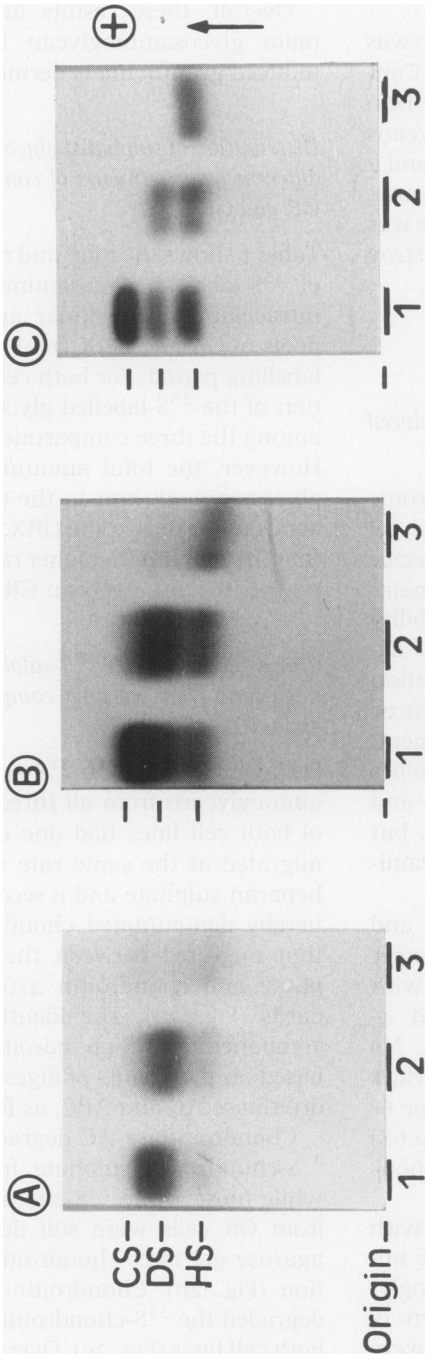

Fig. 1. Agarose gel electrophoresis of the glycosaminoglycans from schistosome-induced chronic granulomas. In A and B, 10 μg of ^{35}S-glycosaminoglycans extracted from schistosome-induced granulomas were incubated with 2, chondroitinase AC-II or 3, chondroitinase ABC in 0.05M ethylenediamine: acetate buffer (pH 8.0) at 37°C for 8h, in a final volume of 50μl. Controls (1) were incubated in the absence of enzyme. After incubation, 10-μl aliquots were applied to 0.5% agarose gels and electrophoresis was carried out in 0.05 M 1,3-diaminopropane: acetate buffer (pH 9.0), for 1 h at 120 V. The glycosaminoglycans in the gel were fixed with 0.1% Cetavlon for 12 h, and stained with 0.1% toluidine blue in acetic acid/ethanol/water (0.1:5:5, v/v) (A). The radioactive bands corresponding to the ^{35}S-sulphated glycosaminoglycans were detected by autoradiography of the fixed and stained gel (B). In C, a mixture of standard glycosaminoglycans containing 10 μg each of chondroitin 4-sulphate (CS), dermatan sulphate (DS) and heparan sulphate (HS) were analysed by agarose gel electrophoresis, 1, before and after incubation with 2, chondroitinase AC or 3, chondroitinase ABC.

Table 1. Distribution of the ^{35}S-labelled glycosaminoglycans in the various compartments of GR and GRX cells. The ^{35}S-labelled glycosaminoglycans from the intracellular, pericellular and extracellular pools were determined as described in Methods. The values in parentheses are the percentages of the total ^{35}S-labelled glycosaminoglycans appearing in each of the three compartments

	^{35}S-labelled glycosaminoglycans (c.p.m./10^6 cells)		
Cell line	Intracellular	Pericellular	Extracellular
GR	35 450 (25%)	73 700 (53%)	30 750 (22%)
GRX	18 270 (19%)	58 370 (63%)	16 060 (18%)

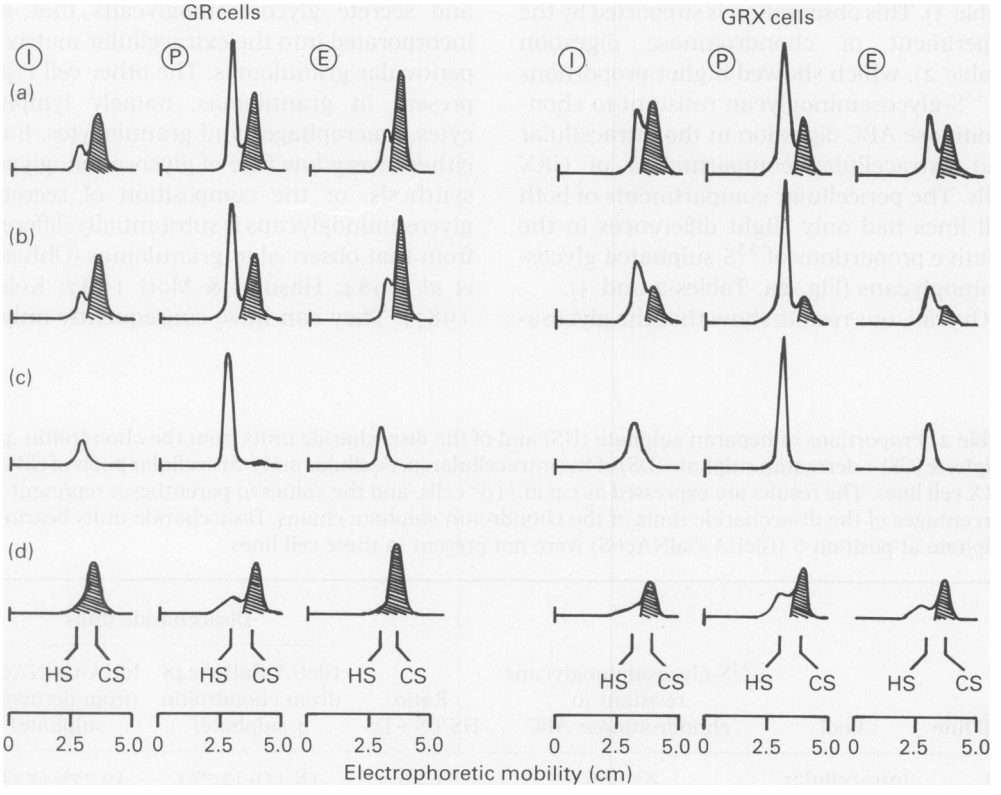

Fig. 2. Densitometry of agarose gel electrophoretograms of the ^{35}S-sulphated glycosaminoglycans from I, the intracellular; P, pericellular; and E, extracellular compartments of GR and GRX cells, a, before and after incubation with b, chondroitinase AC or c, chondroitinase ABC or d, after deaminative cleavage by nitrous acid. The agarose gel electrophoresis was performed as described in the legend of Fig. 1, and the radioactive bands were quantitated by densitometry of the X-ray films. The ^{35}S-chondroitin sulphate peaks are cross-hatched.

iduronic acid units than chondroitin sulphate from GRX cells. In fact, the analysis of the products formed by chondroitinase digestions (Table 2) showed a higher proportion of *Id*UA-GalNAc4S units, derived from dermatan sulphate, in the three compartments of GR cells when compared with the same compartments of GRX cells. In contrast, *Glc*UA-GalNAc4S units, which are derived from chondroitin 4-sulphate, occur in higher proportion in GRX than in GR cells.

Finally, higher proportions of ^{35}S-chondroitin sulphate and lower proportions of ^{35}S-heparan sulphate were found in the intracellular and extracellular compartments of GR cells, when compared with the same compartments of GRX cells (Fig. 2a and Table 3). This observation is supported by the experiment of chondroitinase digestion (Table 2), which showed higher proportions of ^{35}S-glycosaminoglycan resistant to chondroitinase ABC digestion in the intracellular and extracellular compartments of GRX cells. The pericellular compartments of both cell lines had only slight differences in the relative proportions of ^{35}S-sulphated glycosaminoglycans (Fig. 2a, Tables 2 and 3).

Overall, our results show that the glycosaminoglycan composition of the whole granuloma (Fig. 3a) is similar to that observed in the intracellular and extracellular compartments of GR cells (Fig. 3b). It differs substantially from that of the GRX cells (Fig. 3c).

Discussion

Our results suggest that GR cells are the major cell population involved in synthesis and accumulation of glycosaminoglycans in the schistosomal periovular granulomas in liver. The glycosaminoglycan composition of the whole granuloma is similar to that observed in the intracellular and extracellular compartments of the GR cells (Fig. 3), suggesting that GR cells actively synthesize and secrete glycosaminoglycans that are incorporated into the extracellular matrix of periovular granulomas. The other cell types present in granulomas, namely lymphocytes, macrophages and granulocytes, have either a very low rate of glycosaminoglycan synthesis, or the composition of secreted glycosaminoglycans is substantially different from that observed in granulomas (Ohhashi *et al.* 1984; Hasumi & Mori 1987; Kolset 1987). They can have consequently only a

Table 2. Proportions of heparan sulphate (HS) and of the disaccharide units from the chondroitin 4/6-sulphate (CS) + dermatan sulphate (DS) of the intracellular, pericellular and extracellular pools of GR and GRX cell lines. The results are expressed as c.p.m./10^6 cells, and the values in parentheses represent the percentages of the disaccharide units of the chondroitin sulphate chains. Disaccharide units bearing a sulphate at position 6 (GlcUA-GalNAc6S) were not present in these cell lines

Cell line	Pool	^{35}S-glycosaminoglycans resistant to chondroitinase ABC	Ratio HS/CS + DS	Disaccharide units: GlcUA-GalNAc4S (from chondroitin 4-sulphate)	Disaccharide units: IdUA-GalNAc4S (from dermatan sulphate)
GR	Intracellular	87 066	1.28	18 419 (27%)	49 776 (73%)
	Pericellular	255 764	3.60	31 243 (43%)	41 404 (57%)
	Extracellular	45 940	0.82	31 964 (57%)	24 120 (43%)
GRX	Intracellular	42 827	4.04	5301 (50%)	5301 (50%)
	Pericellular	75 599	4.94	9042 (59%)	6267 (41%)
	Extracellular	20 100	2.70	5122 (70%)	2196 (30%)

Table 3. Total and percentage amounts of ^{35}S-labelled glycosaminoglycans from the intracellular, pericellular and extracellular compartments of GR and GRX cell lines. The ^{35}S-glycosaminoglycans were identified by agarose gel electrophoresis (see Fig. 2). The radioactive bands having identical electrophoretic migration to heparan sulphate (HS) and chondroitin sulphate (CS) standards were scraped and radioactivity counted in 10ml of 0.5% PPO/toluene solution. The values in parentheses are the percentages of heparan sulphate or chondroitin sulphate

		^{35}S-labelled glycosaminoglycans (c.p.m./10^6 cells)		
Cell line	Fraction	Intracellular	Pericellular	Extracellular
GR	total	60 597	125 981	52 563
	HS	15 149 (25)	79 368 (63)	14 192 (27)
	CS	45 448 (75)	46 613 (37)	38 371 (73)
GRX	total	12 775	40 811	11 230
	HS	6004 (47)	29 792 (73)	5615 (50)
	CS	6771 (53)	11 019 (27)	5615 (50)

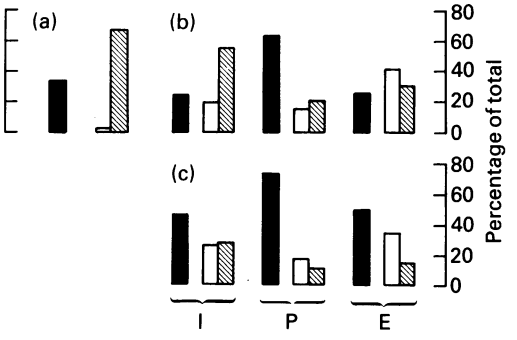

Fig. 3. Proportions of heparan sulphate and chondroitin sulphate isolated from a, schistosome-induced granuloma and from I, the intracellular, P, pericellular and E, extracellular compartments of b, GR and c, GRX cells. The proportions of ■, heparan sulphate were calculated from the data of Table 3, and the proportion of the two disaccharides found in the chondroitin sulphate chains were obtained from the data of Table 2. Data are normalized so that the total glycosaminoglycan content of each compartment equals 100%. □, GlcUA-GalNAc4S, 2-acetamido-2-deoxy-3-O-(β-D-glycopyranosyluronic acid)-4-O-sulpho-D-galactose (disaccharide from chondroitin 4-sulphate). ▨, IdUA-GalNAc4s, 2-acetamido-2-deoxy-3-O-(α-L-idupyranosyluronic acid)-4-O-sulpho-D-galactose (disaccharide from dermatan sulphate).

minor participation in glycosaminoglycan deposition into the granuloma extracellular matrix.

Since the two cell lines studied have a similar pericellular glycosaminoglycans composition, it may be concluded that the conversion of the primary cell line GR into the established GRX cells did not modify the pattern of cell coat glycosaminoglycans. However, it decreased the rate of glycosaminoglycan synthesis and secretion in the three cell compartments, reduced the proportion of iduronic acid units in the chondroitin sulphate and increased the relative proportion of heparan sulphate in intracellular and extracellular pools. The decrease in dermatan sulphate synthesis after establishment of cells in culture has been observed in other studies. Several established cell lines examined previously contain mainly heparan sulphate and/or chondroitin 4/6-sulphate, but no dermatan sulphate (Mourão & Machado-Santelli 1978). In addition, the absence of dermatan sulphate and a slight increase in the amount of heparan sulphate were also observed after several subcultures of other primary cell lines (Mourão & Machado-Santelli 1978; Mourão *et al.* 1980).

Changes in some of the original properties of cells, in relation to the synthesis and secretion of macromolecules, have been reported when cells are grown *in vitro*. In general, mesenchymal cells tend to de-differentiate and acquire a general 'fibroblastoid' morphology and behaviour. Examples are smooth muscle cells in which synthesis of collagen type III decreases in favour of type I collagen, the shift of collagen secretion from type II to types I and III in chondroblasts, and from type IV to type I in endothelial cells, during their de-differentiation *in vitro* (Beldekas *et al.* 1982; Benya *et al.* 1978; Tseng *et al.* 1982). Liver connective-tissue cells in culture acquire the myofibroblastic phenotype typical of several other perivascular cell lineages. This is in agreement with the concept of a single and specific mesenchymal cell type, different from fibroblasts, that differentiates into pericytes and other perivascular cells with myofibroblastic characteristics. These cells have apparently a relatively wide capacity of differentiation and they are reactive in reparative and regenerative processes in response to tissue injuries (Borojevic *et al.* 1985b; Schurch *et al.* 1981).

Glycosaminoglycans are among the major components of extracellular matrix, and their close association with collagen fibres led various authors to propose their participation in the formation, maintenance and regulation of collagen deposits (Scott 1988). The isolation and characterization of a cell line responsible for synthesis of sulphated glycosaminoglycans in schistosome-induced granulomas, as reported in this study, may constitute a useful experimental model for studies on establishment and turn-over of hepatic fibrosis in schistosomiasis, as well as to understand interactions among cell populations in granulomas. It has been shown that proliferation and extracellular matrix secretion in liver connective tissue cells may be modified by cytokines (Zerbe & Gressner 1988). The sensitivity of specific cell populations isolated from granulomas, such as GR, to these factors should be an experimental model closer to the in-vivo situation than studies in which skin fibroblasts are used as target cells. On the other hand, it has been shown that schistosomal granulomas represent unique structures with local production of cytokines that mediate proliferation and differentiation of inflammatory cells (Borojevic *et al.* 1989, 1990b; El-Cheikh *et al.* 1990). In particular, it has been shown that GR cells may represent an extramedullar myelopoietic stroma that may mediate a long-term proliferation of macrophages (Borojevic *et al.* 1990a). Roberts *et al.* (1988) have shown that glycosaminoglycans bound myelopoietic growth factors and presented them to progenitor cells. This molecular mechanism may explain the ability of GR cells to function as myelopoietic stroma, and modulations of glycosaminoglycan synthesis and secretion may be one of the control points of extramedullar amplification of inflammatory cell populations in granulomas. GR cells are thus simultaneously sensitive to cytokines present in granulomas and they may actively modulate the activity of other cell populations through interaction of their glycosaminoglycans with locally secreted cytokines.

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