Secreted and Cellular Proteochondroitin Sulfates of a Human B Lymphoblastoid Cell Line Contain Different Protein Cores

Herbert Butz‡, Helmut W. Stuhlsatz‡, Gernot Maier‡, and Reinhard Schwartz-Albiez‡

From the ‡Institute of Immunology and Genetics and the ‡Institute of Cell and Tumoriology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany and the ‡Department of Clinical Chemistry and Pathobiocmetry, Medical Faculty, Aachen University of Technology (RWTH), D-5100 Aachen, Federal Republic of Germany

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Proteoglycans of the human B lymphoblastoid cell line L1CR-LON-HMy2 were metabolically labeled with 35S-sulfate. High-density fractions of [35S]labeled material separated by CsCl gradient ultracentrifugation were further purified by anion exchange chromatography and gel filtration. Two proteoglycans, isolated from cell lysates and culture supernatants, were characterized by gel filtration and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in combination with enzymatic degradation. Treatment with chondroitinase AC completely degraded the glycosaminoglycan moiety of the proteoglycans. Three to 4 chondroitin sulfate chains (average molecular mass = 26 kDa) were estimated for each of the two proteoglycans. Differences between the proteochondroitin sulfates (CSPG) were observed in the content of N-linked oligosaccharides. After chondroitinase AC treatment the resulting band in SDS-PAGE of the secreted CSPG was sensitive to treatment with endoglycosidase F (Endo F) which further reduced the molecular mass from 30 to 21.5 kDa, whereas the band of the cellular CSPG after chondroitinase AC treatment (molecular mass = 30 kDa) remained resistant to Endo F treatment. The composition of amino acids was different in the protein cores, suggesting differences in the primary structure. Both CSPG contained a high percentage of glycine and serine. For both CSPG a molecular mass of approximately 135 kDa was deduced from the hydrodynamic sizes of the glycosaminoxy oligosaccharide complexes in SDS-PAGE. 75% of all [35S]sulfate-labeled molecules were found in the culture supernatant and 25% in the cellular fraction. 35S-Labeled material in the culture supernatant consisted exclusively of intact CSPG, whereas 35S-labeled molecules in the cellular preparation consisted largely of free chondroitin sulfate chains. Only 8.3% of the cellular material, isolated from the microsomal fraction, was intact CSPG. In pulse-chase experiments maximal secretion of CSPG was found after 4 h, comprising approximately 40% of totally synthesized CSPG. From these experiments we tentatively conclude that a small proportion of CSPG synthesized by L1CR-LON-HMy2 cells is membrane-associated, a larger portion is secreted, and another portion is intracellularly degraded.

Several lines of evidence indicate an involvement of proteoglycans in functions of leukocytes. For example, proteochondroitin sulfates were found in secretory granules of natural killer cells (1), mast cells (2), and granulocytes (3) together with certain proteinases. It was suggested that these proteoglycans, by their binding to proteinases, may prevent autolysis or degradation of cellular components or may allow a more effective packaging of enzymes in the vesicles (4). Secreted proteoglycans of T lymphocytes have been shown to be closely associated with mitogenic factors for B lymphocytes (5). In most cases hematopoietic cells produce substantial amounts of CSPG and no or only small amounts of proteoheparan sulfate (6–10). Recently, two functionally important glycoproteins of lymphocytes, defined by monoclonal antibodies, were found to be expressed also as CSPG. The CSPG form of the major histocompatibility complex class II-associated invariant chain (2), identified both in murine (11) and human lymphocytes (12), may be involved in antigen presentation (13). The lymphocyte homing receptor CD44, first described as a glycoprotein (14), was also isolated as a CSPG (15). Both structures, II and CD44, occur only in minor proportions as proteoglycans. They certainly do not represent the major class of proteoglycans produced by B lymphocytes.

With regard to their potential importance for B lymphocytes, we analyzed the structure and metabolism of the major proteoglycans of a human B cell line. A secreted and a cellular CSPG could be distinguished by the different structures of their protein cores and the content of N-linked oligosaccharides.

EXPERIMENTAL PROCEDURES

Materials—Sepharose CL-4B, DEAE-Sephalac, Superose 6 (HR10/30), and Sephadex G-25 columns (PD10) were from Pharmacia LKB Biotechnology Inc.; Bio-Gel P10, Bioxi Amino SS, and electrophoresis materials were obtained from Bio-Rad; ultra-pure guanidine HCl, urea, PMSF, CHAPS, and NEM were purchased from Sigma; [35S]-labeled HzSO4 (10 mCi/ml) was from Du Pont-New England Nuclear, di-[18]iodide Bolton-Hunter reagent (4000 Ci/mmol) and Na[131]iodide were from Amersham Buchler; chondroitinase ABC, chondroitinase AC, heparinase, heparitinase, and chondroitinase AC.

3The abbreviations used are: CSPG proteochondroitin sulfate; CHAPS, [3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; NEM, N-ethylmaleimide; FPLC, fast performance liquid chromatography; PMSF phenylmethylsulfonl fluoride; CD chondroitin sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Endo F, endoglycosidase F; Endo H, endoglycosidase H.

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† To whom correspondence should be addressed: Inst. of Immunology and Genetics, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, Federal Republic of Germany, Tel: 6221-484-712; Fax: 6221-411-715.
drotic sulfite disaccharide standards were from Seikagaku Koyo; nitrocellulose was from Schleicher and Schuell; Immobilon membrane was from Millipore; endoglycosidase F, endoglycosidase H, endoprotease Arg-C, papain, chymotrypsin, and endoprotease Glu-C (protease V8) were from Boehringer Mannheim; RPMI 1640, fetal calf serum were from Gibco; streptomycin, streptomycin from GIBCO; X-Omat AR films were from Eastman Kodak, and liquid scintillation fluid Hydroluma was from Baker; all other chemicals were of analytical grade and purchased from Merck.

**Cell Culture and Labeling**—The B-lymphoblastoid cell line LIRC-LON-HMy2 (10) was grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum, 2 mM L-glutamine, 10 mM Hepes, penicillin (5 units/ml), and streptomycin (5 units/ml). The cells (10^5/l) were labeled for 24 h in sulfate-free RPMI1640 (MgSO_4_ replaced by MgCl_2_ with 10 μCi/ml 35S-labeled H_2SO_4_ and supplements as described above. In pre-experiments these labeling conditions resulted in maximum incorporation of 35S-labeled H_2SO_4_ into proteoglycans.

**Extraction and Purification of Proteoglycans**—After labeling, cells were centrifuged twice at 400 × g for 10 min and washed with ice-cold phosphate-buffered saline. Culture supernatant and washes were combined, centrifuged for 20 min at 10,000 × g, and freeze-dried. Nonlabeled culture supernatant was concentrated 20-fold by tangential filtration using a Millipore Minipart equipment and was lyophilized. Labeled and nonlabeled culture supernatants were combined in a ratio of 1:10, resuspended in 0.5 M NH_4(HCO_3_), containing 1 mM EDTA, 1 mM PMSF, 1 mM NEM, and aprotinin (1 μg/ml), and freed from contaminating membranes and salts by chromatography on Spin-Gel P10 (6–50 cm). The fractions of the exclusion volume, containing macromolecular material, were combined and lyophilized.

Labeled and nonlabeled cells were also combined in a ratio of 1:10. Proteoglycans were extracted from cells with 10 ml/10^6 cells of 0.1 M Tris/HCl, pH 7.5, containing 4 mM guanidine HCl, 1% Nonidet P-40 (v/v), 1 mM EDTA, 1 mM PMSF, 1 mM NEM, and aprotinin (1 μg/ml), for 24 h at 4 °C under constant stirring. The suspension was centrifuged at 4,000 × g for 20 min, and the resulting supernatant was used for further separation. The cellular extract and macromolecular material of the culture supernatant, resuspended in extraction buffer, were brought to a starting density of 1.45 g/ml of CsCl/ml by addition of solid CaCl_2_. Both preparations were centrifuged at 245,000 × g (40-ml quickseal tubes, rotor VT10, Beckman) for 18 h. Tube contents were fractionated in portions of 2 ml by emptying from the bottom with a syringe. Density was measured by weighing, glucaric acid content was determined according to Bitter and Muir (17), and protein content was assessed by the Bio-Rad colorimetric assay according to the manufacturer's instruction. Fractions of high density with the highest content of radiolabeled macromolecules (see Fig. 1) were freed of salt by repeated precipitations with ice-cold 80% ethanol (v/v). Dried precipitates were resuspended in 1 ml of 0.1 M Tris/HCl, pH 7.5, containing 4 mM guanidine HCl, 1 mM EDTA, 1 mM PMSF, 1 mM NEM, aprotinin (1 μg/ml), and applied to a DEAE-Sephaloc column (bed volume, 5 ml), equilibrated in the same buffer. After sample application, the column was washed with 2 column volumes of buffer and was eluted with a linear gradient (0–1 M NaCl), equilibrated in the same buffer, containing 10% of a marker protein (5000–10,000 cpm) added to the reaction mixture. Fractions were finally purified by chromatography on DEAE-Sephaloc (buffer conditions as described above). Subsequently, samples were digested with chondroitinase ABC and separated on SDS-PAGE with a buffer system according to Laemmli (20).

**Proteinase Susceptibility**—[35S]Sulfate-labeled proteoglycans were hydrolyzed in 6 M HCl for 15 h at 105 °C. The amino acids were determined with an Alpha Plus automated amino acid analyzer (LKB) (22).

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RESULTS

Purification of Proteoglycans from Cell Lysates and Culture Supernatants of Cell Line LICR-LON-HMy-2—For monitoring proteoglycans in all purification steps, cells were labeled with 35S-labeled H2SO4 as indicated under "Experimental Procedures" and mixed with unlabeled cells in a ratio of 1:10.

Purification to homogeneity of proteoglycans derived from the B cell line LICR-LON-HMy-2 was achieved by sequential steps of (i) centrifugation in CsCl density gradients, (ii) ion exchange chromatography on DEAE-Sephacel, and (iii) gel filtration on Sepharose CL-4B. When necessary, the last two steps were repeated in order to gain proteoglycan fractions without contamination of other proteins. Culture supernatants and cellular extracts dissolved in extraction buffer were ultracentrifuged in CsCl at a starting density of 1.45 g of

Proteoglycans and glycosaminoglycans of these fractions were further defined by the content of 35S-labeled proteoglycans was found in the

culture supernatant fraction: 6.14 pg of glucuronic acid/l

cellular fraction: 6.4 pg of glucuronic acid/l

35S-labeled macromolecules of cell lysates separated as two peaks (peak I, Kav = 0.32; peak II, Kav = 0.43), whereas those of culture supernatants separated as one peak identical in its position to that of peak I of cellular material. (Fig. 3). When aliquots of these preparations were labeled with 135I according to Bolton and Hunter (19) and separated on SDS-PAGE, no further protein bands were visible. Both proteoglycan fractions migrated on SDS-PAGE in the range of 200–300 kDa as compared with protein standards (Fig. 4). This material was taken for further structural characterization.

Characterization of the Glycosaminoglycan Chains—Portions of purified proteoglycans and glycosaminoglycans from cell lysate and culture supernatant were chromatographed on Superose 6 before and after digestion with chondroitinase

![Fig. 1. Purification of 35S-labeled proteoglycans by CsCl ultracentrifugation. Separation of 35S-labeled material of concentrated culture supernatant (a) and cellular lysate (b) was monitored by incorporation of 35S (counts/min) (c) and protein content (c) per fraction (2 ml). Density of each fraction (c) was determined by weighing. Fractions used for further purification are indicated by bars.](image1)

![Fig. 2. DEAE-Sephacel chromatography of 35S-labeled proteoglycans of pooled high-density fractions obtained by CsCl ultracentrifugation. The elution profile of material derived from culture supernatant is depicted in this figure. Material of cellular extracts had identical separation characteristics on DEAE-Sephacel. The NaCl gradient was measured by conductivity (O).](image2)

![Fig. 3. Purification of 35S-labeled proteoglycans on Sephrose CL-4B chromatography in 4 M guanidine HCl. High-density proteoglycans obtained by sequential CsCl ultracentrifugation and DEAE-Sephacel chromatography were applied to gel filtration on Sephrose CL-4B. 35S-Labeled material of cellular lysates (O) eluted in two peaks, that of culture supernatants (●) in one peak. V0 and Vf are indicated with arrows.](image3)
beled purified proteoglycans from culture supernatant
I)
preparations showed elution profiles comparable with those
treatment (Fig.
peak 11: cellular material was separated in two peaks (peak I: obtained by Sepharose CL-4B chromatography. Although cell-
glycosaminoglycan moiety of these B cell proteoglycans and glycosaminoglycans from culture supernatant
ABC, AC,
and cell lysate
glycans and glycosaminoglycans from culture supernatant
chondroitinase ABC and chondroitinase AC, whereas heparitinase/keratanase, and alkali
treatment (Fig. 5). Untreated 35S-labeled molecules of both preparations showed elution profiles comparable with those obtained by Sepharose CL-4B chromatography. Although cellular material was separated in two peaks (peak I: $K_w = 0.3$, peak II: $K_w = 0.56$), culture medium proteoglycan migrated in a single peak corresponding to peak I of cellular material. Since glycosaminoglycan chains, obtained after elimination, migrated in the range of peak II we conclude that peak I represents proteoglycan structures and peak II comprises free glycosaminoglycan chains. 35S-Labeled material of both peaks was completely degradable by chondroitinase ABC and chondroitinase AC, whereas heparitinase/heparinase and keratanase treatment (data not shown) had no effect on the elution profiles. The glycosaminoglycan moiety of these B cell proteoglycans therefore exclusively consists of CS chains. The size of the eluted material was compared with that of glycosaminoglycan molecular mass standards (shown in Fig. 7) and was estimated as 80 kDa for the proteoglycan and 26 kDa for single CS chains. Therefore, three to four CS chains seem to be attached to the protein core of both proteoglycans, since the influence of the small protein core on migration of complex proteoglycans can be neglected in gel filtration.
Analysis of chondroitinase digests of the culture supernatant CSPG in comparison with the respective standards resulted in 66.4% chondroitin 4-sulfate, 21.7% chondroitin 0-sulfate, and 11.9% chondroitin 6-sulfate. Dermatan sulfate was not detectable since treatment with chondroitinase ABC and chondroitinase AC resulted in identical disaccharide patterns.
Characterization of the Protein Cores—In order to characterize the protein cores of both proteoglycan entities, purified CSPG were radioiodinated and submitted to SDS-PAGE under reducing conditions before and after treatment with chondroitinase AC. After digestion of the glycosaminoglycan moiety by chondroitinase AC a band of approximately 30 kDa appeared in both preparations. Since no other protein bands were generated by chondroitinase AC treatment we conclude that this band represents the protein core of the respective CSPG. This separation also indicated that in each preparation only one major type of CSPG molecule, defined by this protein core, was present.
The possible attachment of N-linked oligosaccharide chains to these protein cores was investigated by treatment of the 125I-labeled proteins with endoglycosidase F (Endo F) or endoglycosidase H (Endo H) after degradation of CS chains. Endoglycosidase F treatment (with the addition of CHAPS) did not change the migration pattern of the protein core of the cellular CSPG, whereas Endo F treatment reduced the molecular mass of the secreted CSPG from 30 to 27 kDa and, after addition of CHAPS to the reaction mixture, to a major band of 21.5 kDa (Fig. 6). Endo H did not have an effect on the electrophoretic mobility of the proteins (not shown), which indicates the absence of high-mannose-type oligosaccharide chains. We conclude that the protein core of the secreted CSPG contains an N-linked complex-type oligosaccharide moiety of approximately 8.5 kDa, whereas that of the cell CSPG is apparently deficient of additional N-linked oligosaccharide chains. The appearance of an intermediary cleavage product of 27 kDa and the accelerated degradation to the 21.5-kDa band, induced by combined addition of detergents CHAPS and SDS to the Endo F reaction mixture, points to at least two N-glycosylation sites, located on different parts of the protein. The accessibility of endoglycosidase to the second site was facilitated by enforced detergent treatment.
Amino acid analysis of the purified protein cores and the intact proteoglycans revealed differences in the quantitative composition of amino acids between both proteins, although,
as a common feature, both proteins had remarkably high contents of glycine/serine, followed by asparagine/aspartic acid and glutamine/gluamic acid. Aromatic amino acids were only present in low quantities (Table 1).

Taken together, these results demonstrate that LICR-LON-HMy-2 cells produce two CSPG (cell and supernatant acid and glutamine/glutamic acid. Aromatic amino acids were concomitantly display a certain resistance to proteolytic degradation (4, 23). In order to investigate whether the supernatant CSPG shares these characteristics, we applied aliquots of purified supernatant CSPG to Superose 6 chromatography before and after treatment with chymotrypsin and papain. No change in the elution profile was observed after chymotrypsin treatment, whereas papain treatment shifted the peak of the proteoglycan into the range of the 42.5 kDa glycosaminoglycan molecular mass standard (Fig. 7). Since the proteoglycan elutes at approximately 80 kDa and a single glycosaminoglycan chain at 26 kDa, as determined by $\beta$-elimination, one can assume that papain cleaves supernatant CSPG into two peptides which contain two glycosaminoglycan chains each.

**Subcellular Localization of CSPG**—In order to localize the CSPG within the cellular compartments, microsomal and cytosolic fractions of LICR-LON-HMy-2 cells were prepared as described under "Experimental Procedures." Both fractions were chromatographed on Superose 6. The elution profiles show that intact CSPG is primarily present in the microsomal preparation, whereas a minor proportion of intact CSPG, to a larger extent free chondroitin sulfate chains and intermediary products, are included in the cytosolic fraction (Fig. 8). For comparison, $^{35}$S-labeled material of total cell lysates and culture medium of the same preparation were separated. This experiment suggests that the largest portion of intact CSPG derived of cells is associated to plasma membranes or a vesicular compartment. When one compares incorporation of $^{35}$S into macromolecules present in total cell lysate (70,460 cpm, taken as 100%), microsomal (5830 cpm), and cytosolic fraction (61,800 cpm), a proportion of 8.3% of totally incorporated radioactivity was determined for the microsome-associated CSPG. On the other hand, 25% of $^{35}$S-labeled macromolecules were found in the cell fraction and 75% in the culture supernatant fraction (mean values derived from triplicates, deviation was less than 5%). In conclusion, this would mean that only a minor part of approximately 3% of totally synthesized proteoglycan can be found in the microsomal fraction, whereas the major proportion of intact CSPG is secreted.

**Secretion of Supernatant CSPG**—In order to investigate the rate of secretion of supernatant CSPG, we performed pulse-chase experiments. LICR-LON-HMy-2 cells in their logarithmic growth phase were incubated in sulfate-free medium for 30 min and then pulsed in sulfate-free medium containing 10 $\mu$Ci of $^{35}$S-labeled H$_2$SO$_4$ for 60 min. Cultures were washed and chased for up to 24 h. Increasing amounts of radioactively labeled proteoglycans were measured in the culture medium until a plateau value was reached after 3 h of pulse time (Fig. 9). At the same time labeled compounds gradually decreased in the cellular fraction. By comparison of totally incorporated radioactivity and radioactivity determined after 3-h chase in the culture medium fraction, we conclude that a portion of

### Table 1: Amino acid composition of cell and supernatant CSPG

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues/1000</th>
<th>Cell CSPG</th>
<th>Supernatant CSPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine/aspartic acid</td>
<td>75</td>
<td>120</td>
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<td>Threonine</td>
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<tr>
<td>Serine</td>
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<td>110</td>
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<tr>
<td>Glutamine/glutamic acid</td>
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<tr>
<td>Proline</td>
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<tr>
<td>Arginine</td>
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</table>

**Fig. 7.** Superose 6 chromatography of $^{35}$S-labeled proteoglycan from culture supernatant before and after proteinase treatment. Elution profiles of untreated and chymotrypsin digested proteoglycan (□), after papain (○), and alkaline/borohydride (■) treatment. Arrows indicate $V_c$, $V_s$, and glycosaminoglycan standards (trace 1, dermatan sulfate, 42.5 kDa; trace 2, chondroitin sulfate, 26.5 kDa; trace 3, keratan sulfate, 18.5 kDa).

**Fig. 8.** Superose 6 chromatography of $^{35}$S-labeled macromolecules from different cellular compartments. $^{35}$S-labeled macromolecules were prepared from different cellular compartments and culture supernatant. $a$, microsomal fraction; $b$, cytosolic fraction; $c$, total cellular lysate; $d$, culture supernatant. $V_c$, and $V_s$ are indicated with arrows.
approximately 40% of proteoglycans synthesized in the 60-min pulse were secreted within 4 h. The remaining 60% may belong to a pool of proteoglycans being intracellularly degraded since, after 24-h labeling time, only a minor part of cellular 35S-labeled macromolecular material represents the intact, possibly membrane-associated, CSPG, and the greater portion comprises free CS chains or split products as described above.

DISCUSSION

In this report we describe the structures of two high-density CSPG isolated from cell lysate and culture supernatant of the human B-lymphoblastoid cell line LICR-LON-HMy2.

Cell line LICR-LON-HMy2 did not produce proteoheparan sulfate in measurable amounts, since treatment of purified cellular and secreted proteoglycan fractions with heparitinase/heparinase had no effect on the elution profiles in gel filtration. The same was observed when other B cell lines, such as JOK-1, WI-L2HF2, Raji, and Namalwa, were analyzed. Our finding is in accordance with earlier studies which describe the exclusive synthesis of chondroitin sulfate in human chronic leukemias of B cell type (9) and in murine B lymphocytes (24).

The CSPG of cell line LICR-LON-HMy2 could be distinguished by a different amino acid composition and by N-linked oligosaccharides which were only found attached to the protein core of the supernatant CSPG. The core molecules of both CSPG, after treatment with chondroitinase AC, had a molecular mass of 30 kDa. Treatment with Endo F reduced the molecular mass of the core molecule of supernatant CSPG to 21.5 kDa. Therefore the supernatant CSPG protein core contains additionally N-linked oligosaccharide chains of approximately 8.5 kDa. Endo F treatment did not affect the protein core of cell CSPG which points to the absence of N-linked oligosaccharides.

The hydrodynamic size of both CSPG in gelfiltration was determined as 80 kDa both under dissociative and nondissociative conditions and that of single chondroitin sulfate chains as 26 kDa.

Taken these results together, we deduce a molecular mass of approximately 135 kDa for both CSPG. Both CSPG consist of a comparatively small protein core to which four chondroitin sulfate chains are attached.

Cell CSPG was found in cellular extracts, whereas supernatant CSPG was exclusively isolated from the culture medium. Intact cell CSPG was predominantly enriched in microsomal fractions, whereas cytosolic fractions mainly contained free CS chains. It may be that cell CSPG represents either a surface expressed molecule or a constituent of vesicles.

The N-linked oligosaccharides found in supernatant CSPG may play a role in the regulation of proteoglycan secretion. For instance, N-linked oligosaccharides were reported to influence assembly and secretion of human chorionic gonadotropin (hCG) (25). It remains to be clarified why the protein core of supernatant CSPG cannot be detected in cellular extracts. A possible explanation for this phenomenon could be that the precursor molecule of supernatant CSPG migrates in the medium density proteoglycan fractions of the CaCl2 gradient which we did not analyze for their structural properties.

A considerable amount of intracellular 35S-labeled macromolecules consisted of free glycosaminoglycan chains and split products thereof. Analysis of 35S-labeled material in subcellular compartments and pulse-chase experiments revealed that only a very small percentage (2–3%) of totally synthesized proteoglycans was present as intact proteoglycans in the microsomal fraction and that most proteoglycans synthesized (80%) at a given time were internally degraded. It is not clear at this moment whether the pool of proteoglycans internally degraded consists of cell or supernatant CSPG. It has been observed that, in a comparable fashion, human T lymphocytes (26) and the monocytic cell line M1 (27) contain pools of internally degraded proteoglycans.

Protein cores of both CSPG are not identical to those of the invariant chain (li) and the homing receptor CD44 which are expressed on B cells (15). Amino acid composition of the protein cores differed from that of the li and CD44 proteins. In case of supernatant CSPG, a 12-amino acid peptide sequence, produced by proteinase Glu-C degradation of a 10-kDa peptide fragment of the protein core, showed no homology to other known proteins. Our results suggest that cell and supernatant CSPG are macromolecules not yet described for lymphocytes.

However several features point to a possible relationship of the B cell CSPG to the family of Serglycine proteoglycans. Members of this family, such as a rat leukemia proteoglycan (3), the yolk-sac-tumor proteoglycan (28), and the proteoglycan of cell line HL-60 (29), have a high content of serine and glycine in common, often appearing as Ser-Gly repeats. We found a high content of serine and glycine residues in both B cell CSPG.

Second, Serglycine proteoglycans display a certain resistance to proteolysis which affects their migration behavior in gel filtration (23). Supernatant CSPG was tested for this characteristic. Chymotrypsin treatment did not alter migration on Superose 6 chromatography, whereas papain treatment induced a significant decrease in molecular mass from approximately 80 to 40 kDa. Resistance to chymotrypsin cleavage of the supernatant CSPG protein may be explained by a lack (Trp) or low content (Tyr, Phe) of aromatic amino acids as possible cleavage sites. Potential cleavage sites for papain digestion, such as Arg and Lys, occurred in low frequency in the supernatant CSPG protein. Oligosaccharide chains may also impede proteolysis of the supernatant CSPG molecule.

Two other CSPG entities, those of the murine monocytic leukemic cell line M1 (27) and of human T lymphocytes (26), were ascribed to the Serglycine family due to their apparent

FIG. 9. Pulse-chase experiment after labeling with 35S-sulfate. Cells were labeled with [35S]sulfate for 1 h, washed, and pulsed for various times. 35S-Labeled macromolecules were determined in the cellular extract (■) and in the culture supernatant (▲), total incorporated radioactivity is indicated in the upper graph (○). The results shown represent the means of three independent experiments, the variation for each determination was less than 10% in all experiments.

1H. Butz and R. Schwartz-Albiez, unpublished data.
Proteinase resistance. Information on the protein structure was not provided by these authors. The published hydrodynamic size of these molecules (approximately 135 kDa) and the predominant expression of chondroitin 4-sulfate prompt a close relationship to the B cell CSPG described here.

A striking feature of human B cell lines which is shared by other cell types (26, 27) is the abundant secretion of proteoglycans. During a cultivation time of 24 h, free glycosaminoglycan chains or degradation products of supernatant CSPG were not present in the culture medium. This also points to the stability of supernatant CSPG to proteolysis.

It remains to be clarified whether supernatant CSPG of B lymphocytes is secreted as single molecule or, as it has been described for mast cells (21), granulocytes (3), and other cell types (26, 27), as multiple molecules. Information on the protein structure was not provided by these authors. The published hydrodynamic size of these molecules (approximately 340 kDa) and proteinase resistance. The published hydrodynamic size of these molecules (approximately 135 kDa) and the predominant expression of chondroitin 4-sulfate prompt a close relationship to the B cell CSPG described here.

Acknowledgment—We sincerely thank Antje Feldmann for excellent technical assistance.

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