The Complement Component C1s Is the Protease That Accounts for Cleavage of Insulin-like Growth Factor-binding Protein-5 in Fibroblast Medium*

Cultured fibroblasts secrete an 88-kDa serine protease that cleaves insulin-like growth factor binding protein-5 (IGFBP-5). Because IGFBP-5 has been shown to regulate IGF-I actions, understanding the chemical identity and regulation of this protease is important for understanding how IGF-I stimulates anabolic functions. The protease was purified from human fibroblast-conditioned medium by hydrophobic interaction, lectin affinity, and heparin Sepharose affinity chromatography followed by SDS-polyacrylamide gel electrophoresis. An 88-kDa band was excised and digested with lysyl-endopeptidase. Sequencing of the high pressure liquid chromatography-purified peptides yielded the complement components C1r and C1s. To confirm that C1r/C1s accounted for the proteolytic activity in the medium, immunoaffinity chromatography was performed. Most of the protease activity adhered to the column, and the eluant was fully active in cleaving IGFBP-5. SDS-polyacrylamide gel electrophoresis with silver staining showed two bands, and IGFBP-5 zymography showed a single 88-kDa band. Amino acid sequencing confirmed that the 88-kDa band contained only C1r and C1s. C1r in the fibroblast medium underwent autoactivation, and the activated form cleaved C1s. C1s purified from the conditioned medium cleaved C4, a naturally occurring substrate. The purified protease cleaved IGFBP-5 but had no activity against IGFBP-1 through -4. C1 inhibitory substrate. The purified protease cleaved IGFBP-5 but had no activity against IGFBP-1 through -4. C1 inhibitor, a protein known to inhibit activated C1s, was shown to inhibit the cleavage of IGFBP-5 by the protease in the conditioned medium. In summary, human fibroblasts secrete C1r and C1s that actively cleave IGFBP-5. The findings define a mechanism for cleaving IGFBP-5 in the culture medium, thus allowing release of IGF-I to cell surface receptors.

Insulin-like growth factor-I (IGF-I) is a potent trophic factor for multiple cell types (1). The mitogenic potential of IGF-I is controlled by a family of high affinity IGF binding proteins (IGFBPs) that are ubiquitously present in interstitial fluids (2). The concentrations of IGFBPs and their affinity constants are such that at equilibrium most of the IGF-I is bound (3). Factors that disrupt this binding, such as proteolysis, result in the release of IGF-I to receptors (4, 5).

IGFBP-5 has been shown to be an important regulator of IGF-I actions in mesenchymal cell types (6). Both cultured human fibroblasts and smooth muscle cells secrete an IGFBP-5 protease that is specific for IGFBP-5 and cleaves it into a 22-kDa fragment, which has more than 1000-fold reduction in its affinity for IGF-I (7, 8). Studies utilizing a protease-resistant IGFBP-5 mutant have shown that high concentrations of the mutant IGFBP-5 (at least a 5-fold molar excess over IGF-I) completely inhibited IGF-I-mediated receptor activation (9). In contrast, if a 1:1 molar ratio of native IGFBP-5 to IGF-I is added to extracellular matrix, IGFBP-5 can act to potentiate the mitogenic effect of IGF-I (10). Therefore, the factors that control proteolytic cleavage of IGFBP-5 represent an important mechanism for controlling the amount of IGF-I that is available to interact with receptors. Although some broad spectrum proteases, such as plasmin, thrombin, or matrix metalloproteases-2 and -9, have been shown to cleave several forms of IGFBPs, the identity of IGFBP protease activities that are specific for a single form of IGFBP has been difficult to determine (9, 11–14). Recently, a partially purified fraction of fibroblast-conditioned medium containing the pregnancy-associated plasma protein-A protease was shown to specifically cleave IGFBP-4 (15). However, a homogeneous preparation of a protease that specifically cleaves IGFBP-5 has not been reported. For these reasons, we purified and characterized the IGFBP-5-specific protease that is present in fibroblast-conditioned medium.

EXPERIMENTAL PROCEDURES

Cell Culture—Human dermal fibroblasts (GM 10) were purchased from Coriell Institute (Camden, NJ). The cells were grown to confluence in 175-cm² tissue culture flasks (Falcon Labware, Fairfield, NJ) in minimum essential media (Life Technologies, Rockville, MD) supplemented with 10% bovine serum (Colorado Serum Co., Denver, CO). To collect conditioned medium, the monolayers were washed three times with PBS, and 150 ml of serum-free minimum essential medium was added per flask. The medium was collected after 48 h, centrifuged at 16,000 × g for 20 min to remove cellular debris, and then stored at −70 °C.

Protein Purification—Ammonium sulfate (Mallinckrodt, Baker, Paris, KY) was added to 12 liters of conditioned medium to 85% satu-
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roration, and the mixture was stored for 14 h at 4 °C, then centrifuged at 23,500 × g for 1 h. The pellet was extracted with 600 ml of 20 mM Tris, 4 mM CaCl₂, 10 mM NaCl, pH 7.4. The extract was adjusted to 1.0 M ammonium sulfate, stirred for 2 h at 4 °C, and centrifuged at 23,500 × g for 45 min. The supernatant was applied to a 4.4 × 3.5 cm butyl-Sepharose column (Amersham Pharmacia Biotech) that had been equilibrated with 20 mM Tris, 4 mM CaCl₂, pH 7.2 (7). The fractions of the product were analyzed by SDS-PAGE, with immunoblotting using a specific antibody, as described previously (16). The eluant, approximately 350 ml, was applied directly to a wheat germ-agarose column (Amersham Pharmacia Biotech) that had been equilibrated with 20 mM Tris, 4 mM CaCl₂, 0.4 mM NaCl, pH 7.2. The fractions were eluted with the same buffer containing 0.5 mM N-acetyl-g-glucosamine. The fractions containing proteolytic activity were determined as described above, and then the fractions with the greatest amount of activity were pooled (approximately 100 ml of eluant) and diluted 1:1 with 20 mM Tris, 4 mM CaCl₂, 2 mM MnCl₂, 0.4 mM NaCl, pH 7.2. This solution was used to prepare the cell lysis procedure described below.

Immunoaffinity Chromatography—Antiserum to C1r and C1s was prepared using synthetic peptides that contained the following sequences: C1r (CYPKEHKQANSNLVDVPGLHTVNEE) and C1s (CVEGNREPTMYGVSSTVQTSRLARK). The peptides (6.1 mg of C1r and 8.8 mg of C1s) were each conjugated to 4 mg of maleimide-activated KLH (Pierce, Rockford, IL) per mg of peptide. Each peptide was linked with KLH in 1.4 ml of 0.83 mM Na₂HPO₄, pH 7.2, containing 0.9 mM NaCl, 0.35 mM EDTA. Following dialysis in the same buffer without EDTA, the mixture was lyophilized. New Zealand White rabbits were immunized with 1 mg of linked KLH peptide in complete Freund’s adjuvant. The rabbits were injected with 0.5 mg of KLH peptide in incomplete Freund’s adjuvant at monthly intervals. To prepare an affinity column, 6 ml of C1r and C1s antisera were each diluted to 12 ml with 20 mM NaH₂PO₄, pH 7.0, and each was linked to 4 mg of maleimide-activated CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech) for 24 h at 4 °C, then washed. Approximately 10 ml of the pool of active fractions that had been eluted from heparin-Sepharose was diluted 1/10 with 0.05 M Tris, 4 mM CaCl₂, 0.5 mM EDTA, pH 7.4. The extract was adjusted to 1.0 M Tris to pH 7.2. The purity of the IgG was 95–99% based on SDS-PAGE with silver staining. 15.4 mg of the anti-C1r and 15.4 mg of anti-C1s affinity column (1.5 cm in diameter) were run in parallel lanes to confirm that the antibodies recognized proteins of the correct molecular weight. For immunoblotting, 15.4 mg of CaCl₂ was used at a 1:500 dilution. For immunoblotting for IGFBP-1, -2, -3, and -4, the specific antisera utilized and the conditions used have been described previously (7).

IGFBP-5 Zymography—Seven micrograms of IGFBP-5 was mixed with 4.0 ml of acrylamide gel solution (10% gel), and the gel was polymerized (5). The IGFBP-5 protease-containing fractions were concentrated 5–20× by centrifugation using Ultrafree 0.5 ml centrifugal filters. The filter was exposed to 10 μl of 3% Laemmli sample buffer, 400 ng of IGFBP-5, and 100 ng of lysyl-endopeptidase (Wako Bioproducts, Richmond, VA) for 14 h at 37 °C to cleave IGFBP-5. The gel was washed in 2.5% Triton X-100 at 4 °C for 1 h, then washed with distilled water. The gel was incubated in 0.05 M Tris, 4 mM CaCl₂, pH 7.4, overnight at 37 °C to allow for proteolysis and for capillary transfer to a polyvinylidene difluoride membrane (9). The membrane was immunoblotted using a 1:1000 dilution of IGFBP-5 antisera as described above. The electroblotting was performed on the membrane that was detected with the mouse monoclonal antibody to IGFBP-5 and a rabbit polyclonal antibody to IGFBP-5 that was precipitated with prestained molecular weight standards (Life Technologies).

Analysis of Protease Activation—To determine if the purified C1r could undergo autoactivation, 500 ng of material was incubated in 0.025 M MES, 125 mM NaCl, 2 mM EGTA, pH 7.2, for 15–90 min. The products of the reaction were then analyzed by SDS-PAGE (9% gel) using reducing conditions, 0.1 M dithiothreitol, followed by immunoblotting for C1r and C1s. To determine the ability of the purified C1s to be cleaved by C1r, 400 ng of the highly purified protease was exposed to 800 ng of C1r in 30 μl of the buffer listed previously, incubated at 37 °C for 2 h, and then analyzed by SDS-PAGE (9% gel) under reducing conditions, followed by immunoblotting for C1s. To determine if the purified IGFBP-5 protease had activity in cleaving C₁r, 180 or 540 ng of the highly purified protease was incubated with 600 ng of C₁ (Calbiochem) in 25 μl of 25 mM MES, 125 mM NaCl, 2 mM EGTA, pH 7.2, for 18 h and the products analyzed by SDS-PAGE (7%) gel with immunoblotting for C₁. Duplicate tubes containing 200 or 500 ng of pure C₁s (Enzyme Research) were also analyzed. To determine the ability of the purified IGFBP-5 protease to cleave IGFBP-5, between 3 and 50 ng of protein was incubated with 100 ng of IGFBP-5 in 60 μl of 0.05 M Tris, 10 mM CaCl₂, 50 mM NaCl, 0.1 M EDTA, pH 7.4. To determine the effects of protease inhibitors on the ability of IGFBP-5 protease in conditioned media to cleave IGFBP-5, 25 μl of fibroblast conditioned media was incubated with 100 ng of IGFBP-5 for 16 h at 37 °C in the buffer listed previously. The following inhibitors were analyzed, PA-145 (10⁻⁷ M), a synthetic peptide with sequence similarity to anti-thrombin III (20), hirudin co-factor II (10⁻⁷ M), antithrombin III.
Fig. 1. Properties of IGFBP-5 protease purified through the heparin-Sepharose step. Material that had been purified through the heparin-Sepharose step, as described under “Experimental Procedures,” was analyzed by the following methods. A, silver stain analysis. Lane 1, pure C1r and bovine serum albumin standards; lane 2, 2 μl of the active fraction; lane 3, 4 μl of the active fraction. The arrows denote the positions of the four bands that were detected. For sequencing, 250 μl of the active fraction was run in two lanes, that were stained with Coomassie Brilliant Blue R250. The four same bands that had been detected by silver stain analysis were excised, digested, and sequenced. B, immunoblotting for C1r and C1s. The fraction that was analyzed by silver staining was also analyzed by immunoblotting for C1r (lanes 2 and 6) and C1s (lanes 4 and 8) under non-reducing (lanes 1–4) and reducing (lanes 5–8) conditions. Pure C1r standard (lanes 1 and 5) and pure C1s standard (lanes 3 and 7) were also analyzed. The single arrow (lanes 1–4) denotes the positions of C1r (lanes 1 and 2) or C1s (lanes 3 and 4) (non-reduced). The arrows shown for lanes 5–8 denote the positions of intact C1r and C1s, and their two major proteolytic fragments that are detected after reduction. C, IGFBP-5 zymography. A pool of active heparin-Sepharose-purified material (lane 1) and immunoaffinity-purified material (lane 2) were analyzed. The results show IGFBP-5 proteolytic activity with molecular mass estimates of 88 and 190 kDa (lane 1) and 88 kDa (lane 2). D, IGFBP-5 protease activity. Increasing concentrations (3.5–50 ng) of IGFBP-5 for 1.5 h (Fig. 2) and 25 ng (Fig. 3). These corresponded to size estimates of pure activated C1r. Immunoblotting for C1s showed prominent bands with molecular mass estimates of 88 and >200 kDa (non-reduced) and 86, 68, and 28 kDa (following reduction). IGFBP-5 zymography of this same material showed a prominent band of activity with an 88-kDa molecular mass estimate, and a less intense band was detected that had an estimated molecular mass of 190 kDa (Fig. 2C). Four independently run gels, followed by excision of the 88-kDa band and sequence analyses, showed only C1r and C1s, and no other proteins were present in this band. When IGFBP-5 protease activity was analyzed, 25 ng of this material (400 ng/ml) cleaved 100 ng of IGFBP-5 in 1.5 h (Fig. 2D). There was a concentration-dependent increase in activity, and cleavage was detected using protease concentrations as low as 3.5 ng (60 ng/ml).

Because the heparin-Sepharose-purified material had several bands that were detected by silver staining, the material was further purified by immunoaffinity chromatography. Silver stain analysis of the material that was eluted showed three detectable bands (Fig. 3A). Sequence analysis of the 88-kDa band confirmed the presence of C1r and C1s, and no other peptide sequences were detected. Sequence analysis of the band that did not enter the gel revealed thrombospondin-1. The 160-kDa band yielded rabbit IgG, suggesting that this band

(10−7 m). In addition, experiments, the ability of the purified protein to cleave other forms of IGFBPs was determined by incubating 50 ng of purified protease with 150 ng of pure IGFBP-1, -2, -3, and -4 as stated for IGFBP-5. The products of the reactions were then analyzed by SDS-PAGE (12.5% gel) followed by immunoblotting for each specific IGFBP.

RESULTS

Silver stain analysis of the material purified through the heparin-Sepharose step showed a prominent 88-kDa band, a high molecular weight band that did not enter the gel, and two other bands with molecular mass estimates of 180 and 280 kDa (Fig. 1A). Minor bands were detected at 92 and 240 kDa. Amino acid sequence analyses, showed only C1r and C1s, and no other proteins were present in this band. When the faint band that was detected at 92 kDa was carefully excised from the gel and separated from the lower 88-kDa band, sequences corresponding to C1r only were obtained. The 180-kDa band gave the sequences of the α1 and α2 chains of collagen type VI. The 280-kDa band contained two peptides with sequences corresponding to tenasin-C, and the band that did not enter the gel contained multiple peptides with sequences encoding collagen type VI or thrombospondin-1. Both tenasin-C and thrombospondin-1 have been shown to bind to IGFBP-5 (21, 22). Immunoblotting of the identical material with anti-C1r antiserum showed a prominent 88-kDa band that co-migrated with the intact C1r standard and a second band that had a molecular mass estimate of >200 kDa (Fig. 1B). When analyzed following reduction, the major C1r band was detected that had a molecular mass estimate of 94 kDa, and less abundant bands were detected at 62 and 38 kDa (Fig. 1B). These corresponded to size estimates of pure activated C1r. Immunoblotting for C1s showed prominent bands with molecular mass estimates of 88 and >200 kDa (non-reduced) and 86, 68, and 28 kDa (following reduction). IGFBP-5 zymography of this same material showed a prominent band of activity with an 88-kDa molecular mass estimate, and a less intense band was detected that had an estimated molecular mass of 190 kDa (Fig. 2C). Four independently run gels, followed by excision of the 88-kDa band and sequence analyses, showed only C1r and C1s, and no other proteins were present in this band. When IGFBP-5 protease activity was analyzed, 25 ng of this material (400 ng/ml) cleaved 100 ng of IGFBP-5 in 1.5 h (Fig. 2D). There was a concentration-dependent increase in activity, and cleavage was detected using protease concentrations as low as 3.5 ng (60 ng/ml).
Fig. 3. Purity and protein characteristics of immunoaffinity purified proteases. The heparin-Sepharose-purified material was further purified by immunoaffinity chromatography with a column that had been prepared with anti-C1r- and -C1s antisera. The pool of the most active fractions was then analyzed by the methods listed. A, Coomassie stain analysis. Lanes 1 and 2 contain 250 and 125 μl of the eluted pool of fractions from the immunoaffinity column. These bands were subsequently excised, digested, and sequenced. The protein staining bands are noted with arrows. A non-reduced, pure C1s standard and BSA are shown in lane 3. The arrows denote the positions of the three detectable bands, B, C1r and C1s immunoblotting. The material that was eluted from the immunoaffinity column was immunoblotted separately for C1r and C1s. Lane 1, C1r, non-reduced; lane 2, C1r, reduced; lane 3, C1r, non-reduced. C, IGFBP-5 protease activity. The capacity of the purified material to degrade IGFBP-5 was determined as described under “Experimental Procedures.” The arrows denote the position of intact IGFBP-5 and its major proteolytic 22-kDa fragment. Lane 1, material that was loaded onto the column; lane 2, material excluded from the column; lane 3, 4 μl of eluant; lane 4, 1 μl of eluant; lane 5, 0.25 μl of eluant; lane 6, 0.05 μl of eluant; lane 7, conditioned medium; lane 8, IGFBP-5 standard.
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We have reported previously that human fibroblasts secrete an IGFBP-5 protease that cleaves IGFBP-5 into predominantly a 22-kDa fragment, and this fragment has a low affinity for IGF-I (8). This proteolytic activity is a serine protease and is specific for IGFBP-5 (7). Because IGFBP-5 is an important modulator of IGF-I bioactivity, the factors that regulate the activity of this protease have the potential to regulate the ability of IGFBP-5 to modulate IGF-I actions. For that reason, we were interested in determining the molecular identity of IGFBP-5 protease activity in fibroblast-conditioned medium. To that end, we purified a large quantity of IGFBP-5 protease activity from human fibroblast-conditioned medium and subjected it to amino acid sequence analysis. Sequence analysis yielded several peptides that encoded C1r and C1s. Because these proteins could not be separated, it is not possible to definitively determine which protease is actually cleaving IGFBP-5; however, C1s is known to have a much broader range of substrates (25). Furthermore, a very low concentration (60 ng/ml) of the C1r/C1s mixture cleaved IGFBP-5 rapidly. In contrast, IGFBP-5

DISCUSSION

C1s activation (25), was the most potent inhibitor of the IGFBP-5 protease activity contained in the fibroblast-conditioned medium. The other inhibitors that had less of an effect on C1s activation (data not shown) also had less of an effect on IGFBP-5 proteolysis.

Activiation of C1r and activation of C1s. To determine if the C1r in the purified protease fraction could autoactivate and if its activation correlated with C1s activation, the heparin-Sepharose-purified material was incubated for 15 min, 45 min, and 90 min, and the same samples were analyzed for both C1r (lanes 1–4) and C1s (lanes 5–8) activation using reducing conditions. Lanes 1 and 5, no incubation; lanes 2 and 6, 15 min; lanes 3 and 7, 45 min; lanes 4 and 8, 90 min. The results show that C1r is autoactivated, and C1s is also activated over the same time course.

Inhibition of IGFBP-5 proteolysis. Aliquots of fibroblast conditioned media that were prepared as described under “Experimental Procedures,” were incubated overnight with IGFBP-5 in the presence or absence of protease inhibitors, and the amount of proteolysis was determined by immunoblotting. Lane 1, control IGFBP-5; lane 2, medium, no inhibitor; lane 3, PB-145; lane 4, heparin co-factor II; lane 5, anti-thrombin III; lanes 6–8, C1 inhibitor (lane 6, 10−8 M; lane 7, 10−6 M; lane 8, 10−4 M). Arrows denote positions of intact IGFBP-5 and its major proteolytic fragment.

Cleavage of C4. Pure C4, 600 ng, was incubated with the highly purified protease or C1s for 18 h at 37 °C. Lane 1, C4 control, no incubation; lanes 2–6, C4, 600 ng; lane 2, 18-h incubation with no protease; lane 3, C1s, 200 ng; lane 4, C1s, 500 ng; lane 5, purified protease, 90 ng; lane 6, purified protease, 270 ng. The upper arrow denotes intact C4; the lower arrow denotes its major fragment.

Autoactivation of C1r and activation of C1s. To determine whether this C1r was fully active, 40 ng was incubated with C1s (200 ng), and the fragments were analyzed as in panel a. Lane 2, no incubation; lane 3, 1-h incubation.

Specificity of IGFBP-5 protease. The material that had been immunoaffinity purified (50 ng) was tested for its capacity to cleave other forms of IGFBPs, as described under “Experimental Procedures.” Each form of IGFBP (150 ng) was incubated with the protease for 16 h at 37 °C, and then each was analyzed by immunoblotting using specific antisera. The preparation and specificity of the antisera that were used to detect IGFBP-1 through -4 have been described previously (7). No fragments were detected except when IGFBP-5 was used as a substrate.
cleavage by C1r required a much higher concentration, proceeded slowly, and yielded fragments that had size estimates that were different from those that were detected after cleavage by the purified IGFBP-5 protease. These results do not exclude the possibility that C1r may be cleaving IGFBP-5 to some extent in the medium, but they suggest that C1s accounts for the major portion of the IGFBP-5 protease activity. Because C1s is secreted at least in part as an inactive zymogen, the primary role of C1r may be to cleave and activate C1s, which subsequently cleaves IGFBP-5. This conclusion is also supported by the known specificity of both enzymes for specific recognition sequences (25).

That these enzymes were the predominant protease activity for IGFBP-5 in fibroblast-conditioned medium is proven by several points. First, during purification, other proteases that have been shown to cleave IGFBP-5, such as ASP-5 and MMP-2 and -9 were removed by various chromatographic steps; however, most of the proteolytic activity was retained. Furthermore, when pure MMP-2, -9, or ASP-5 are incubated with IGFBP-5, the rate of proteolysis is slow and the fragment sizes that are generated are distinct from those generated by the IGFBP-5 protease activity (7). Finally, MMP-2 and -9 are not specific for IGFBP-5 and degrade other forms of IGF binding proteins. In contrast, the proteolytic activity in fibroblast media and the immunoaffinity-purified material were specific for IGFBP-5 and did not cleave other forms of IGF binding proteins. Second, immunoaffinity chromatography of partially purified conditioned medium showed that most of the IGFBP-5 protease activity in the medium could be accounted for by C1r and C1s that adhered to the antibody affinity column. Third, the specific C1s protease inhibitor, C1 inhibitor, inhibits IGFBP-5 cleavage by crude conditioned medium, and its ability to inhibit C1r and C1s activation correlates with its ability to inhibit IGFBP-5 proteolysis. Furthermore, extensive sequencing of all of the protein bands that were detected in the most highly purified material did not yield sequences corresponding to any other protease. Following immunoaffinity chromatography of fibroblast medium, approximately 84% of the IGFBP-5 proteolytic activity adhered to the column (data not shown). Taken together, these data strongly suggest that C1s is the predominant protease cleaving IGFBP-5 in fibroblast medium and C1r is responsible for its activation.

Recently, it was shown that a part of the IGFBP-5 protease in fibroblast medium could be ascribed to PAPP-A, which is a metalloprotease (15). However, in that study PAPP-A was not purified to homogeneity, and therefore the results did not exclude the possibility that other proteases were present in fibroblast medium that could potentially degrade IGFBP-5. Furthermore, the extent to which any of these proteases is active is determined not just by the concentration of protease, but whether it is secreted as the zymogen, the percentage activation and whether protease inhibitors are present. When the secretion of C1r and C1s was analyzed using several test conditions, partial activation of these proteases was noted. Therefore, partially activated forms exist in fibroblast medium, making it more likely that they account for IGFBP-5 protease activity.

The physiologic significance of activated C1r/C1s in fibroblast medium and their roles in modulating IGF-I actions remains to be determined. However, we have shown that a protease-resistant form of IGFBP-5 inhibits IGF-I actions (11). Therefore, the fact that these proteases are present in the medium in partially activated forms that can cleave IGFBP-5 into fragments with very low affinity suggests that they are capable of modulating IGF-I bioactivity. Furthermore, inhibition of their activity with a specific inhibitor (e.g. C1 inhibitor) results in inhibition of IGFBP-5 cleavage, suggesting that activation can be modulated in physiologic fluids that contain this inhibitor. In additional studies, we have detected C1 inhibitor in fibroblast-conditioned medium by immunoblotting (data not shown). Therefore, the variables that regulate C1r and C1s synthesis and activation, as well as the secretion of C1 inhibitor, have the potential to alter IGFBP-5 cleavage and thereby modulate IGF-I actions.

Other investigators have reported that activated C1r and C1s occur at sites outside the liver (25, 26) and that they have proteolytic functions other than complement activation (27–30). Specifically, pleural fluid and joint fluid contain activated C1r and C1s, as do cell culture supernatants from glial cells (25, 31). Several central nervous system cell types have been shown to contain C1s peptide and messenger RNA. Spleen, liver, brain, and kidney have been shown to contain C1r and C1s mRNA (25). Several studies have also shown that C1r and C1s activation may lead to cleavage of peptides other than the complement components (27–30), and several of the proteins that have been shown to be substrates for C1s are not traditional components of the complement pathway. Taken together, these findings suggest that C1r and C1s may have roles other than complement activation.

For several years, investigators have hypothesized a linkage between inflammation (i.e. acute complement activation during injury) and subsequent cellular repair processes. Because IGF-I and IGFBP-5 are secreted by several cells, such as macrophages or fibroblasts, which are involved in repair that occurs in response to injury, and both peptides are present in pericellular fluids (32), on cell surfaces, and in extracellular matrix of several mesenchymal cell types (33), it is possible that there is a coordinated linkage between activation of C1r and C1s during acute injury and the subsequent release of IGF-I to receptors that are present on the surface of cell types that are involved in tissue repair. IGFBP-5, which is also secreted by the connective tissue cell types that are involved in tissue repair (34, 35), could be an important component for controlling the amount of IGF-I that can be released to receptors after C1r and C1s activation (35). Whether such a linkage exists deserves further exploration. In summary, we have determined that the predominant protease component of fibroblast-conditioned medium is ascribable to the complement subcomponents C1r and C1s. C1s is the major protease that cleaves IGFBP-5, but its full activation requires the presence of activated C1r. Further studies are necessary to determine the physiologic role of these proteases in activating a cascade of events leading to IGF-I receptor stimulation.

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