Induction of Chondroitin Sulfate Proteoglycan Synthesis and Secretion in Lymphocytes and Monocytes

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ABSTRACT   The ability of mononuclear leukocytes to synthesize and secrete proteoglycans was evaluated. Using radiolabeling with H_2 $^{35}SO_4$, it is shown that peripheral blood mononuclear cells (PBMC) and their major subpopulations (B cells, T cells, and monocytes), as well as mouse spleen cells, all secreted easily detectable proteoglycan. After 24-h labeling periods, 90% of macromolecular ^{35}S could be detected in culture media. This material was primarily (>95%) chondroitin-4-sulfate proteoglycan (CSPG). Production and secretion of CSPG could be stimulated more than 200% in PBMC and 300% in T cell populations by high concentrations of concanavalin A and phorbol 12-myristate-13-acetate; lipopolysaccharide induced a small (twofold) but reproducible increase in CSPG secretion by adherent mononuclear leukocytes. The CSPG secreted by PBMC was relatively small in size compared to chondrocyte CSPG (130,000 daltons vs. 2-4 million daltons) but possessed similar sizes of glycosaminoglycan chains and greater solubility in low ionic strength solutions. This sulfated polyanion, which was produced endogenously by leukocytes and was actively secreted, might function as a co-mediator or "second messenger" in certain immune responses.

Sulfated proteoglycans (PG)[1] are complex macromolecules consisting of polysaccharide chains containing uronic acid (D-glucuronic or L-iduronic acids) and N-acetylamino sugars covalently linked to a protein core (1, 2). The best characterized PG are the chondroitin sulfate proteoglycans (CSPG) of cartilage. These molecules usually form large, matrix-bound aggregates with hyaluronic acid and link protein (3–5).

Chondroitin sulfate proteoglycan has been isolated from a large number of cells and tissues other than cartilage, is a major component of virtually all connective tissues, and is synthesized by many nonconnective tissue cells (6–17). In some instances, CSPG isolated from noncartilage cells can form macro-aggregates with hyaluronic acid, and may even share core protein antigenic identities with cartilage CSPG (18–20).

The potential to produce chondroitin sulfate (CS) exists in virtually all cells. Either D-xylose or β-D-xylosides can substitute for both the core protein and first xylose residue and

permit cells to express their biosynthetic potential for CS chain production in the absence of core protein (21–25). Cells producing minimal quantities of CSPG appear to lack the capacity for translating a core protein acceptor but do possess the multienzyme glycosyl transferase complexes capable of creating CS chains linked to xyloside acceptors. These CS chains are shorter than CS chains linked to core protein (24). It has also been demonstrated that CSPG produced by non-cartilage cells is smaller than cartilage CSPG (26). In some instances, only 1–2 CS chains (compared with 25–40 in cartilage) are linked to core protein, yielding PG with molecular weights between 70,000 and 170,000 daltons (18).

Although sulfated proteoglycans can be produced by several types of cells, the function(s) of these macromolecules, apart from their structural roles in connective tissues, are still unclear. Low molecular weight chondroitin 4-sulfate isolated from human serum inhibits the hemolytic activity of Clq (27). Alterations in CSPG content and metabolism occur during distinct stages of organogenesis and may effect cell migration and tissue morphology (28).

The immune system is composed of networks of interacting cells and molecules where specific humoral or cellular functions can be easily analyzed. While the effects of cartilage proteoglycans on immune responses have not been well char-

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; CS, chondroitin sulfate; CSPG, chondroitin sulfate proteoglycan; Δ Di-0S, disaccharide o-sulfate; Δ Di-4S, disaccharide 4-sulfate; Δ Di-6S, disaccharide 6-sulfate; GAG, glycosaminoglycan; LPS, *E. coli* 055:BS lipopolysaccharide; PBMC, peripheral blood mononuclear cells; V_o, void volume; V_t, total volume.

acterized (29, 30), it is apparent that certain sulfated poly-anions can exert significant influences on immune responses (31, 32) and may even alter hematopoiesis (33). Since non-cartilage cells can synthesize PG, it seemed possible to us that polyanions endogenously produced by leukocytes themselves might act as intermediaries in the regulation of various immune responses. As the first step towards analyzing potential effects of PG on the immune system, we evaluated its presence in white blood cells. We report here that low molecular weight CSPG are ubiquitously synthesized and secreted by normal human and mouse lymphocytes and monocytes. More important, T lymphocytes, the major immunoregulatory cells in mammals, can be selectively stimulated by specific compounds to increase their synthesis and secretion of CSPG.

MATERIALS AND METHODS

Materials

RPMI-1640 and pokeweed mitogen (PWM) were purchased from Gibco Laboratories (Grand Island, NY), fetal bovine serum from K. C. Biologicals, Inc. (Lanexa, KA), concanavalin A (Con A), aminoethylisothiouronium bromide (AET), nonspecific protease, soy bean trypsin inhibitor, phenylmethyl-sulfonyl fluoride (PMSF), e-aminocaproic acid and phorbol 12-myristate 13-acetate (TPA) from Sigma Chemical Co. (St. Louis, MO), phytohemagglutinin M (PHA-M), and *Escherichia coli* 055:B5 lipopolysaccharide (LPS) from Difco Laboratories (Detroit, MI), sheep erythrocytes from Flow Laboratories (McLean, VA), H_2 $^{35}SO_4$ (43 Ci/mg) and thymidine (methyl-^{3}H) (6.7 Ci/mmol) from New England Nuclear (Boston, MA), Sepharose 6B, Sephacryl S-200, Sephadex G-50, G-10 and Ficoll-isopaque from Pharmacia Fine Chemicals (Piscataway, NJ), bovine testicular hyaluronidase from Leo (Helsingborg, Sweden), chondroitinase AC II, chondroitinase ABC, and standard disaccharides (Δ Di-OS, Δ Di-4S and Δ Di-6S) from Miles Laboratories (Elkhart, IN). BALB/c mice were purchased from Cumberland View Farms (Clinton, TN) and Jackson Laboratories (Bar Harbor, ME).

Cell Lines

Cell lines were derived from peripheral blood cells of normal, immunodefi-cient, and autoimmune individuals and individuals with lymphoid malignan-cies by infecting B cells with Epstein-Barr virus (34). The 7-0Z/3 mouse cell line and its subclones 70Z/3.1.4 and 4.1. were obtained from Dr. Michael Loken (University of Chicago). Cell lines were cultured in RPMI-1640 media containing 10% fetal bovine serum, 5 × 10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (complete media). Cells were fed every 2-3 d by splitting 1:4 with complete medium.

Separation of Peripheral Blood Mononuclear Cell Fractions

Heparinized venous blood obtained from normal volunteers was separated on Ficoll-hypaque cushions (35). Monocytes were depleted from peripheral blood mononuclear cells (PBMC) with Sephadex G-10 (36). Monocyte-enriched cells (70-80% nonspecific esterase positive) were obtained by washing the G-10 beads with 100% fetal bovine serum at 4°C for 60 min.

To separate B cell-enriched fractions from T cells, monocyte-depleted PBMC were incubated with AET-treated sheep erythrocytes (37). The B cell fraction contained 50-60% surface Ig$^+$ cells, 15-25% esterase positive cells, 10-25% null cells, and 3-6% OKT3$^+$ cells. The T cell fraction possessed 95-98% T cells determined by immunofluorescent staining with the monoclonal pan T cell reagent OKT3 (38).

Isolation of Mouse Spleen Cells

Spleens were removed from adult (3-6 mo) BALB/c female mice, then dissociated into single cell suspensions using sterile frosted glass slides or wire mesh (Cellector, Bellco Glass Co., Vineland, NJ). Erythrocytes were hypotoni-cally lysed and debris was removed by filtering cells through nylon mesh. Spleen cells prepared in this manner were >92% viable (determined by exclusion of 0.2% trypan blue dye) and contained an average of 45% surface Ig$^+$ B cells and 35% thy 1.2$^+$ T cells by fluorescence microscopy and 10% esterase-positive macrophages.

Radiolabeling of Sulfated Proteoglycans and DNA

Cells were cultured in complete media containing different concentrations of mitogen (dose ranges of mitogens: (a) Con A: 1-100 μg/ml; (b) LPS: 5-100 μg/ml; (c) pwm: 1/100-1/800 (vol/vol); (d) PHA: 1-100 μl/ml; (e) Staphylo-coccus aureus Cowans I (SA): 1:1,000-1:20,000 (vol/vol); (f) TPA: 0.5-25 ng/ml) and 25 μCi/ml H_2 $^{35}SO_4$ for 24-120 h. In some experiments, 24 h before harvest 5 μCi/ml [^{3}H]thymidine were added to duplicate cultures to analyze for DNA synthesis. Cells were harvested onto glass fiber filters using a multiple automated sample harvester (Flow Laboratories, McLean, VA); filters were dried and counted in a Packard 3000 TriCarb Liquid Scintillation Counter.

Analysis of Proteoglycans

MEDIA: Radiolabeled-culture medium was dialyzed against 20 liters of H_2O, then lyophilized. The sample was dissolved in 0.05 M sodium acetate buffer, pH 6.8, containing either 0.15 M NaCl or 4 M guanidine HCl plus proteolytic inhibitors PMSF (2 mM), e-aminocaproic acid (1 mM), and soybean trypsin inhibitor (1 mg/ml). To determine the size of intact PG, samples were chromatographed on a 1.5 cm × 50 cm column of Sepharose 6B or a 1 cm × 85 cm column of Sephacryl S-200 or Sephadex G-50 equilibrated with 0.05 M sodium acetate buffer, pH 6.8, plus 0.15 M NaCl. Radioactivity in 1-ml fractions was determined by liquid scintillation counting.

CELLS: After washing with 0.15 M NaCl, cells were lysed with borate-saline buffer, pH 8.2 containing 0.02 M KCl, 0.5% Na deoxycholate, 0.5% Nonidet P-40, 0.1% NaN$_3$, and proteolytic inhibitors for 30 min on ice. Debris was sedimented for 10 min at 10,000 g and the supernatant was dialyzed against 20 l of H_2O, then treated the same as the culture media.

Quantitation and Analysis of Sulfated Glycosaminoglycans

Incorporation of H_2 $^{35}SO_4$ into glycosaminoglycans was determined for both media and cell lysates. After dialysis against H_2O and lyophilization, material from media and cells was resuspended in phosphate-buffered saline (pH 7.4) and digested with 1.5 mg/ml of autodigested protease for 3 h at 37°C. Glycos-aminoglycans were isolated from digests using 10% cetylpyridinium chloride (39), and radioactivity was measured by liquid scintillation counting. The capacity of cells to produce sulfated glycosaminoglycan chains was determined using 4-methylumbelliferyl-β-D-xyloside as previously described (22).

The size of sulfated glycosaminoglycans was analyzed after their release from core protein using 0.5 N NaOH for 18 h at room temperature. After being neutralized with 6 N HCL, the digests were chromatographed on Sepharose 6B or Sephacryl S-200 and the size of labeled material was calculated according to Wasteson (40).

The percentage of chondroitin sulfate in each sample was determined by digestion of sulfate-labeled material larger than the total volume V_t with chondroitinase ABC or AC II to distinguish between chondroitin 4- and 6-sulfate and dermatan sulfate. The percentage of heparan sulfate in culture media was analyzed after treating the samples with nitrous acid (41-42). In both instances, released or digested ^{35}S-labeled material was analyzed on Sephadex G-50 columns eluted with 0.15 M NaCl.

Analysis of 4- Vs. 6-Sulfation

Sulfate-labeled material derived from culture media or cell lysates of PBMC or spleen cells was initially separated on Sephadex G-50. Radioactivity migrat-ing in the void volume V_o was digested with chondroitinase AC, then rechro-matographed on Sephadex G-50. Counts isolated in the V_t were concentrated by lyophilization, then separated by descending paper chromatography (43) using standard disaccharides to determine distances of migration. Radioactivity was eluted from serially sliced 2-cm × 2-cm pieces of paper using 0.15 M NaCl, then counted. In some instances, chromatograms were exposed to Kodak XAR x-ray film to determine the migration patterns of released disaccharides.

RESULTS

Synthesis of PG and Sulfated Glycosaminoglycan (GAG) by Lymphoblastoid Lines

To assess whether lymphoid cells could produce proteogly-cans, cell lines possessing phenotypic similarities with normal B cells at various stages of development were radiolabeled with H_2 $^{35}SO_4$, and relative quantities of PG were measured.

All cell lines that were evaluated synthesized and secreted sulfated PG (Table I). <10% of incorporated ^{35}S remained cell-associated after the 24-h labeling period. The capability of each cell line to produce sulfated glycosaminoglycan chains was explored by adding the galactose acceptor 4-methylum-belliferyl-β-D-xyloside to replace core protein (22–25). Lymphoblastoid lines could increase synthesis of GAG 1.4–5-fold above endogenous levels (Table I), confirming the substantial biosynthetic potential for sulfated polysaccharides present in each B cell line.

Interestingly, the mouse "pre-B" cell line 70Z/3 also secreted sulfated proteoglycans (Table II). Two clones of 70Z/3, 70Z/3.1.4, and 70Z/3.4.1 were labeled with H_2 [35]SO_4 for 24 h in the presence or absence of LPS. LPS induces surface IgM expression in 70Z/3.1.4 cells, but not in 70Z/3.4.1 cells. Three- to fivefold increases in ^{35}S incorporation occurred in the presence of LPS inboth cell lines, while increased GAG secretion in the presence of β-D-xyloside was consistently greater for the 70Z/3.4.1 cell line. Thus, synthesis and secretion of PG did not correlate with induction of surface Ig expression in these cell lines.

Normal Lymphocytes Secrete PG

Since lymphoblastoid cell lines produced sulfated PG, we determined whether normal lymphocytes produced and secreted these macromolecules. Both total peripheral blood

TABLE I

Secretion of Sulfated Proteoglycans by Human Lymphoblastoid Cell Lines

Cell line*	CPM ^{35}S secreted/10^6 cultured cells[‡]	
	Unstimulated	With xyloside
LBW-8	630	1,230
Nalm-6	2,500	10,940
Daudi	4,300	7,130
DB	18,545	78,253
F4	8,150	44,120
LBW-17	13,580	18,140
Stern	12,430	ND
LBW-25	11,471	26,895

* Cell lines were loosely classified according to B cell phenotype as: LBW-8: null; Nalm-6: pre-B; Daudi, DB, F4: B lymphoblast; LBW-17, Stern, LBW-25: plasmacyte.
‡ Nondialyzable, water-soluble radioactivity was isolated from culture media after 24-h incubation with 10 μCi/ml H_2 [35]SO_4 as described in Materials and Methods. For xyloside stimulation, 10^{-4} M 4-methylumbelliferyl-β-D-xyloside was added at the initiation of culture. Specific activities of H_2 [35]SO_4 used for labeling were the same in all experiments and were corrected for radioactive decay.

TABLE II

Stimulation of Proteoglycan Secretion by LPS in 70Z/3 Mouse Cell Lines

Cell line*	LPS[‡]	CPM ^{35}S secreted/10^6 cultured cells[§]	
		Unstimulated	With xyloside
70Z/3.1.4	−	1,680	3,520
	+	6,860	9,140
70Z/3.4.1	−	1,520	2,790
	+	5,230	13,930

* 70Z/3.1.4 can be induced to express surface IgMk with LPS. 70Z/3.4.1 is unable to express surface IgM after LPS stimulation.
‡ 25 μg/ml.
§ See ‡, Table I.

TABLE III

Cell-associated and Secreted Sulfated PG Produced by Human PBMC Populations

Exp. no.		CPM S[35]/10^6 cells*			
		PBMC	B cells	T cells	Monocytes
1	Cells	2,305	1,115	2,005	3,210
	Media	27,700	35,270	21,500	32,250
2	Cells	1,900	870	1,875	3,220
	Media	20,805	16,360	25,620	26,210
3	Cells	2,290	1,630	1,325	3,800
	Media	30,805	15,000	21,820	19,520

* Cultures were incubated for 4 d in media containing 25 μCi/ml H_2 [35]SO_4. Media were removed and dialyzed against 20 l of H_2O. Cells were washed, then lysed. Debris was sedimented at 10,000 g and the supernatant was dialyzed against H_2O. Results are expressed as sulfate incorporation into nondialyzable water-soluble material per 10^6 cultured cells.
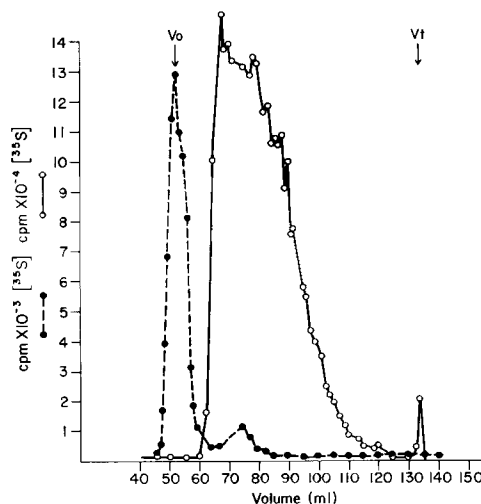

FIGURE 1 Migration of proteoglycans isolated from media of mononuclear leukocyte cultures on Sephacryl S-200. Sulfate-labeled proteoglycans were separated on 1-cm × 85-cm columns of Sephacryl S-200. Large molecular weight material (●) was treated with 0.5 N NaOH and rechromatographed on the same column (O). Vo, void volume; Vt, total volume.

mononuclear cells and mononuclear cell subpopulations secreted easily detectable sulfated PG (Table III, see below). Further, the three major subpopulations of PBMC, i.e., monocytes, B lymphocytes, and T lymphocytes, secreted approximately equivalent quantities of these molecules after pulse labeling with H_2 [35]SO_4. Most of the sulfate-labeled material was actively secreted from cells, since <10% of the ^{35}S counts were cell-associated after the 4-d culture period (Table III).

Characterization of Sulfated PG

We next evaluated the characteristics and types of sulfated material synthesized and secreted by human PBMC. Undigested, secreted material from both lymphocyte and monocyte populations was completely soluble in water, distinguishing it from matrix-bound CSPG made by chondrocytes. It migrated in the V_o of Sephadex G-50 and Sephacryl S-200 (Fig. 1). Chromatography on Sepharose 6B revealed a relatively polydisperse molecule with a K_{av} of 0.23 (Fig. 2). This elution pattern was consistent with an approximate molecular weight of 130,000, according to the estimates of Kanwar et al. (26) for similar material extracted from glomerular basement
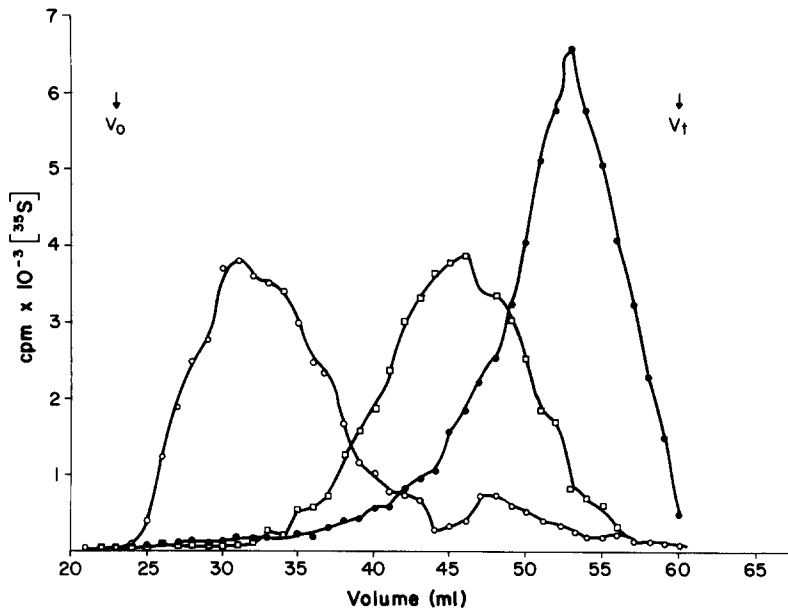
FIGURE 2 Migration of proteoglycans isolated from media of mononuclear leukocytes on Sepharose 6B. Proteoglycans isolated from media of normal PBMC (O) or PBMC incubated with 4-methylumbelliferyl-β-D-xyloside (●) were separated on a 1.5-cm × 50-cm Sepharose 6B column. Material migrating between 25 and 43 ml were treated with 0.5 N NaOH, then rechromatographed on the same column (□). Vo, void volume. Vt, total volume.
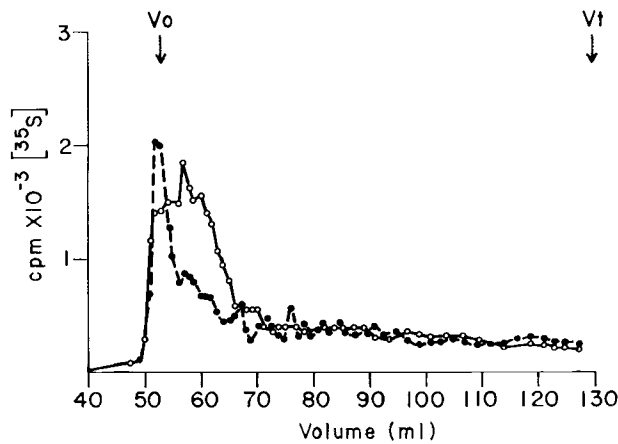

FIGURE 3 Migration on Sephacryl S-200 of proteoglycans isolated from the cells of cultured PBMC. Sulfate-labeled PBMCs were washed with 0.15 M NaCl then lysed with NP-40-deoxycholate buffer. Debris was removed by high speed centrifugation. The supernatant was dialyzed against elution buffer, then separated on a 1-cm × 85-cm column of Sephacryl S-200 (●). Material migrating between fractions 50 and 70 was treated with 0.5 N NaOH, then rechromatographed on the same column (O). Vo, void volume. Vt, total volume.

membrane. The elution patterns of PG were identical whether 0.15 M NaCl or 4 M guanidine HCl were employed as solvent, indicating that proteoglycans were not secreted as aggregates that were dissociated at high salt concentrations.

Sulfate-labeled molecules were extracted from washed cells after lysis with detergent buffer. Following centrifugation, soluble material was dialyzed against H_2O, lyophilized, then dissolved in either 0.15 M NaCl or 4 M guanidine HCl. Chromatography of this cell-associated PG on Sepharose 6B revealed a far more polydisperse pattern than its counterpart from medium; this ^{35}S-labeled material migrated primarily in the V_o of Sephacryl S-200 but could also be detected in the included fractions (Fig. 3).

Since water-soluble macromolecules that can be pulse-labeled with H_2 [35]SO_4 are generally considered to be proteoglycans (2), we explored which specific PGs were produced and secreted by PBMCs. Large molecular weight sulfate-

labeled material collected from either Sepharose 6B or Sephacryl S-200 columns was treated with HNO_3 (41, 42); the amount of released ^{35}S constituted the percentage of heparan sulfate in the sample. As shown in Fig. 4 and Table IV, <5% of ^{35}S from the media of Con A-stimualted PBMC was detected in the V_t of Sepharose 6B columns. The amount of chondroitin 4/6 sulfate and dermatan sulfate was determined by digesting the included material from 6B or S-200 columns with chondroitinase AC or ABC (44). Fig. 4 and Table IV demonstrate that an average of 98% of the ^{35}S radioactivity appeared in the V_t after chondroitinase AC or ABC treatment, suggesting that virtually all of the sulfated material was either chondroitin 4 or 6 sulfate. Sulfate-labeled material from media of unstimulated cultures of PBMC, B cells, or T cells consisted of 75 chondroitin 4/6 sulfate and 20% HNO_3-sensitive heparan sulfate (Table IV).

~75–80% of ^{35}S radioactivity was digested by chondroitinase AC in material isolated from cell lysates of Con A-stimulated mononuclear leukocytes, indicating that most of the intracellular PG possessed chondroitin sulfate polysaccharide chains. Another 15–20% of the radioactivity was degraded after incubation with HNO_3, demonstrating the presence of heparan sulfate in mononuclear cells. Between 5 and 10% of the sulfated material associated with cells could not be characterized and may be composed of sulfoglycoproteins. In cell lysates from unstimulated cultures, >90% of PG was heparan sulfate, chondroitin sulfate comprising only 5–10% of sulfate-labeled material (Table IV).

To evaluate the size of chondroitin sulfate chains produced normally by PBMC and spleen cells, CSPG from the V_o of Sephadex G-50 was either digested for 8 h with nonspecific protease or treated with 0.5 N NaOH to release polysaccharide chains from o-serine linkages. Released material migrated with a K_{av} of 0.54 on Sepharose 6B (Fig. 2) and an approximate molecular weight of 25,000, they were also included on Sephacryl S-200 columns (Figs. 1 and 3). As previously shown, chondroitin sulfate chains secreted by lymphocytes incubated with β-D-xylosides were somewhat shorter than chains released from normal proteoglycans and exhibited a molecular weight of 15,000 (Fig. 2). Paper chromatography of chondroitinase digests of ^{35}S-labeled CSPG revealed that virtually all of the radioactivity migrated identical to the Δ-Di-4-S

standard, indicating that only chondroitin 4-sulfate was produced by these immunocytes.

Mitogens Stimulate T Lymphocytes to Secrete More CSPG

We evaluated alterations of CSPG production in blood mononuclear cells treated with different "activators" to determine whether consistent changes in either biosynthetic rates or patterns of this molecule occurred. As shown in Fig. 5, both Con A and tetraphorbol acetate (TPA) induced the greatest increase in CSPG secretion by PBMC and T cells (Fig. 5 a and b). B cell populations were poorly stimulated to produce PG by any mitogen we used (Fig. 5 c) while monocytes were enhanced 1.5-2-fold by PWM and twofold by LPS (Fig. 5 d). No synergism between Con A and TPA for CSPG synthesis could be detected, suggesting that both molecules may induce the same population of cells.

The time and dose dependence of Con A-induced CSPG secretion by T cells was also evaluated (Fig. 6). A threefold increase in CSPG secretion was discernable by 3 d in vitro. Although the absolute amount of CSPG in media increased over the next 2 d of culture, the relative degree of stimulation remained approximately the same. To determine whether continued stimulation by Con A was required for enhanced

PG secretion, PBMC were incubated with 25 μg/ml Con A for 3 d, then labeled for 24 h in the presence or absence of Con A. A threefold decrease in the amount of CSPG secreted by cells labeled in the absence of Con A occurred (12,063 $\pm$ 355 cpm $^{35}SO_4$ vs. 37,255 $\pm$ 1,560 cpm $^{35}SO_4$), suggesting that continued physical contact with the cell surface is necessary for Con A to exert its effect on CSPG production.

The correlation between DNA synthesis and CSPG secretion induced by Con A was poor; while [^{3}H]thymidine incorporation generally displayed maximum activity at 5-10 μg/ml Con A and decreased with higher doses of this mitogen, CSPG secretion was greatest when >10 μg/ml were added to cultures and did not diminish at 50 μg/ml Con A (Fig. 7).

Mouse Spleen Cells Also Secrete CSPG

Since responses of murine lymphocytes to various mitogens have been well characterized, we investigated production of CSPG by spleen cells from these animals. When cells from BALB/c mice were cultured and labeled with H_2 $^{35}SO_4$, secretion of sulfated PG occurred rapidly. This material chromatographed identical to CSPG from human PBMC and was degraded by the same enzymatic treatment; therefore, the sulfated PG produced by mouse lymphocytes was also chondroitin sulfate proteoglycan. Spleen cells could be stimulated
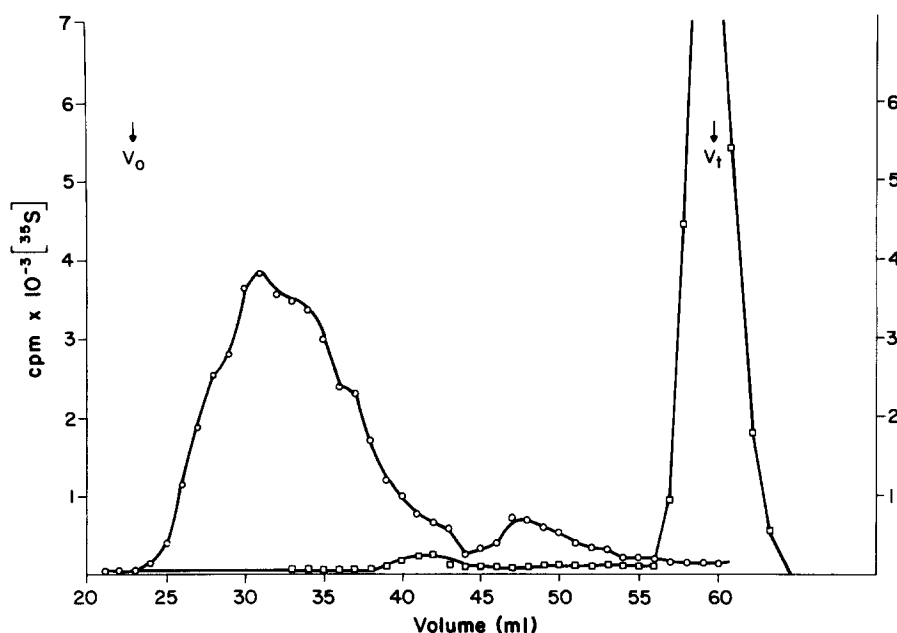
Figure 4   Digestion of PG by chondroitinase and HNO_3. Sulfate-labeled PG isolated from culture media of PBMC was separated on Sepharose 6B. Material migrating between 25 and 55 ml was concentrated and treated with either HNO_3 (O) to assess the quantity of heparan sulfate or with chondroitinase AC ($\square$) to analyze chondroitin sulfate content. V_o, void volume. V_t, total volume.

Table IV
Specific Degradation of Sulfated Proteoglycans from Peripheral Blood Mononuclear Cells

	Percentage of ^{35}S-labeled Material							
	Undigested*				Digested‡			
	Media		Cells		Media		Cells	
Treatment	U§	S§	U	S	U	S	U	S
HNO₃ (for heparan sulfate)	80	96	19	73	20	4	81	27
Chondroitinase ABC (for chrondroitin 4/6 sulfates and dermatan sulfate)	25	2	95	22	75	98	5	78
Chondroitinase AC (for chrondroitin 4/6 sulfates)	22	0.5	93	20	78	99.5	7	80

* Undigested = fractions 25-55 on Sepharose 6B column.
‡ Digested = fractions 57-65 on Sepharose 6B column.
§ U, unstimulated cultures; S, cells stimulated with 25 μg/ml Con A.

to secrete greater quantities of CSPG by Con A, predominantly a T cell mitogen, and not by LPS, a B cell activator (Table V); these findings were similar to the results from the human cultures.
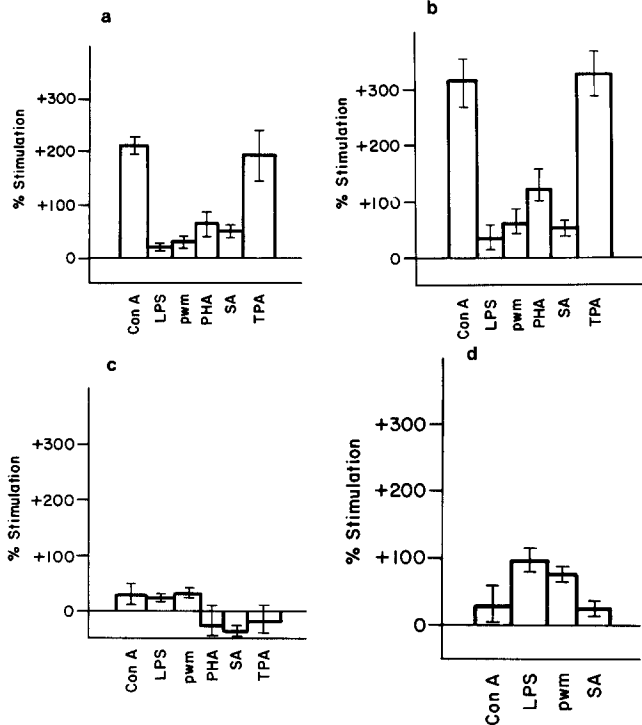
FIGURE 5   Stimulation of PG secretion by lympohcyte activators. PBMCs or mononuclear cell subpopulations were incubated with various concentrations of activators and 25 μCi/ml H_2 $^{35}SO_4$. After 4 d, culture media were harvested and PG determined. The concentrations of activators ultimately added to cultures were (a) Con A = 25 μg/ml, (b) LPS = 50 μg/ml, (c) pwm = 1/400 (vol/vol), (d) PHA = 25 μl/ml, (e) SA = 1:8,000 (vol/vol), (f) TPA = 10 ng/ml. Results are expressed as % change from cultures lacking these activators. (a) PBMC. (b) T cells. (c) B cell population. (d) Adherent cells.
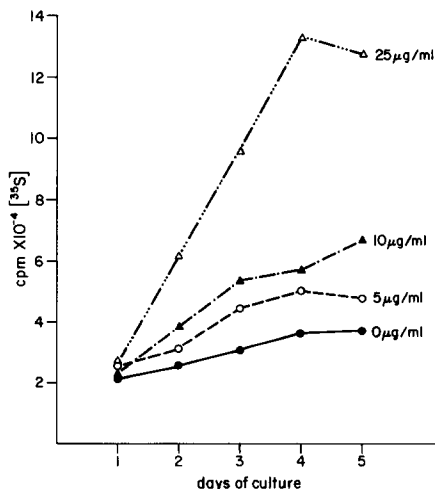
FIGURE 6   Dose and time course of Con A-stimulated PG secretion. 10^6 cells were incubated with Con A at the indicated concentrations plus 25 μCi/ml H_2 $^{35}SO_4$. Cultures were harvested on d 1–5 and PG quantitated in culture media.
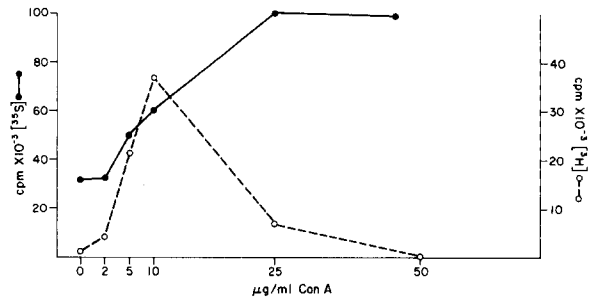
FIGURE 7   Proliferation vs. PG secretion induced by Con A. 10^6 PBMCs were stimulated with various concentrations of Con A. 25 μCi/ml H_2 $^{35}SO_4$ or 5 μCi/ml [^{3}H]thymidine were added to cultures on day 0 and day 3 respectively. Cultures were harvested on day 4; media were analyzed for PG secreted (●) and cells assessed for thymidine incorporation into DNA (O).

TABLE V

Secretion of Sulfated Proteoglycans by Mouse Spleen Cells

	cpm ^{35}S secreted/10^6 cells*		
Exp. no.	None	Con A	LPS
1	7,350	37,225	9,730
2	5,100	32,250	3,960
3	10,090	49,330	7,150

* 10^6 spleen cells/ml were cultured for 4 d with (a) no added stimuli, (b) 5 μg/ml Con A, or (c) 25 μg/ml LPS. Media were harvested and analyzed for sulfated proteoglycans as described in Materials and Methods.

DISCUSSION

Although chondroitin sulfate proteoglycans are macromolecules normally associated with cartilage, our results conclusively demonstrate that mononuclear cells from the immune systems of both humans and mice synthesize and secrete chondroitin sulfate linked to a core protein. However, there appear to be major differences between the two types of CSPG. The PG secreted by chondrocytes aggregates with link protein and hyaluronic acid to help form an insoluble matrix (3–5); it is a large macromolecule with a molecular weight between 2 and 3 million daltons (1, 2). The size of cartilage-specific core protein has been estimated to be 200,000–300,000 daltons (45, 46).

The CSPG derived from lymphocytes and monocytes is soluble in media, appears to be nonaggregated, and is ~1/20 the size of CSPG from cartilage. The CS chains are similar in size to those found in cartilage (54) (25,000 daltons; range 12–40,000 daltons) and preliminary gel filtration data suggest that the core protein is 30,000–35,000 daltons (D. Levitt and P.-L. Ho, unpublished observations). Thus, an average of only four CS chains per core protein molecule would be expected, which could help account for the high solubility of this protein-polysaccharide complex.

Chondroitin sulfate has been previously isolated from leukocytes obtained from human peripheral blood (6, 7) or rabbit bone marrow (8). In these studies, the quantities of CS, cells involved in its production, size of the PG molecule, and secretion of these sulfated polysaccharides were not determined. Our findings indicate that CSPG is actively secreted by both lymphocytes and monocytes; after only a 24-h labeling period, 90% of incorporated $^{35}SO_4$ can be found in the culture media. Unstimulated culture supernatants contained both chondroitin 4-sulfate and heparan sulfate in a 3:1 ratio

(based upon $^{35}SO_4$ incorporation). After stimulation with either Con A or TPA, the percentage of chondroitin sulfate increased to >90% of the isolated GAG. Further, heparan sulfate comprised >90% of the sulfated GAG from "resting" PBMC lysates and only 20% of sulfate-labeled material in cell lysates from stimulated cultures. Thus, the ratio of different types of sulfated GAG vary in mononuclear leukocytes depending upon their state of activation. Some shedding of heparan sulfate, a PG normally associated with the cell surface, (50–53) occurs in vitro, but CSPG is the proteoglycan that is actively secreted by mononuclear leukocytes. Such cells may be responsible for producing the chondroitin-4 sulfate present in serum (27).

Low molecular weight CSPG can be produced and secreted by noncartilage cells; many of these cells, however, are found primarily in connective tissues (10–15, 26, 43). Chondrocytes themselves appear to be capable of producing such low molecular weight molecules, especially when expression of their differentiated phenotype is inhibited (47). It is not known how synthesis of this smaller PG is controlled.

Our findings demonstrate that synthesis and secretion of CSPG can be modulated by at least two substances in PBMC, primarily in T lymphocytes. Both Con A and TPA stimulate two- to fivefold increases in CSPG secretion with the response to the stimuli occurring fairly rapidly. Each of these molecules effectively induces formation of interleukins, especially interleukin-2, by T lymphocytes (48). The synthesis of these two molecules (CSPG and interleukin-2) after induction by the same compounds may imply a functional relationship. In comparison to mononuclear leukocytes, our preliminary data (D. Levitt, L. Wagner-Weiner, and P.-L. Ho, unpublished data) indicate that polymorphonuclear leukocytes synthesize and secrete similar amounts of PG as mononuclear leukocytes; none of this material appears to be CSPG. Also, these cells are not stimulated to produce more PG by either the same compounds that enhance PG synthesis by PBMC, or by substances that alter polymorphonuclear leukocyte functions, a situation we are currently exploring.

The function of sulfated polysaccharides in normal cell physiology or immune regulation is unknown. In the immune system, fractions of CSPG isolated from human cartilage may alter mitogenic responses of human PBMC to PHA (30). The nonsulfated GAG hyaluronic acid suppresses mitogenic responses by immune lymphocytes (29, 49). Heparan sulfate on the surface of normal and transformed cells may mediate intercellular interactions and cell growth (50–53). As yet, no studies have evaluated the functional roles of secreted, low molecular weight CSPGs. It seems reasonable that sulfated polyanions could electrostatically bind cationic substances which are secreted by cells or are adherent to cell surfaces. In this way, the polyanions might serve as ligands that promote intercellular communications. The polyanionic CSPG might also interact with lymphocyte mediators and either protect them from proteolytic destruction or assist their association with receptor sites on target cell surfaces. Since we have recently purified the soluble CSPG secreted by T cells, it is now possible to explore its effects on specific functions of the immune system.

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