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A Cellulose-Binding Domain-Fused Recombinant Human T Cell Connective Tissue-Activating Peptide-III Manifests Heparanase Activity

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The chemokine connective tissue-activating peptide (CTAP)-III, which belongs to the leukocyte-derived growth factor family of mediators, was previously shown to be mitogenic for fibroblasts. However, it has recently been shown that CTAP-III, released from platelets, can act like a heparanase enzyme and degrade heparan sulfate. This suggests that CTAP-III may also function as a proinflammatory mediator. We have successfully cloned CTAP-III from a λgt11 cDNA library of PHA-activated human CD4$^+$ T cells and produced recombinant CTAP-III as a fusion protein with a cellulose-binding domain moiety. This recombinant CTAP-III exhibited heparanase activity and released degradation products from metabolically labeled, naturally produced extracellular matrix. We have also developed polyclonal and monoclonal antibodies, and these antibodies against the recombinant CTAP-III detected the CTAP-III molecule in human T cells, polymorphonuclear leukocytes, and placental extracts. Thus, our study provides tools to examine further immune cell behavior in inflamed sites rich with extracellular moieties and proinflammatory mediators.

Chemokines are a recently characterized family of pro-inflammatory mediators that function biologically in both blood vessels and in extracellular matrix sites to promote immune cell-adhesion and migration (1-4). One member of the C-X-C chemokine sub-family, connective tissue-activating peptide (CTAP)-III, is mitogenic for connective tissue cells, stimulates glucose uptake, glycosaminoglycan synthesis, proliferation of fibroblasts and synovial cells (5,6), induces the release of histamine from basophils (7), and induces plasminogen activator activity (8). Detection of CTAP-III in wound fluid (9), in the synovium in osteoarthritis, and in the sera of rheumatoid arthritis patients (10,11) suggests that CTAP-III may also be involved in inflammation. CTAP-III is a N′-truncated derivative of the leukocyte-derived growth factor (LDGF) molecule (12,13). Cleavage of LDGF (a 128-amino acid protein) at its Arg-Asn site generates CTAP-III, a 85-amino acid protein with a molecular weight of 9278 Da (13). CTAP-III has been identified in platelets (6), activated macrophages, neutrophils, and more recently, also in T lymphocytes (12), all of which are involved in inflammatory reactions.

In addition to the stimulatory activities of CTAP-III, heparanase-like heparan sulfate-degrading activity of platelet-derived CTAP-III has been recently reported (14). Specifically, a heparan sulfate-degrading molecule was purified from human platelets. N-terminal sequencing revealed that this enzymatic activity was due mainly to CTAP-III and neutrophil-activating peptide (NAP)-2, and partially to platelet basic protein (PBP), all of which are derivatives of the LDGF gene. This heparanase activity is also present in commercially prepared CTAP-III isolated from human platelets (14), but not in commercially prepared recombinant NAP-2.

Heparan sulfate proteoglycans (HSPG) are associated with cell surfaces and the extracellular matrix (ECM; 15) and bind growth factors and cytokines. Cleavage of the heparan sulfate moieties of HSPG in

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Abbreviations used: CTAP-III, connective tissue activating peptide-III; LDGF, leukocyte derived growth factor; NAP-2, neutrophil activating peptide-2; HSPG, heparan sulfate proteoglycans; ECM, extracellular matrix; CBD, cellulose binding domain.

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the ECM by heparanase facilitates cell movement and migration through extravascular tissues (16). Heparanase activity has been found in various activated blood-borne cells, such as macrophages, neutrophils, activated $CD4^+$ T cells, mast cells and platelets (17-20). Partially purified heparanase obtained from human platelets, melanoma cells, and placenta has been used in many studies (18,21,22), but the effects of contaminants in these preparations cannot be ruled out. Despite much effort, an active recombinant immune cell-derived heparanase has not yet been reported, and only bacterial heparinases have been produced as recombinant enzymes (23). Recently, another human platelet heparanase has been described, although its amino acid sequence has not yet been identified (24).

Although many recombinant proteins have been successfully expressed in *Escherichia coli* (*E. coli*), small recombinant polypeptides (<10 kDa) may be unstable and degrade rapidly in the bacteria. Therefore, fusion proteins containing the desired small polypeptides have an advantage when producing such polypeptides (25). A cellulose-binding protein (CBPA) was isolated from the cellulolytic bacterium *Clostridium cellulovorans* by Shoseyov and Doi (26). This protein, which is a major subunit of the bacterial cellulase complex, binds to cellulose, but does not express any hydrolytic activity. The gene (*cbp*A) for the CBPA protein was cloned and sequenced (27). Subsequently, the cellulose-binding domain (*cbd*) of *cbpA* was purified using PCR primers flanking the *cbd*. Cloning of this construct into an over-expression vector enabled overproduction of the 17-kDa cellulose binding domain (CBD), which exhibits a strong affinity for cellulose (28). CBD was found useful as affinity tag for the production of recombinant proteins (29,30). Here, we describe the production of a recombinant human T cell-derived CTAP-III fused to CBD and its heparan sulfate degrading activity.

MATERIALS AND METHODS

Animals. BALB/c mice (8-week-old females) and New Zealand rabbits (8-week-old females) were obtained from the Experimental Animal Center of the Weizmann Institute of Science.

Reagents. Reagents were obtained as follows: β-Thromboglobulin (Calbiochem, San Diego, CA); Phytohemagglutinin (PHA) -activated $CD4^+$ T cell cDNA λgt11 library (Clontech, Inc., Palo Alto, CA); cellulose (Sigmacell-20), protein A-conjugated agarose beads, dextran T-70, heparin, Sepharose 6B, pepstatin A, and leupeptin (Sigma Chemical Co., St. Louis, MO); Jetsorb kit (Genomed, Bad-Oeynhausen, Germany); pET-34b and pET-3c plasmids (Novagen, Madison, WI); complete Freund's adjuvant (Difco Lab., Detroit, MI); CM-Sepharose (Kabi-Pharmacia, Piscataway, NJ); high flash-point liquid scintillation cocktail (Packard Instrument Co., Meriden, CT).

Cloning of CTAP-III. DNA primers for PCR were synthesized according to a previously published cDNA sequence of CTAP-III (6) (5′-AAAACCATGGTTAACTTGGCGAAAGGC-3′ and 5′-AAAAGGATCCTAATCAGCAGATTCATC-3′). A PHA-activated $CD4^+$ T cell cDNA λgt11 library was used as a template. The PCR reaction was continued for 40 cycles (94°C for 0.5 min, 55°C for 0.5 min and 72°C for 1 min for each cycle; annealing temperature of 37°C for the first five cycles; the reaction buffer contained 3 mM $MgCl_2$). The PCR products were separated by 2% agarose gel electrophoresis. An ethidium bromide-stained band of about 260 bp was excised and purified by Jetsorb. The isolated DNA was digested with *Nco*I/*Bam*HI and ligated into either a pET-34b plasmid in frame with *cbd* to create pET-CBD-CTAP-III or into pET-3c without *cbd* to create pET-CTAP-III. *E. coli* XL1 blue competent cells were used for transforming and identifying the correct clones. DNA sequencing was performed using an automatic DNA sequencer (Applied Biosystems DNA Sequencer 373; Perking Elmer, Forest-city, CA).

Production of recombinant CTAP-III. *E. coli* BL21(DE3) strain was transformed with the pET-CBD (27), pET-CBD-CTAP-III or pET-CTAP-III plasmids. Clones harboring the plasmids were cultured (orbital shaker at 250 rpm, 37°C) in 40 ml TB medium (1.2% Bacto tryptone, 2.4% yeast extract, 0.4% glycerol, 17 mM KH_2PO_4 and 72 mM K_2HPO_4) containing kanamycin (0.1 mg/ml) for the pET-CBD and pET-CBD-CTAP-III clones and ampicillin (0.1 mg/ml) for pET-CTAP-III. Once the cultures reached the log phase, over-expression of the recombinant proteins was induced by 0.5 mM Isopropyl-1-thio-β-D-galactopyranoside (IPTG), and the cultures were further incubated overnight. The cells were then centrifuged (2,000 g, 10 min, 25°C), resuspended in 20 mM Tris pH 6 (5 ml), and disrupted by sonication. Inclusion bodies were obtained by centrifugation (8,000 × g, 10 min, 25°C), washing (by resuspension and centrifugation) once with 1% Triton X-100, and twice with 50 mM Tris-HCl, pH 6. The purified inclusion bodies were dissolved (final concentration of 1 mg protein/ml) in 20 mM Tris, pH 6.0, containing 6M guanidine-HCl, 100 mM NaCl, 20 mM $CaCl_2$, and 20 mM β-mercaptoethanol. Then the mixtures were stirred at room temperature for 2 h and dialyzed overnight at 4°C, twice, against 33 volumes of 20 mM Tris, pH 5.5, containing 50 mM NaCl, 20 mM $CaCl_2$, and 5 mM EDTA. Subsequently the dialyzed solutions, which contained the recombinant proteins, were centrifuged (8,000 × g, 10 min, 25°C). Soluble preparations of the recombinant proteins were stored at 4°C.

Immobilization of recombinant CBD and CBD-CTAP-III. To immobilize the recombinant proteins, cellulose (Sigmacell-20) was added to the soluble preparations of CBD or CBD-CTAP-III (1 mg cellulose per 10 μg protein). The suspensions were stirred at 4°C for 4 h, centrifuged (800 × g, 5 min, 25°C), and the resulting pellets resuspended in 50 mM sodium acetate buffer, pH 5.5 (NaAc buffer), containing 150 mM NaCl, 10 mM $CaCl_2$, and 10 mM $MgCl_2$. Further purification was achieved by washing the cellulose once with NaAc buffer containing 1 M NaCl, and twice with NaAc buffer.

Production of antibodies. Polyclonal antibodies (pAb) against CBD-CTAP-III were generated by immunizing 3 New Zealand rabbits with CBD-CTAP-III (100 μg soluble protein immobilized on 20 mg cellulose) in complete Freund's adjuvant. Booster injections were given 3 and 6 weeks after the first injection. The rabbits were bled prior to the first immunization and 7 weeks later. Sera prepared from these blood were stored at 4°C.

Monoclonal antibodies (mAb) were produced by the Department of Biological Services at the Weizmann Institute, as previously described (31). Briefly, BALB/c mice (8-week-old females) were injected i.p. with 50 μg of the recombinant CBD-CTAP-III in complete Freund's adjuvant. Booster injections were given 2 and 4 weeks after the first injection. Using polyethylene glycol the spleen cells were fused with myeloma cells, and the hybridomas were selected on hypoxanthine/aminopterin/thymidine medium. Supernatants of the growing cells were screened by ELISA (32). Afterwards, antibody secreting lines that specifically reacted with CBD and CTAP-III were selected and analyzed by ELISA and Western blot techniques (32). Positive hybridomas were cloned twice by limiting dilution. Consequently, two clones were chosen, one that recognized CBD (clone number 340) and one that recognized CTAP-III (clone number 792).

Preparation of human T cells homogenates. Human T cells were purified from the peripheral blood of healthy donors according to a previously described procedure (18). The cultured cells (about 2.5×10^8 cells) were washed with 50 mM Tris, pH 6.8, containing 10 mM EDTA, centrifuged (120 × g, 10 min, 25°C), and the pellet stored at −20°C. Homogenates of human T cells were obtained by 5 cycles of freezing and thawing T cell pellets in 2 ml of 50 mM Tris, pH 6.8, containing 1 mM EDTA, pepstatin A (1 μg/ml) and leupeptin (2 μg/ml; buffer A). The resulting homogenate was centrifuged (750 × g, 10 min, 25°C) and the supernatant filtered through a 0.2μ cellulose acetate filter.

Preparation of supernatants from PMN lymphocytes. Human PMNs were purified from the peripheral blood of healthy donors. Briefly, fresh blood (~30 ml) was incubated (1 h, room temperature) with 0.9% NaCl (75 ml) and 6% dextran T-70 (50 ml). Equivalent amounts of the mixture were then placed in four 50 ml plastic tubes and centrifuged (190 × g, 10 min). Afterwards the supernatants were discarded, and the pellets resuspended in 6% KCl (3 ml) and centrifuged (190 × g, 10 min, 25°C). The supernatants were again discarded, and the neutrophils thus purified were resuspended in PBS containing 0.01% $CaCl_2$ and 0.01% $MgCl_2$ to a final dilution of 5×10^6 cells/ml and incubated overnight on ice. Subsequently the cells were centrifuged (750 × g, 20 min, 25°C) and the supernatants (PMN supernatants) used for the immunoprecipitation.

Immunoprecipitation of CTAP-III from T cell homogenates and PMN supernatants. T cells supernatant, corresponding to $\sim 5 \times 10^7$ cells, and the volume was increased to 10 ml with buffer A. Polyclonal antibodies (pAb) (50 μl serum) was added and the mixtures rotated overnight at 4°C. Protein A-conjugated agarose beads (200 μl) were added to each tube and the mixtures rotated again overnight at 4°C. For the immunoprecipitation of CTAP-III from PMN supernatants, aliquots (50 μl) of protein A-conjugated agarose beads were rotated (4°C, overnight) in the presence of 100 μl rabbit anti-CBD-CTAP-III antibodies, PBS, or non-related rabbit pAb. After centrifugation (270 × g, 10 min, 25°C), a supernatant of PMN (1.5 ml, the equivalent of $\sim 7 \times 10^6$ cells) was added to each sample and the tubes were rotated (4°C, overnight).

All immunoprecipitation mixtures were centrifuged (270 × g, 10 min, 25°C) and the pellets washed once with 50 mM TRIS pH 6.8 containing 200 mM NaCl and twice with 50 mM TRIS pH 6.8. Then, the supernatants were removed and the pellets resuspended in 40 μl of 10 mM sodium acetate buffer, pH 5, containing 2 M NaCl and 1 mM DTT, and centrifuged (800 × g, 5 min, 25°C). Finally, the supernatants were tested for heparanase activity.

For Western blotting, 5 μl samples of the immunoprecipitated proteins were subjected to 15% SDS-PAGE and transferred to a cellulose nitrate membrane. The primary antibody was anti-CTAP-III mAb (hybridoma culture supernatant designated clone 792; diluted 1:5 in 20 mM Tris, pH 7.6, containing 80 mM NaCl, 5 mM HCl, 0.1% Tween-20 containing 7.5% milk powder) and goat anti-mouse pAb, conjugated to alkaline phosphatase (diluted 1:10,000) was used as a secondary antibody.

Coating of tissue culture dishes with ECM. ^{35}S-labeled bovine corneal endothelial cell ECM-coated plates were prepared as previously described (33). The ECM thus exposed remained intact, free of cellular debris, and firmly attached to the entire area of the tissue culture plates (33).

Preparation of soluble high molecular weight sulfate-labeled proteoglycans. ^{35}S-labeled soluble high molecular weight proteoglycans (peak I) were prepared as previously described (34).

Heparanase activity assay. Heparanase activity was determined by measuring the degradation of either immobilized ^{35}S-labeled ECM or soluble ^{35}S-labeled heparan sulfate proteoglycans. For immobilized preparations, aliquots (1 ml of ~150 μg CBD-CTAP-III or CBD immobilized on 80 mg of cellulose) in 80 mM phosphate-citrate buffer, pH 5.5, containing 50 mM NaCl and 1 mM $CaCl_2$ were added to ^{35}S-labeled ECM-coated plates with or without 50 μg/plate heparin. The plates were then incubated (37°C, 18 h), the ^{35}S-labeled material that was released into the incubation medium was collected and clarified by centrifugation (13,000 × g, 5 min, 25°C).

In addition, heparanase activity was evaluated by adding 40 μl of the Immunoprecipitates derived from human T cells and PMN to ^{35}S-labeled peak I material (10–20 μl; ~20,000 cpm) in 20 mM phosphate citrate buffer, pH 6.2 (1 ml), and the reaction mixtures were incubated (37°C, 18 h). Both degradation results were analyzed by gel filtration on a Sepharose CL-6B column (0.9x25 cm) equilibrated with PBS. Fractions (0.3 ml) were eluted with PBS (flow rate of 15 ml/h) and their radioactivity was determined. The excluded (Vo) and total included (Vt) volumes were marked by blue dextran and phenol red, respectively.

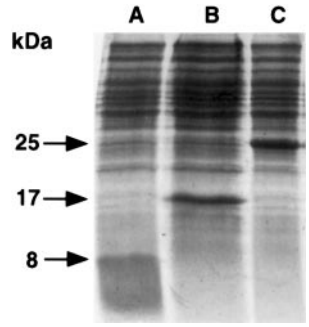

FIG. 1. Overexpression of recombinant proteins. CBD (lane B; ~17 kDa), CBD-CTAP-III (lane C; ~25kDa), and CTAP-III (lane A; ~8 kDa) over-expressed in BL21(DE3) *E. coli* containing the pET-CBD, pET-CBD-CTAP-III, and pET-CTAP-III plasmids, respectively. Fifteen μl of the cell lysates were subjected to 15% SDS-PAGE.

RESULTS

Cloning of human T cell CTAP-III in E. coli expression vectors. In order to clone the T cell CTAP-III, a λgt11 cDNA library of PHA-activated human $CD4^+$ T cells was used as a template for the PCR reaction, using CTAP-III-specific primers. The 260 bp PCR product was cloned into the pET-3c plasmid. Sequence analysis revealed that the T cell CTAP-III cDNA sequence was identical to that of the human platelet CTAP-III cDNA (13). The pET-CBD, pET-CTAP-III and pET-CBD-CTAP-III expression vectors were constructed and used to over-express the CBD, CTAP-III, and the CBD-CTAP-III fusion proteins in *E. coli.* SDS-PAGE analysis of total extracts of *E. coli* (Figure 1) revealed only low quantities of CTAP-III, which appeared as a broad band of MW ranging from 5 to 9 kDa, which indicated its degradation. In contrast, large amounts of CBD-CTAP-III (> 0.5 g/L of fermentation broth) were obtained, as were large quantities of CBD (> 0.5 g/L of fermentation broth).

The CBD-CTAP-III thus obtained was used to produce antibodies and for the heparanase activity assays, since large amounts of the peptide could be obtained, and the protein appeared to be protected from degradation in the *E. coli* cells. Moreover, the CBD-fused

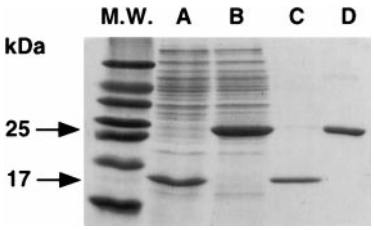

FIG. 2. Purification of the recombinant proteins. CBD (lanes A and C) and CBD-CTAP-III (lanes B and D). SDS-PAGE (12.5%) of purified inclusion bodies (lanes A and B) and the immobilized proteins (lanes C and D). MW of CBD ~17 kDa, and of CBD-CTAP-III ~25kDa.

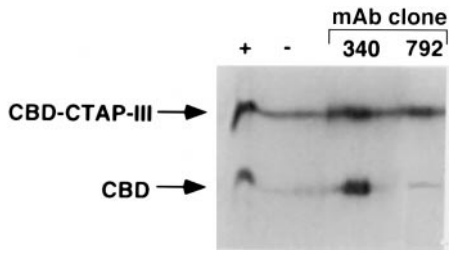

FIG. 3. Mouse mAb exhibit CBD and CBD-CTAP-IIII specific recognition. Western blot of recombinant CBD and CBD-CTAP-III (~50 μg each/slot). Primary antibodies: mouse anti-CBD-CTAP-III pAb (+), no serum (−), mouse anti-CBD-CTAP-III mAbs (clone 340; clone 792). Secondary antibody: goat anti-mouse antibody.

protein could be purified on a cellulose-containing affinity column (29,30).

CTAP-III contains four cysteines, which form two disulfide bonds. A refolding-oxidation protocol that enabled the production of soluble CBD-CTAP-III was developed, since substantial oxidation of the recombinant protein failed to occur in the bacterial cytoplasm and most of the protein accumulated in non-soluble inclusion bodies. In this protocol, the bacterial cells were disrupted by sonication, and the washed inclusion bodies were solubilized using guanidine-HCl, and allowed to oxidize slowly during gradual dialysis. Finally, the recombinant CBD-CTAP-III was affinity purified on cellulose (Figure 2).

Immunoprecipitation of heparanase activity from human T cells and PMN. Immunoprecipitation of heparan sulfate-degrading activity from T cells and PMN, using anti-CBD-CTAP-III antibodies, would be consistent with CTAP-III being a T cell heparanase. For this purpose, the fusion protein CBD-CTAP-III was used to immunize rabbits and mice. The fusion protein was immobilized on cellulose, which facilitated its purification. For production of polyclonal Abs, rabbits were injected with either the immobilized CBD-CTAP-III or with the soluble form after its release from cellulose. For production of mAb, BALB/c mice were injected with the soluble CBD-CTAP-III.

Screening by Western blot analysis (Figure 3) of the mAb indicated that clone number 340 was specific for CBD, whereas mAb clone number 792 identified CBD-CTAP-III but not CBD. Thus, antibodies specific for CTAP-III could be generated and used to clarify the biological role of this molecule.

Immunoprecipitates derived from the human T cells and PMN using rabbit sera (anti-CBD-CTAP-III, preimmune) and protein-A-conjugated agarose beads were subjected to Western blot analysis. Immunoprecipitation of CTAP-III occurred only when the polyclonal anti-CBD-CTAP-III Abs were used (Figure 4). The two bands of about 25 and 55 kDa, which appeared with both the anti-CBD-CTAP-III and preimmune (control) sera, are caused by antibody chains that cross-react with the goat anti-mouse IgG used as the secondary antibody. CTAP-III was also identified in the heparanase preparation of human placeta when anti-CBD-CTAP-III pAb was used for the immunoprecipitation assay (data not shown). Finally, when mouse mAbs were used to immunoprecipitate T cell lysates, the anti-CTAP-III mAb (clone 792), but not the anti-CBD mAb (clone 340), precipitated CTAP-III (data not shown). The presence of platelets CTAP-III in the T cell lysates used throughout our studies was minimized by washing the purified T cells several times at low speed, also in the presence of EDTA, thus eliminating the binding of platelets to T cells by cation bridges. Thus, both pAb and mAb against CTAP-III appear to recognize, bind, and precipitate human CTAP-III derived from human peripheral blood immune cells, as well as from human placenta.

Determination of heparanase activity in the immunoprecipitates and recombinant CBD-CTAP-III. Heparanase activity was determined by measuring the degradation of either soluble ^{35}S-labeled proteoglycans, designated peak I, or immobilized ^{35}S-labeled ECM. Immunoprecipitates released from the agarose beads degraded soluble ^{35}S-labeled heparan sulfate proteoglycans only when the anti-CBD-CTAP-III pAb was used for the immunoprecipitation (Figures 5A and 5B).

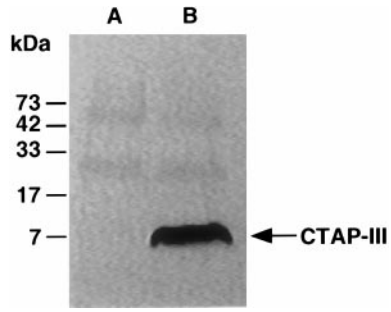

FIG. 4. Immunoprecipitation by anti-CTAP-III Abs of CTAP-III from human T cell lysates. Precipitates obtained from T cell lysates using preimmune rabbit sera (lane A) and rabbit anti-CBD-CTAP-III (lane B) were subjected to 15% SDS-PAGE and Western blotting with anti-CTAP-III mAb (clone 792) and goat anti-mouse antibodies.

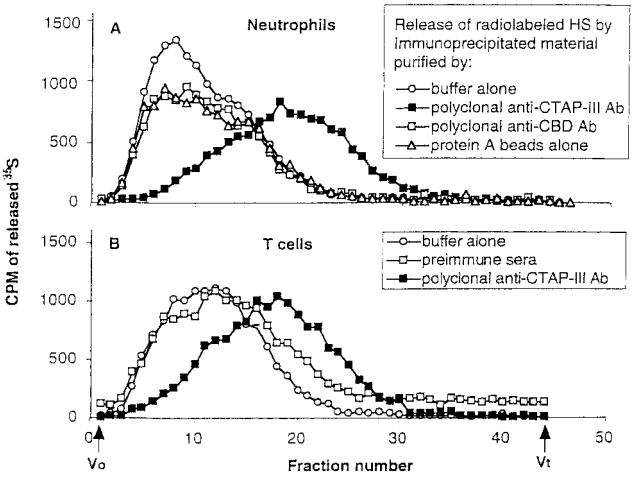

FIG. 5. Degradation of soluble ^{35}S-labeled heparan sulfate proteoglycans by solubilized immunoprecipitates obtained with anti-CTAP-III antibodies. (A) Activated human neutrophils supernatants, (B) T cell lysates. Gel filtration chromatography of the sulfate-labeled substrate and its degradation products (V_o-excluded volume; V_t-total included volume).

Because the heparanase activity immunoprecipitated with CTAP-III, we examined the heparanase activity of the recombinant CBD-CTAP-III. CBD-CTAP-III or CBD (added as control) immobilized on cellulose were added to ^{35}S-labeled ECM-coated plates, with or without heparin, a non-radiolabeled substrate of heparanase. Gel filtration chromatography of the reaction mixtures revealed a second peak of low molecular weight ^{35}S-labeled degradation products only when CBD-CTAP-III was added (Figure 6A). This second peak was not produced when heparin was included together with the CBD-CTAP-III preparation (Figure 6B). Soluble CBD and CBD-CTAP-III before immobilization did not show heparan sulfate-degrading activity (data not shown). Thus, CBD-CTAP-III exhibited heparan sulfate-degrading activity reminiscent of heparanase preparations from different sources (17–19).

DISCUSSION

In this study we have cloned CTAP-III from a λgt11 cDNA library of PHA-activated human CD4$^+$ T cells. The cDNA sequence of this chemokine was found to be identical to that previously published for human platelet-derived CTAP-III (13). Moreover, in agreement with our finding, it has been recently reported that CTAP-III is expressed by human T cells (12).

When CTAP-III and the fusion protein CBD-CTAP-III were over-expressed in *E. coli*, the fusion protein, but not CTAP-III, was produced in large quantities and appeared resistant to proteolysis in the bacteria. The apparent resistance from degradation of CBD-CTAP-III was probably due to its accumulation within inclusion bodies.

Immunoprecipitation of heparanase activity from human T cells and PMN with anti-CTAP-III antibodies, (Figure 5) substantiates the report of Hoogewerf *et al* (14) that native CTAP-III, purified from human platelets, exhibits heparanase activity. Moreover, the possibility that the platelet-derived heparanase was co-purified with native CTAP-III has been ruled out by our present production of recombinant heparanase.

It is highly unlikely that the heparanase activity, manifested by the cloned CTAP-III, was due to the actual existence in *E.coli* of molecules capable of degrading heparan sulfate since: (1) all cytosolic-soluble material and membrane fragments were separated from the insoluble inclusion bodies by washing with a detergent. (2) the recombinant CBD-CTAP-III and CBD were affinity-bound to cellulose and the columns were washed with high stringency (the buffer contained 1M NaCl). And, finally, (3) CBD was expressed as a control for the recombinant CBD-CTAP-III; CBD was treated the same as CBD-CTAP-III, but showed no heparanase activity. Therefore, our results clearly demonstrate that the heparan sulfate-degrading protein expressed by T cells is actually the chemokine CTAP-III.

The identification of CTAP-III in heparanase preparations from human placenta may indicate that CTAP-III is also at least one of the heparanases found in the human placenta.

Heparanase plays an important role in the extravasation of blood-borne tumor cells and of activated cells of the immune system and hence in tumor metastasis, wound healing, and inflammation (35). This study proves that at least one of the heparanases found in T cells and PMN is CTAP-III and suggests that CTAP-III is also at least one of the heparanases found in the placenta. However, whether CTAP-III can express its

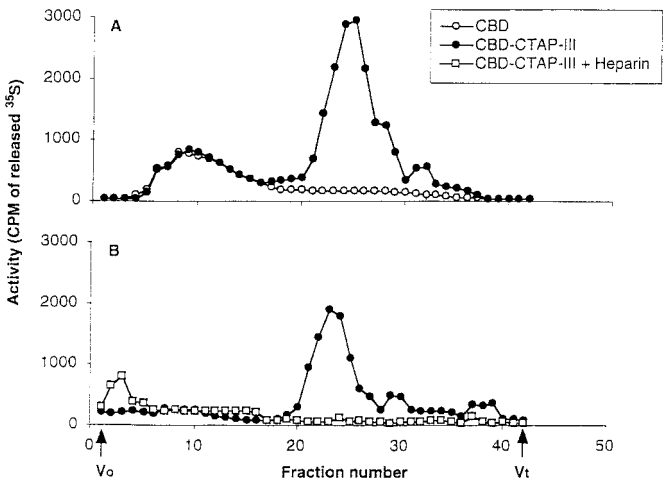

FIG. 6. Degradation of ^{35}S-labeled ECM by the recombinant proteins. CBD (○) and CBD-CTAP-III (●) (~150 μg immobilized on cellulose) in the presence (□) and absence (●) of heparin (50 μg/ml). Gel filtration chromatography of the reaction media (V_o-excluded volume; V_t-total included volume).

heparanase activity only when found in the tetrameric form (which is required for its chemokine activity) is not yet known.

Production of a recombinant CBD-CTAP-III exhibiting heparanase activity not only proves that CTAP-III has heparanase activity, but should further elucidate the multiple activities of this sub-family of chemokines. The antibodies produced and characterized herein may be studied for their ability to inhibit the enzymatic or chemokinic activity of CTAP-III *in-vivo* in inflammatory reactions in laboratory animals, as well as their application in staining tissues obtained from mammals undergoing wound healing, inflammation, or tumor growth. Our findings may thus lead to additional studies, not only supporting the versatility of chemokines and ECM-degrading enzymes, but also regarding the clinical significance of these mediators.

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