Characterization of a Human Eosinophil Proteoglycan, and Augmentation of Its Biosynthesis and Size by Interleukin 3, Interleukin 5, and Granulocyte/Macrophage Colony Stimulating Factor*

Marc E. Rothenberg, Joel L. Pomerantz, William F. Owen, Jr., Shalom Avraham, Roy J. Soberman, K. Frank Austen, and Richard L. Sievens

From the Department of Medicine, Harvard Medical School and the Department of Rheumatology and Immunology, Brigham and Women’s Hospital, Boston, Massachusetts 02115

Human eosinophils were cultured for up to 7 days in enriched medium in the absence or presence of recombinant human interleukin (IL) 3, mouse IL 5, or recombinant human granulocyte/macrophage colony stimulating factor (GM-CSF) and then were radiolabeled with [35S]sulfate to characterize their cell-associated proteoglycans. Freshly isolated eosinophils that were not exposed to any of these cytokines synthesized $M_r$ ~80,000 Pronase-resistant $^{35}$S-labeled proteoglycans which contained $M_r$ ~8,000 glycosaminoglycans. RNA blot analysis of total eosinophil RNA, probed with a cDNA that encodes a proteoglycan peptide core of the promyelocytic leukemia HL-60 cell, revealed that the mRNA which encodes the analogous molecule in eosinophils was ~1.3 kilobases, like that in IL-60 cells. When eosinophils were cultured for 1 day or longer in the presence of 10 pm IL 3, 1 pm IL 5, or 10 pm GM-CSF, the rates of $[^{35}S]$sulfate incorporation were increased ~2-fold, and the cells synthesized $M_r$ ~300,000 Pronase-resistant $^{35}$S-labeled proteoglycans which contained $M_r$ ~30,000 $^{35}$S-labeled glycosaminoglycans. Approximately 93% of the $^{35}$S-labeled glycosaminoglycans bound to the proteoglycans synthesized by noncytokine- and cytokine-treated eosinophils were susceptible to degradation by chondroitinase ABC. As assessed by high performance liquid chromatography, 6–16% of these chondroitinase ABC-generated $^{35}$S-labeled disaccharides were disulfated disaccharides derived from chondroitin sulfate E; the remainder were monosulfated disaccharides derived from chondroitin sulfate A. Utilizing GM-CSF as a model of the cytokines, it was demonstrated that the GM-CSF-treated cells synthesized larger glycosaminoglycans onto $\beta$-D-xylolside than the noncytokine-treated cells. Thus, IL 3, IL 5, and GM-CSF induce human eosinophils to augment proteoglycan biosynthesis by increasing the size of the newly synthesized proteoglycans and their individual chondroitin sulfate chains.

Recently, we developed in vitro methods for maintaining the viability of human peripheral blood eosinophils for at least 7 days by culturing these cells in enriched medium (RPMI 1640 supplemented with 100 units/ml penicillin, 109 $\mu$g/ml streptomycin, 10 $\mu$g/ml gentamicin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, and 10% (v/v) fetal calf serum) that contains endothelial cell-conditioned medium (1) or human recombinant granulocyte/macrophage colony stimulating factor (GM-CSF) (2), interleukin (IL) 3 (3), or IL 5 (4). Culture in the presence of any one of these three cytokines causes the eosinophils to undergo a change in their sedimentation characteristics such that they will be recovered in a less dense region of a discontinuous metrizamide gradient. These eosinophils also exhibit an augmented capacity for killing antibody-coated Shistosoma mansoni larvae and generate more leukotriene C4 when activated with calcium ionophore than freshly isolated cells. Upon short-term exposure to these cytokines, the cells respond with the same increases in function but without an appreciable change in their density sedimentation characteristics. These postmitotic changes in the human eosinophil are induced by those cytokines (IL 3, IL 5, and GM-CSF) that cause progenitor cells to proliferate and differentiate along an eosinophil lineage (5); they are not induced by IL 1a, IL 2, IL 4, tumor necrosis factor, basic fibroblast growth factor, or platelet-derived growth factor (3).

In the present study we demonstrate that human eosinophils cultured in enriched medium supplemented with IL 3, IL 5, or GM-CSF incorporate ~2-fold more $^{35}$Sulfate into cell-associated proteoglycans than freshly isolated eosinophils. Whereas freshly isolated eosinophils synthesize $M_r$ ~80,000 $^{35}$S-labeled chondroitin sulfate E proteoglycans, the cells that are exposed to either one of these cytokines for 1 day or more synthesize $M_r$ ~300,000 $^{35}$S-labeled proteoglycans which contain substantially larger chondroitin sulfate E glycosaminoglycans. We also report that the gene that encodes the proteoglycan peptide core of human promyelocytic leukemia HL-60 cells (6) is expressed in human eosinophils.

**EXPERIMENTAL PROCEDURES**

**Materials**—RPMI 1640, fetal calf serum, L-glutamine, nonessential amino acids, penicillin, and streptomycin (GIBCO); heparin, salmon acid)-4,6-di-O-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4-ene)-pyranosyluronic acid)-4,6-di-O-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4-ene)pyranosyluronic acid)-4-O-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4-ene)pyranosyluronic acid)-4-0-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4-ene)pyranosyluronic acid) acid)-6-O-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4-ene)pyranosyluronic acid) acid)-4,6-di-O-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4-ene)pyranosyluronic acid) acid)-6-O-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4-ene)pyranosyluronic acid) acid)-4,6-di-O-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4-ene)pyranosyluronic acid) acid)-6-O-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4-ene)pyranosyluronic acid) acid)-4,6-di-O-sulfo-2-acetamido-2-deoxy-3-O-(~-~-gluco-4

1 The abbreviations used are: GM-CSF, granulocyte/macrophage colony stimulating factor; cDNA-H4, the HL-60 cell-derived cDNA that contains endothelial cell-conditioned medium (1) or human recombinant granulocyte/macrophage colony stimulating factor (GM-CSF) (2), interleukin (IL) 3 (3), or IL 5 (4). Culture in the presence of any one of these three cytokines causes the eosinophils to undergo a change in their sedimentation characteristics such that they will be recovered in a less dense region of a discontinuous metrizamide gradient. These eosinophils also exhibit an augmented capacity for killing antibody-coated Shistosoma mansoni larvae and generate more leukotriene C4 when activated with calcium ionophore than freshly isolated cells. Upon short-term exposure to these cytokines, the cells respond with the same increases in function but without an appreciable change in their density sedimentation characteristics. These postmitotic changes in the human eosinophil are induced by those cytokines (IL 3, IL 5, and GM-CSF) that cause progenitor cells to proliferate and differentiate along an eosinophil lineage (5); they are not induced by IL 1a, IL 2, IL 4, tumor necrosis factor, basic fibroblast growth factor, or platelet-derived growth factor (3).

In the present study we demonstrate that human eosinophils cultured in enriched medium supplemented with IL 3, IL 5, or GM-CSF incorporate ~2-fold more $^{35}$Sulfate into cell-associated proteoglycans than freshly isolated eosinophils. Whereas freshly isolated eosinophils synthesize $M_r$ ~80,000 $^{35}$S-labeled chondroitin sulfate E proteoglycans, the cells that are exposed to either one of these cytokines for 1 day or more synthesize $M_r$ ~300,000 $^{35}$S-labeled proteoglycans which contain substantially larger chondroitin sulfate E glycosaminoglycans. We also report that the gene that encodes the proteoglycan peptide core of human promyelocytic leukemia HL-60 cells (6) is expressed in human eosinophils.

**EXPERIMENTAL PROCEDURES**

**Materials**—RPMI 1640, fetal calf serum, L-glutamine, nonessential amino acids, penicillin, and streptomycin (GIBCO); heparin, salmon...
spern DNA, fatty acid-free bovine albumin, and p-nitrophenyl-β-D-
Xylose (Sigma); Prons and Zwittergent 3-12 (Calbiochem); blue
xanth, Sephadex G-25/20-PD-10 gel filtration columns, and Sepharose
CL-6B (Pharmacia LKB Biotechnology Inc.); [35S]sulfate (4,000 Ci/
mmol) and [2-14C]deoxy-D-glucose (55 mCi/mmol) (Du Pont New
England Nuclear). Nonspecific binding was determined in each labora-
tory, human recombinant IL-1 (Collaborative Research, Bedford, MA),
human umbilical cord endothelial cells (line CRL-1739) and HL-60 cells
(line CCL-240) (American Type Culture Collection, Bethesda, MD); hu-
man recombinant IL-2 (Cetus Corp., Emeryville, CA); a Cos cell
supernatant of human recombinant IL-4 (DNAx, Palo Alto, CA); a
Cos cell supernatant of human recombinant IL-3 (7) and purified
human recombinant GM-CSF (8) (Genetics Institute, Cambridge,
MA); and mouse IL-5 (purified from the conditioned medium of the
helper T cell line, D10.G4.1) (9) were obtained as noted. [35S]-Labeled
chondroitin sulfite diB/heparin proteoglycans (10), chondroitin sul-
fate E proteoglycan (11), and chondroitin sulfate 5 proteoglycans
(12) were extracted and purified from [35S]-labeled rat basophilic leu-
kemia 1 cells, mouse bone marrow-derived mast cells, and human
foreskin fibroblasts, respectively.

Isolation and Culture of Human Eosinophils—Human eosinophils
were isolated from the peripheral blood of seven different donors, none
of whom were ingesting corticosteroids, aspirin, or other non-
steroidal anti-inflammatory drugs. Two of these donors had no diag-
nosed clinical disorder and had normal white blood cell counts and
differentials. The other five donors were diagnosed as having allergic
rhinitis, allergic conjunctivitis, and/or asthma; 2–10% of their white
blood cells were eosinophils. The isolation procedure used to obtain these eosinophils was performed as described (2, 13).
Residual contaminating erythrocytes in the initial eosinophil prepa-
ratios were eliminated by hypotonic lysis. The purity of the starting
population of normodense human eosinophils was 94 ± 9% (mean ±
S.D., n = 20) as assessed by Wright’s and Giemsa staining. The eosino-
phils from the seven donors all had a normodense phenotype and behaved similarly not only in this study but also in previous
studies of other functional parameters (1–4). Neutrophils were essen-
tially the only leukocyte contaminant. These contaminating neutro-
phils do not survive under the culture conditions described below (1–
3), and pure populations of eosinophils were routinely obtained after
2 days of culture in the presence of IL-3, IL-5, or GM-CSF. For the
RNA blot hybridization experiment described below, eosinophils from
the 21/22% metrizamide interface were isolated to a purity of ≥99%
from a patient with the idiopathic hypereosinophilic syndrome (4).
Freshly isolated eosinophils were routinely suspended at a density
determined with a Coulter counter (RPMI 1640 containing 100 units
ml penicillin, 100 µg/ml streptomycin, 10 µg/ml gentamicin, 2 mM l-glutamine, 0.1 mM nonessential amino acids, and
19% (v/v) fetal calf serum) in the absence or presence of 10 µM
prostaglandin E1. Cells were cultured for up to 7 days at 37 °C in a humidified atmosphere of 5% (v/v) CO2. The culture medium
containing the suspension of eosinophils was aspirated
every 48 h. The eosinophils were centrifuged at 250 × g for 10
min at room temperature, resuspended in fresh enriched medium
containing the appropriate cytokine, and added back to the original
culture dish. In one experiment, eosinophils (5 × 105 cells) were
incubated in 1.5-ml polypropylene tubes in a final volume of 0.3 ml
containing 3 × 105 eosinophils in glucose-free Dulbecco’s phosphate-
buffered saline containing 0.1% (w/v) fatty acid-free bovine albumin,
0.9 mM Ca++, and 0.5 mM Mg++. Eosinophils were preincubated
at 37 °C for 15 min in buffer lacking or containing GM-CSF (10−8-
10−6 M), IL-5 (5 × 10−10–10−8 M), or IL-5 (10−12–10−8 M). [2-14C]Deoxy-
D-glucose (0.5 µCi) was added, and the cells were incubated for an
additional 60 min. The uptake of the radiolabeled carbohydrate was
stopped by the addition of 1.0 ml of 4 °C phosphate-buffered saline
and centrifugation at 5000 × g for 20 s at 4 °C. One ml of 4 °C
phosphate-buffered saline was added, the cells were centrifuged again,
and the amounts of 14C radioactivity associated with the cell pellets
were quantitated by β-scintillation counting.

RNA Blot Analysis—Total RNA was prepared by the method of
Chirgwin et al. (21) from HL-60 cells (2.0 × 106 cells), human
foreskin fibroblasts, respectively. RNA blot hybridization, a sample containing 10 µg of total RNA, was performed in the
presence of 10 µM GM-CSF for 4 days, eosinophils (1.8 × 106 cells) that had been cultured in the presence of 10 µM
GM-CSF for 1 day, eosinophils (1.8 × 106 cells) that had been cultured in the presence of 10 µM GM-CSF for 1 day, and freshly
isolated eosinophils (6.0 × 106 cells) from a patient with the idiopathic
hypereosinophilic syndrome (4).

Uptake of 2-[14C]Deoxy-D-glucose by Human Eosinophils—The
take of 2-[14C]deoxy-D-glucose was measured by a modification of a
previously described technique (20). Triplicate assays were performed in 1.5-ml polypropylene tubes in a final volume of 0.2 ml
containing 3 × 105 eosinophils in glucose-free Dulbecco’s phosphate-
buffered saline containing 0.1% (w/v) fatty acid-free bovine albumin,
0.9 mM Ca++, and 0.5 mM Mg++. Eosinophils were preincubated
at 37 °C for 15 min in buffer lacking or containing GM-CSF (10−8-
10−6 M), IL-5 (5 × 10−10–10−8 M), or IL-5 (10−12–10−8 M). [2-14C]Deoxy-D-
Glucose (0.5 µCi) was added, and the cells were incubated for an
additional 60 min. The uptake of the radio labeled carbohydrate was
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and centrifugation at 5000 × g for 20 s at 4 °C. One ml of 4 °C
phosphate buffered saline was added, the cells were centrifuged again,
and the amounts of 14C radioactivity associated with the cell pellets
were quantitated by β-scintillation counting.

DNA Blot Analysis—Total RNA was prepared by the method of
Chirgwin et al. (21) from HL-60 cells (2.0 × 106 cells), human
foreskin fibroblasts, respectively. A sample containing 10 µg of total RNA was performed in the presence of 10 µM GM-CSF for 4 days, eosinophils (1.8 × 106 cells) that had been cultured in the presence of 10 µM GM-CSF for 1 day, and freshly
isolated eosinophils (6.0 × 106 cells) from a patient with the idiopathic
hypereosinophilic syndrome (4). Samples of RNA were electropho-
resed on 1% agarose-agarose gels and transferred to Nytran
membranes. The RNA blots were incubated at 43 °C for 48 h in 50%
(v/v) formamide, 0.75 M NaCl, 75 mM sodium citrate, 2 ×

Radio labeling of Human Eosinophils and Isolation and Character-
ization of These [35S] Labeled Proteoglycans—Freshly isolated and
cytokine-treated eosinophils were incubated for 1–17 h with 0–200 µCi/
ml [35S]sulfate in enriched medium and in cytokine-supplemented
enriched medium, respectively, in the absence or presence of 0.01–0.1
pmol nitrophenyl-β-D-xylose (prepared as a stock solution of 80
mg/ml in dimethyl sulfoxide). After washing the cells, a radiolabeled cord endothelial cell pellet was removed for analysis of released [35S]-labeled proteoglycans. The cells were sedimented at 250 × g. The supernatants were removed, and samples were retained for analysis. The pellet [35S]-labeled cells were lysed (11) by incubating the cells is 50–100 µl of
0.1% (w/v) Zwittergent 3-12 containing protease inhibitors (14) for
30 min on ice. The cell lysates were clarified by the addition of 1.0 ml of TSG buffer: 0.2 M Tris-HCl, 0.1 M sodium sulfate, and 4 M guanidine HCl, pH 7.0. One hundred µg of heparin and 100 µg of chondroitin sulfate A
glycosaminoglycan carriers were added separately, and the samples were disrupted further by sonication. Samples of the radiolabeled supernatants and the cell lysates were analyzed by Sephadex G-25/20-PD-10 chromatography for the incorporation of [35S]sulfate into released and cell-associated [35S]-labeled macromolecules, respectively, but which had filtered through the exclusion volume of the column. The two-tailed Student’s t test was used to compare differences in the incorporation of [35S]sulfate into macromolecules by freshly isolated cells and by IL-3-treated cells. The [35S]-labeled macromolecules in the remaining of the cell lysates were purified further by CsCl density gradient centrifugation (11, 15). The bottom fractions were dialyzed against 0.1 M ammonium bicarbonate, resuspended in 0.4–0.8 ml of water, and stored at −20 °C for later analysis.

Samples of the [35S]-labeled macromolecules from each preparation were diluted to 0.2–0.5 ml with TSG buffer and were applied to 1 ×
110-cm Sepharose CL-6B columns that had been equilibrated in TSG
buffer. To measure their hydrodynamic sizes, the void and total
volumes of the columns were determined with blue dextran and [35S]
sulfate, respectively; mouse bone marrow-derived mast cell chondro-
A
Denhardt's buffer, 0.1% (w/v) sodium dodecyl sulfate, 1 mM EDTA, 100 μg/ml salmon sperm DNA carrier, and 10 mM sodium phosphate containing 32PO4-labeled cDNA-H4 (the cDNA that encodes the proteoglycan peptide core of HL-60 cells (6)). After the blots were washed under conditions of high stringency (55°C; 30 mM NaCl, 3 mM sodium citrate, 0.1% sodium dodecyl sulfate, 1 mM EDTA, and 10 mM sodium phosphate), autoradiography was performed with Kodak XAR film.

RESULTS

Radiolabeling of Human Eosinophils—The incorporation of [35S]sulfate into macromolecules increased linearly for at least 17 h when freshly isolated eosinophils were incubated in IL 3-supplemented enriched medium containing 25, 50, or 100 μCi of [35S]sulfate/ml (data not shown). Thus, in all subsequent experiments eosinophils were radiolabeled for 17 h with 25–100 μCi/ml [35S]sulfate. When eosinophils were cultured in the presence of IL 3 for 24 h before being radiolabeled, their incorporation of [35S]sulfate into cell-associated macromolecules increased to approximately 150% of that of freshly isolated noncytokine-treated cells (Fig. 1). In all experiments, eosinophils that had been cultured for 1–7 days in the presence of IL 3 had a statistically significant enhancement in their incorporation of [35S]sulfate into macromolecules relative to the incorporation by freshly isolated cells (p < 0.01, n = 4) (Fig. 1). After the 17-h radiolabeling period, 76 ± 8% (mean ± S.D., n = 4) and 94 ± 13% of the [35S]-labeled macromolecules synthesized by freshly isolated eosinophils and 7-day IL 3-treated eosinophils, respectively, remained in a cell-associated pool. Because ~80% of the [35S]-labeled macromolecules remained cell-associated and because IL 3 treatment did not change the relative release of these proteoglycans, only the cell-associated [35S]-labeled proteoglycans were structurally characterized. When replicates eosinophils were cultured for 7 days in the presence of IL 5 or GM-CSF, the respective rates of [35S]sulfate incorporation were 240 ± 140% (mean ± range, n = 2) and 114 ± 26% (mean ± S.D., n = 4) of that of freshly isolated cells. In contrast, the incorporation of [35S]sulfate into macromolecules by eosinophils that had been cultured in the presence of IL 1a (1–10 units/ml; n = 1), IL 2 (102–104 units/ml; n = 1), or IL 4 (a 10−1–10−4 dilution of the Cos supernatant; n = 1) for 1 day was not significantly different from that of freshly isolated cells.

Hydrodynamic Size and Pronase Susceptibility of Human Eosinophil 35S-Labeled Proteoglycans—After CsCl density gradient centrifugation, 78 ± 9% (mean ± S.D., n = 5) of the [35S]-labeled macromolecules synthesized by freshly isolated eosinophils were recovered in the high density fraction, consistent with the preferential incorporation of [35S]sulfate into proteoglycans. As shown in the representative experiment in Fig. 2A, the [35S]-labeled proteoglycans synthesized by eosinophils that had not been exposed to any cytokine other than those in the fetal calf serum were smaller in hydrodynamic size than those produced by eosinophils that had been cultured in the presence of IL 3 for 7 days. In five separate experiments with cells from different donors (Fig. 3), the [35S]-labeled proteoglycans synthesized by freshly isolated eosinophils that were radiolabeled for 17 h in the absence of IL 3 filtered on Sepharose CL-6B columns with a Kav = 0.28 ± 0.04 (mean ± S.D.), whereas replicate cells radiolabeled for 17 h in the presence of IL 3 synthesized proteoglycans that possessed a Kav = 0.23 ± 0.01. Eosinophils from the same donors that were exposed to IL 3 for 1 day or more before being radiolabeled synthesized even larger [35S]-labeled proteoglycans which filtered with a Kav = 0.15 ± 0.01 (mean ± S.D.) (Figs. 2A and 3). In one experiment, the proteoglycans released into the culture medium were found to be the same size as those remaining cell-associated for both the freshly isolated eosinophils (~80,000 and ~80,000, respectively) and the 7-day IL 3-treated eosinophils (~300,000 and ~300,000, respectively). As shown in the representative experiments depicted in Fig.

![Fig. 1](image1.png)

**Fig. 1.** Effect of IL 3 on the [35S]sulfate incorporation into macromolecules by human eosinophils. On day 0, freshly isolated eosinophils were incubated with [35S]sulfate for 17 h in the absence of IL 3. In all other experiments, eosinophils were pretreated with 10 pm IL 3 for 0–7 days before being radiolabeled for 17 h in the presence of 10 pm IL 3. The incorporation of [35S]sulfate into macromolecules is expressed as cpnm/106 viable cells/25 μCi/ml of [35S]sulfate. Each symbol represents an experiment with eosinophils from a different donor.

![Fig. 2](image2.png)

**Fig. 2.** Effect of IL 3, IL 5, and GM-CSF on the hydrodynamic sizes of the [35S]-labeled proteoglycans synthesized by human eosinophils. Eosinophils were radiolabeled before (●) or after (○) 7 days of culture in enriched medium supplemented with 10 pm IL 3 (A), 1 pm IL 5 (B), or 10 pm GM-CSF (C); the [35S]-labeled macromolecules were chromatographed on Sepharose CL-6B columns. Vc and Vt indicate the void and total volumes of the columns, respectively. In all experiments, the recovery of [35S]-labeled proteoglycans from the Sepharose CL-6B columns was ~90%.
Human Eosinophil Proteoglycan

![Image](image.png)

**Effect of the duration of exposure of human eosinophils to IL 3 on the hydrodynamic size of their newly synthesized $^{35}$S-labeled proteoglycans.** On day 0, freshly isolated eosinophils were incubated with $[^{35}$S]sulfate for 17 h in the absence of IL 3. In all other experiments, eosinophils were radiolabeled for 17 h in the presence of 10 pm IL 3 after pretreatment with 10 pm IL 3 for 0–7 days. Each symbol represents an experiment with eosinophils from a different donor. The arrows indicate the $K_w$ values of the reference $M_r$ of $80,000$, $300,000$, and $300,000$ (Table I).

**Estimated $M_r$ of the $^{35}$S-labeled proteoglycans synthesized by freshly isolated and cytokine-treated eosinophils.**

The $M_r$ of the $^{35}$S-labeled proteoglycans were estimated based on their gel filtration properties on Sepharose CL-6B columns. The columns were calibrated with $^{35}$S-labeled proteoglycans from rat basophilic leukemia cells ($M_r$ of $100,000$) and from mouse bone marrow-derived mast cells ($M_r$ of $200,000$) (11). In each experiment, the cytokine-treated eosinophils were exposed to the cytokine for 1–7 days before the 17-h radiolabeling period.

| Donor | Cytokine | $M_r$ of $^{35}$S-labeled eosinophil proteoglycans |
|-------|----------|---------------------------------------------------|
|       |          | Freshly isolated | Cytokine-treated |
| 1     | IL-3     | 50,000           | 250,000          |
| 2     | IL-3     | 150,000          | 350,000          |
| 3     | IL-3     | 100,000          | 350,000          |
| 4     | IL-3     | 50,000           | 250,000          |
| 5     | IL-5     | 80,000           | 300,000          |
| 6     | IL-5     | 100,000          | 350,000          |
| 7     | IL-5     | 120,000          | 325,000          |
| 1     | GM-CSF   | 80,000           | 300,000          |
| 2     | GM-CSF   | 50,000           | 350,000          |
| 3     | GM-CSF   | 50,000           | 350,000          |
| 7     | GM-CSF   | 80,000           | 300,000          |

TABLE I

2, B, and C, eosinophils that had been cultured for 7 days in the presence of IL-5 or GM-CSF, respectively, also synthesized substantially larger $^{35}$S-labeled proteoglycans than the freshly isolated cells. Based on the $K_w$ value of the $M_r$ of $200,000$ chondroitin sulfate E proteoglycan from mouse bone marrow-derived mast cells and the $M_r$ of $100,000$ chondroitin sulfate diB/heparin proteoglycan from rat basophilic leukemia cells, the respective average hydrodynamic sizes of the proteoglycans synthesized by the freshly isolated ($n$ = 7), IL-3-treated ($n$ = 6), IL-5-treated ($n$ = 2), and GM-CSF-treated ($n$ = 4) eosinophils were approximately $M_r$ of $80,000$, $300,000$, $300,000$, and $300,000$ (Table I).

To determine if the smaller hydrodynamic size of the freshly isolated eosinophil proteoglycan was a consequence of the initial metrizamide isolation procedure used to purify these cells from peripheral blood, eosinophils that had been cultured for 7 days in the presence of IL-3 were incubated with $[^{35}$S]sulfate before and after centrifugation on metrizamide gradients ($n$ = 1). The $^{35}$S-labeled proteoglycans produced by freshly isolated and IL-3-cultured eosinophils filtered with respective hydrodynamic sizes of $M_r$ of $80,000$, $300,000$, and $300,000$. When replicate cultured eosinophils were recentrifuged on metrizamide gradients and then radiolabeled, their $^{35}$S-labeled proteoglycans filtered with a hydrodynamic size of $M_r$ of $300,000$ (data not shown).

The ability of Pronase to degrade the $^{35}$S-labeled proteoglycans synthesized by human eosinophils was assessed by Sepharose CL-6B chromatography of the digests. There was no detectable degradation of the partially purified $M_r$ of $80,000$ $^{35}$S-labeled proteoglycans synthesized by freshly isolated eosinophils ($n$ = 3) or of the $M_r$ of $300,000$ $^{35}$S-labeled proteoglycans synthesized by IL-3-treated ($n$ = 2), IL-5-treated ($n$ = 1), or GM-CSF-treated ($n$ = 1) eosinophils under conditions in which Pronase fully degraded human fibroblast $^{35}$S-labeled proteoglycans (data not shown).

Analysis of the $^{35}$S-Labeled Glysosaminoglycans Bound to Human Eosinophil $^{35}$S-Labeled Proteoglycans—The $^{35}$S-labeled glycosaminoglycans bound to the proteoglycans synthesized by freshly isolated eosinophils filtered with $K_w$ of $0.43$ (Fig. 4B). In three experiments (including that depicted in Fig. 4) the $^{35}$S-labeled glycosaminoglycans produced by the starting cells filtered with $K_w$ of $0.58$ ± $0.03$ (mean ± range). In an experiment in which freshly isolated eosinophils synthesized glycosaminoglycans with a $K_w$ of $0.45$, eosinophils that were cultured for 7 days in the presence of IL-5 synthesized $^{35}$S-labeled glycosaminoglycans with a $K_w$ = $0.43$. In two other similar types of experiments in which freshly isolated eosinophils synthesized glycosaminoglycans with a $K_w$ of $0.58$ ± $0.03$ (mean ± range), eosinophils that had been cultured for 7 days in the presence of GM-CSF synthesized $^{35}$S-labeled glycosaminoglycans filtering with a $K_w$ =
The 35S-labeled proteoglycans synthesized by eosinophils that had been cultured in the absence or presence of different cytokines were incubated with chondroitinase ABC, and the net percentages of the total radioactivities that were degraded to 35S-labeled disaccharides were quantitated by Sephadex G-25/PD-10 chromatography. Freshly isolated eosinophils and IL-3-treated eosinophils synthesized 35S-labeled proteoglycans that were 91 ± 12% (mean ± S.D., n = 3) and 94 ± 1% (mean ± S.D. n = 5) degraded by chondroitinase ABC, respectively. When the chondroitinase ABC-generated unsaturated 35S-labeled disaccharides from freshly isolated eosinophils (Fig. 5A) and from eosinophils cultured for 7 days in the presence of IL-3 (Fig. 5B) were analyzed by HPLC, two peaks of radioactivity were obtained which had retention times corresponding to ADi-4S and ADi-diSE. In separate experiments, freshly isolated eosinophils and eosinophils that had been exposed to IL-3 for 1, 3, or 7 days synthesized 35S-labeled chondroitin sulfate proteoglycans in which 6-9% (for untreated cells), and 9, 11, and 16% (for IL-3-treated cells) of their total chondroitinase-generated 35S-labeled disaccharides were ADi-diSE, respectively; the remainder of the 35S-labeled disaccharides in each instance were ADi-4S. In other experiments, 92 ± 1% (mean ± range, n = 2), 98% (n = 1), and 96% (n = 1) of the total 35S-labeled macromolecules that were produced by freshly isolated eosinophils, 7-day IL-3-treated eosinophils, and 7-day GM-CSF-treated eosinophils, respectively, were found to be chondroitin sulfate proteoglycans. HPLC analysis of the chondroitinase ABC digests revealed that GM-CSF-treated eosinophils synthesized chondroitin sulfate in which 12 and 88% of the disaccharides were ADi-diSE (4S), and ADi-diSE (diE), respectively (Fig. 5C).

Effect of β-D-Xyloside on Proteoglycan and Glicosaminoglycan Biosynthesis by Eosinophils—Because IL-3, IL-5, and GM-CSF similarly induced human eosinophils to increase the size of the 35S-labeled glycosaminoglycans that were bound to their proteoglycans, we arbitrarily chose to use cells that had been cultured with GM-CSF to study the effect of p-nitrophenyl-β-D-xyloside on the biosynthesis of 35S-labeled macromolecules. In a representative dose-response study with 0, 0.01, 0.033, and 0.1 mM β-D-xyloside, freshly isolated eosinophils incorporated 1.3 × 104, 1.7 × 104, 2.3 × 104, and 2.4 × 104 cpm of radioactivity into macromolecules/106 cells, respectively, whereas replicate eosinophils that were also cultured in the presence of GM-CSF for 7 days incorporated 3.1 × 104, 3.9 × 104, 6.4 × 104, and 8.4 × 104 cpm/106 cells, respectively, β-D-Xyloside maximally increased the incorporation of 35S-sulfate into macromolecules by 108 ± 40% (mean ± S.D., n = 3) and 152 ± 47% for freshly isolated and replicate 7-day GM-CSF-treated eosinophils, respectively, compared to non-β-D-xyloside-treated cells. β-D-Xyloside treatment (0.1 mM) of freshly isolated eosinophils increased the percent of 35S-labeled macromolecules in the medium pool from 21 ± 2 to 37 ± 6% (mean ± S.D., n = 3). After 7 days of culture in GM-CSF, β-D-xyloside increased the release of the 35S-labeled macromolecules from 19 ± 16 to 34 ± 9%.

To determine the size of the 35S-labeled glycosaminoglycans...
proteoglycans that were tively. These proteoglycans contained 35S-labeled glycosaminoglycans of M, ~80,000 and ~300,000, respectively. These proteoglycans contained 35S-labeled glycosaminoglycans of M, ~12,000 and 32,000, respectively (data not shown). GM-CSF-treated eosinophils exposed to 0.01 mM β-D-xyloside (Fig. 6B) and freshly isolated eosinophils exposed to 0.033 mM β-D-xyloside (Fig. 6C) synthesized ~18,000 and ~12,000 35S-labeled glycosaminoglycans onto the exogenous acceptor, respectively. At the highest dose of β-D-xyloside (0.1 mM), both populations of eosinophils synthesized M, ~12,000 glycosaminoglycans onto the exogenous acceptor (data not shown).

Uptake of 2-[14C]deoxy-D-glucose into Eosinophils—To determine if cytokine exposure increased the rate of transport of glucose into the eosinophils, freshly isolated eosinophils were preincubated with various concentrations of GM-CSF, IL 3, or IL 5 for 15 min, and the uptake of 2-[14C]deoxy-D-glucose was assessed during a subsequent 60-min incubation. As shown in Fig. 7, exposure of these eosinophils to GM-CSF resulted in a dose-dependent increase in the uptake of this radiolabeled carbohydrate. In three experiments (including the one in Fig. 7), eosinophils exposed to 10^{-5} M GM-CSF had a 241 ± 151% (mean ± S.D.) increase in the uptake of the radiolabeled carbohydrate compared to that by noncytikine-treated cells. Eosinophils that were exposed to incremental concentrations of IL 3 (10^{-14}-10^{-10} M; n = 1) or IL 5 (10^{-10}-10^{-11} M; n = 1) took up 3.6- and 5.8-fold more 2-[14C] deoxy-D-glucose, respectively, at the maximal cytokine concentration than the freshly isolated cells.

RNA Blot Analysis—To determine if the mRNA that encodes the HL-60 cell proteoglycan peptide core is expressed in human eosinophils, total RNA was extracted from freshly isolated eosinophils (≥99% purity) as well as from eosinophils that had been depleted of their contaminating neutrophils by a 2-day culture with IL 3. Total RNA from two preparations of ~6 × 10^6 eosinophils, ~1 × 10^6 HL-60 cells, and ~1 × 10^6 endothelial cells was electrophoresed in separate lanes of the same agarose gel. Although the 28 S rRNA and the 18 S rRNA were clearly detectable by ethidium bromide staining in those lanes that contained HL-60 cell RNA and endothelial cell RNA, no 28 S or 18 S rRNA was detected in either lane that contained human eosinophil RNA (data not shown). Nevertheless, when the RNA blot was probed with cDNA-H4 under conditions of high stringency, HL-60 cells (lane 2), freshly isolated eosinophils from a patient with hypereosinophilia (lane 3), and human eosinophils that had been exposed to 10 pM IL 3 for 2 days (lane 4). The origin and the positions of the 28 and 18 S ribosomal RNAs are indicated.

![Fig. 6. Sepharose CL-6B chromatography of the 35S-labeled macromolecules synthesized by human eosinophils that were radiolabeled in the absence (A) or in the presence of 0.01 mM (B) and 0.033 mM (C) β-D-xyloside. Eosinophils were radiolabeled with 35S]sulfate for 17 h before (●) and after (○) 7 days of culture in the presence of GM-CSF.](image)

![Fig. 7. GM-CSF-dependent enhancement of 2-[14C]deoxy-D-glucose uptake by freshly isolated human eosinophils. Eosinophils were pretreated with various concentrations of GM-CSF for 15 min and then incubated with 2-[14C]deoxy-D-glucose for 60 min. The uptake of the radiolabeled carbohydrate into the cells was assessed by β-scintillation counting of each washed cell pellet. The data are from a representative experiment done in triplicate and are expressed as the mean ± S.D.](image)

![Fig. 8. RNA blot analysis of total RNA from human umbilical cord endothelial cells (lane 1), HL-60 cells (lane 2), freshly isolated eosinophils from a patient with hypereosinophilia (lane 3), and human eosinophils that had been exposed to 10 pM IL 3 for 2 days (lane 4). The origin and the positions of the 28 and 18 S ribosomal RNAs are indicated.](image)
results were obtained when total RNA was prepared from eosinophils that were cultured in GM-CSF for 4 days (data not shown).

**DISCUSSION**

It has been reported (23) that human eosinophils from patients with hypereosinophilia synthesize $M_r \sim 60,000$ $^{35}$S-labeled chondroitin sulfate proteoglycans when radiolabeled in the absence of any human cytokine. We demonstrate that when freshly isolated normal eosinophils from mastotic or mildly atopic donors are radiolabeled in the absence of any cytokine other than those in fetal calf serum, they synthesize cell-associated proteoglycans (Figs. 2 and 3, Table I) that contain $M_r \sim 8,000$ $^{35}$S-labeled glycosaminoglycans (Fig. 4, Table II). More than 90% of the glycosaminoglycans bound to these $^{35}$S-labeled proteoglycans were chondroitin sulfate. As assessed by its HPLC retention time and its susceptibility to chondro-6-sulfatase, 6–9% of the unsaturated disaccharides generated by chondroitinase ABC treatment were $\Delta$Di-diSa, indicating that these glycosaminoglycans were chondroitin sulfate E (Fig. 5). Inasmuch as human lung mast cells (24), basophilic leukocytes from patients with myelogenous leukemia (25), and rodent mast cells (11, 26) contain chondroitin sulfate E proteoglycans in their secretory granules, it seemed likely that this unusual cell- associated proteoglycan also resided in a granule compartment in the eosinophil.

The $^{35}$S-labeled proteoglycans synthesized by human eosinophils were found to be resistant to degradation by Pronase E as is also characteristic of the intragranular proteoglycans of mast cells. This finding is most likely a consequence of the unique region of the peptide core where the glycosaminoglycans are attached; this region has been shown to be rich in serine and glycine in rodent mast cells (27–29). Based on the deduced amino acid sequence of their respective cDNA, the peptide cores of the proteoglycans that are synthesized by rat L2 yolk sac tumor cells (30) and rat basophilic leukemia cells (31, 32) are the same; both have a proteoglycan peptide core that contains a 49-amino acid region of alternating serine and glycine. A human analogue of this gene has been isolated from a cDNA library prepared from the promyelocytic leukemia cell line, HL-60 (6). The presence of relatively high levels of an $\sim$1.3-kilobase species of RNA that hybridized under conditions of high stringency to the cDNA that encodes this HL-60 cell proteoglycan peptide core was demonstrated in eosinophils of $\geq 99\%$ purity (Fig. 8). Thus, although mature eosinophils contain low amounts of total RNA, they contain abundant amounts of an mRNA that encodes a specific granule-localized proteoglycan peptide core. The HL-60 cell-derived cDNA-H4 encodes a $M_r 17,600$ proteoglycan peptide core containing an 18-amino acid region that consists primarily of alternating serine and glycine with eight possible sites for glycosaminoglycan attachment. Therefore, it is likely that all of the glycosaminoglycan attachment sites in the $M_r \sim 80,000$ proteoglycan that is synthesized by freshly isolated eosinophils are occupied with $M_r \sim 8,000$ chondroitin sulfate E chains.

Exposure of mature eosinophils to the cytokines (IL 3, IL 5, and GM-CSF) which induce hematopoietic progenitor cells to proliferate and differentiate into eosinophils (5) also causes peripheral blood-derived eosinophils to undergo postmitotic phenotypic changes (1–4). We have demonstrated that upon exposure to each of these cytokines, the eosinophils altered their biosynthesis of proteoglycans. Human eosinophils that were exposed to IL 3 (Fig. 1), GM-CSF, or IL 5 for 1 day or longer incorporated $\sim$2-fold more $^{35}$S-sulfate into proteoglycans than freshly isolated noncytokine-treated cells. Neither IL 1a, IL 2, nor IL 4 stimulated $^{35}$S-sulfate incorporation into macromolecules. Upon exposure to GM-CSF, IL 3, or IL 5 for 1 day or more, eosinophils synthesized $M_r \sim 300,000$ $^{35}$S-labeled proteoglycans (Figs. 2 and 3, Table I) that contained $M_r \sim 30,000$ glycosaminoglycans (Fig. 4, Table II), compatible with the utilization of the eight glycosaminoglycan attachment sites. As assessed by their susceptibility to degradation by chondroitinase ABC and by the chromatography of the chondroitinase ABC digests on HPLC columns, the cytokine-treated eosinophils synthesized $^{35}$S-labeled chondroitin sulfate E glycosaminoglycans onto their peptide cores that had a type of sulfation similar to that of the noncytokine-treated cells (Fig. 5). Thus, although the cytokine-treated cells and the freshly isolated cells synthesize proteoglycans that have a similar number of chondroitin sulfate E chains, these glycosaminoglycans are substantially larger when the cells are exposed to IL 3, IL 5, or GM-CSF. After establishing that IL 3, IL 5, or GM-CSF each induced eosinophils to increase the size of the $^{35}$S-labeled glycosaminoglycans bound to their proteoglycans, we chose one of these cytokines (GM-CSF) to investigate its effect on the biosynthesis of $^{35}$S-labeled glycosaminoglycans onto p-nitrophenyl-$\beta$-d-xyloside. Fresh eosinophils and GM-CSF-treated eosinophils incorporated $\sim 110$ and $\sim 150\%$, respectively, more $^{35}$S-sulfate into macromolecules when cultured in the presence of $\beta$-d-xyloside than in the absence of the exogenous glycosaminoglycan acceptor. This finding indicated that the freshly isolated eosinophils and the GM-CSF-treated cells could synthesize more glycosaminoglycans than required, most likely because the amount of peptide core that reached the Golgi was rate-limiting. Additionally, the ability of the GM-CSF-treated eosinophils to synthesize more glycosaminoglycans in the absence and also the presence of $\beta$-d-xyloside indicated that the GM-CSF-treated eosinophils had an increased biosynthetic capacity compared to noncytokine-treated cells. Noncytokine-treated cells synthesized $M_r \sim 12,000$ $^{35}$S-labeled glycosaminoglycans onto the exogenous acceptor (Fig. 6), whereas the glycosaminoglycans of the GM-CSF-treated cells were $M_r \sim 18,000$. The ability of GM-CSF to induce the synthesis of larger glycosaminoglycans onto $\beta$-d-xyloside indicated that the cytokine effect on proteoglycan biosynthesis was in part independent of the amount of peptide core.

Previous biosynthetic studies with mesenchymal and epidermal cell lines (33) have revealed that the size of the glycosaminoglycans bound to their constitutively secreted proteoglycans can be increased by treatment of these cells with transforming growth factor-$. In other studies on the constitutively secreted proteoglycans synthesized by chondrocytes, the length of the chondroitin sulfate chain bound to the proteoglycan has been shown to be increased $\sim 250\%$ after cycloheximide treatment (34–36) or $\sim 30\%$ after insulin treatment (37). In the chondrocyte studies, it was proposed that the rate of proteoglycan peptide core being translated, the speed by which the peptide core of the proteoglycan moves through the Golgi, and the available pool size of the UDP-sugars all influence the length of the chondroitin sulfate side chain. Although UDP-GalNAc and UDP-GlcUA pool sizes were not measured in the present study, the finding that the uptake of 2-$[^{14}$C]$\text{D}-\text{glucose}$ was substantially greater in the eosinophils exposed to cytokines for 60 min in phosphate-buffered saline than in the noncytokine-exposed cells (Fig. 7) suggests that the cytokine-induced endocytosis of glucose may be a factor in the regulation of the size of eosinophils proteoglycans when cells were cultured in enriched medium with fetal bovine serum and cytokine. It is also possible that
cytokine treatment of eosinophils increases the pool size of phosphoadenosine-phosphosulfate by increasing the transport of sulfate and/or cysteine.

We have found that the protease-resistant cell-associated chondroitin sulfate E proteoglycan synthesized by human eosinophils can be dramatically increased in size by treatment of these cells with IL 3, IL 5, and GM-CSF; this effect is primarily due to the increased size of their glycosaminoglycans. These results provide biochemical evidence that mature human eosinophils undergo postmitotic phenotypic changes when exposed to the cytokines which also regulate their proliferative differentiation.

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