An α_2-Macroglobulin Receptor-dependent Mechanism for the Plasma Clearance of Transforming Growth Factor-β1 in Mice

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Abstract

Radioiodinated transforming growth factor-β1 (TGF-β1) bound to the plasma proteinase inhibitor, α_2-macroglobulin (α_2M), as determined by chromatography on Superose-6 and native polyacrylamide gel electrophoresis. When α_2M conformational change was induced with methylamine, ^{125}I-TGF-β1 binding significantly increased. Intravenously injected ^{125}I-TGF-β1 cleared from the circulation of mice rapidly at first; however, intravascular radioactivity stabilized near 20% of the initial level. At necropsy, radioactivity was recovered predominantly in the liver (65%); however, the density of radioactivity (disintegrations per minute/g organ wt) was highest in the lungs. Markedly different results were obtained with purified ^{125}I-TGF-β1-α_2M-methylamine complex. Clearance of the complex occurred as a first-order process with a $t_{1/2}$ of 4 min. Greater than 90% of the radioactivity was recovered in the liver. The clearance and distribution of ^{125}I-TGF-β1-α_2M-methylamine were equivalent to those observed with ^{125}I-α_2M-methylamine and ^{125}I-α_2M-trypsin. The latter two radioligands clear via specific α_2M receptors in the liver. Large molar excesses of α_2M-trypsin or α_2M-methylamine competed with ^{125}I-TGF-β1-α_2M-methylamine for plasma clearance. Native α_2M, which does not bind to the α_2M receptor, did not compete. The receptor binding domain of α_2M-methylamine was blocked by chemical modification or enzyme treatment. The resulting α_2M preparations still bound ^{125}I-TGF-β1; however, the complexes did not clear when injected intravenously in mice. The studies presented here demonstrate that α_2M can mediate the plasma clearance of a growth factor via the α_2M receptor system. We propose that α_2M, the α_2M receptor, and proteinases may function as a concerted system to regulate TGF-β1 activity and the activity of related factors in vivo. (*J. Clin. Invest.* 1991. 87:39–44.) Key words: growth factor • proteinase • receptor • hepatocyte • liver

Introduction

Transforming growth factor-β1 (TGF-β1)[1] is a 25-kD disulfide linked homodimer (1). The active form of TGF-β1 is a multifunctional regulator of cell growth demonstrating both proliferative and antiproliferative activities (2). TGF-β1 modulates the immune response, regulates cellular secretion of extracellular matrix proteins, and affects cellular production of proteinases and proteinase inhibitors (2). TGF-β1 activity results from interaction with specific cell surface receptors, the most important of which is probably a 53-kD glycoprotein (3, 4). Since numerous normal and neoplastic cells secrete TGF-β1 and express the TGF-β receptor (2, 5), it is not clear whether physiologic mechanisms exist to direct TGF-β1 to specific target cells.

TGF-β1 is secreted as an inactive high molecular weight precursor with an NH_2-terminal proregion (5–7). The activity of TGF-β1 may be regulated in part by molecules that influence the rate of precursor activation. Plasmin and cathepsin-D both react with the TGF-β1 precursor yielding active 25-kD dimer (7). The dimers then bind to cell surface receptors or to certain soluble proteins including betaglycan (8), fibronectin (9) and α_2-macroglobulin (α_2M) (10–12). These TGF-β1 carrier molecules may transfer TGF-β1 from one tissue to another or direct the activity to a specific cell type. In the plasma, essentially all of the TGF-β1 is associated with α_2M (10).

α_2M is a 718-kD plasma glycoprotein that inhibits numerous proteinases from all four major classes (13). Reaction of α_2M with proteinase causes a major conformational change in the inhibitor (14). As a result, the proteinase is trapped and a nondissociable complex is formed. Primary amines such as methylamine react with α_2M thiol ester bonds causing a conformational change similar to that induced by proteinase (14). In either reaction (proteinase or amine), the change in α_2M structure generates a site that binds to specific cell surface receptors on hepatocytes, macrophages, and fibroblasts (15). The native form of α_2M is not recognized by the α_2M receptor.

α_2M receptors have been studied extensively using in vivo model systems (15–18). In mice, receptor binding and endocytosis cause the rapid plasma clearance of α_2M-proteinase complexes and α_2M-methylamine ($t_{1/2}$ 3–5 min). The major organ responsible for α_2M-proteinase clearance is the liver (15); the primary cell type is the hepatocyte (19). Native α_2M has a relatively long circulating half-life due to the inability to interact with α_2M receptors (15). The clearance of α_2M-proteinase complex in the mouse is species independent; α-macroglobulins from multiple species (including human and murine α_2M) compete similarly for α_2M receptors in vivo (15, 17).

The nature of the α_2M/TGF-β1 interaction has not been completely elucidated; however, TGF-β1 binds preferentially to some forms of α_2M that have undergone conformational change, such as α_2M-methylamine (11) and α_2M-plasmin (unpublished data). Since TGF-β1–α_2M complexes do not interact with TGF-β receptors (10, 11), α_2M may serve as an "intercellular shuttle," mediating the clearance of TGF-β1 from the circulation or directing the cytokine to specific cell types that express α_2M receptors (macrophages and hepatocytes). In this study, we have tested this hypothesis using the established in vivo mouse model system. Our results indicate that stable α_2M-

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1. *Abbreviations used in this paper:* α_2M, α_2-macroglobulin; α_2M-M_{Pt}, α_2M-methylamine treated with *cis*-DPP; *cis*-DPP, *cis*-dichlorodiammineplatinum (II); PNPGB, p-nitrophenyl p'-guanidinobenzoate HCl; TGF-β1, transforming growth factor-β1.

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methylamine–TGF-β1 complexes can be formed and that these complexes are cleared from the murine circulation by α_2M receptors.

Methods

Proteins and reagents. Papain, p-nitrophenyl p'-guanidinobenzoate HCl (PNPGB), trypsin, and methylamine-HCl were purchased from Sigma Chemical Co., St. Louis, MO. The concentration of active enzyme in trypsin preparations was determined by the method of Chase and Shaw (20). *Cis*-dichlorodiammineplatinum (II) (*cis*-DDP) was purchased from Aldrich Chemical Co., Milwaukee, WI.

α_2M was purified from human plasma by zinc chelate and gel filtration chromatography as described by Imber and Pizzo (16). α_2M-trypsin complex was prepared by reacting α_2M with a twofold molar excess of active trypsin. The bound trypsin was then inactivated with PNPGB. Highly purified ^{125}I-TGF-β1 (> 97%) was purchased from R&D Systems, Minneapolis, MN, or prepared by the method of Ruff and Rizzino (21). The sp act was between 30 and 100 μCi/μg. Equivalent results were obtained with both preparations of ^{125}I-TGF-β1.

Chemical modification of α_2M. α_2M-methylamine was prepared by dialyzing purified α_2M against 300 mM methylamine, 50 mM Tris-HCl, pH 8.2, for 8 h at 25°C. The preparation was then dialyzed exhaustively against 20 mM sodium phosphate, 150 mM NaCl, pH 7.4 (PBS). In a native gel electrophoresis system, α_2M-methylamine migrated in a single band with increased mobility compared with native (unreacted) α_2M. This change in electrophoretic mobility confirmed that reaction was complete and that α_2M conformational change occurred (22).

α_2M-methylamine was reacted with 1.67 mM *cis*-DDP in PBS for 6 h at 37°C and then dialyzed against PBS at 4°C. Under these conditions, the *cis*-DDP reacts with a critical methionine residue in the α_2M receptor recognition site, forming a stable complex (α_2M-M_{Pt}) that does not bind to α_2M receptors (23). Each α_2M-M_{Pt} preparation was screened using an in vitro rat hepatocyte binding assay as described elsewhere (24). α_2M-M_{Pt} did not displace ^{125}I-α_2M-methylamine from hepatocyte receptors at 4°C.

α_2M-methylamine was treated with papain at pH 5.0 as previously described (25). Papain digests α_2M-methylamine into one major fragment (600 kD) and four equivalent 18-kD peptides representing the COOH termini of the four α_2M subunits (25). The 600-kD fragment retains the basic structural features of α_2M-methylamine as shown by electron microscopy (24) but lacks the receptor binding domains (25). The 600-kD fragment was purified by chromatography on Ultrogel AcA-22. In hepatocyte binding assays, 100 nM 600-kD fragment did not displace ^{125}I-α_2M-methylamine (1 nM) from receptors at 4°C.

Formation of α_2M-TGF-β1 complexes. Radioiodinated TGF-β1 was incubated with native α_2M, α_2M-methylamine, α_2M-M_{Pt}, or the 600-kD fragment. The α_2M and TGF-β1 concentrations were 1.4 μM and 1.2 nM, respectively. Incubations were conducted at 37°C for 2 h or the specified time. TGF-β1-α_2M complexes were separated from free TGF-β1 by FPLC on prepacked Superose-6 columns (Pharmacia LKB, Piscataway, NJ). The flow rate was 0.4 ml/min. The extent of binding was determined by the radioactivity coeluting with the α_2M in an early peak.

Electrophoresis and autoradiography. Radioiodinated TGF-β1 (5.0 nM) was incubated with the various forms of α_2M (0.3 μM) for 1.5 h at 37°C. PAGE was then performed on 5% slabs using the nondenaturing buffer system described by Van Leuven et al. (26). Binding of ^{125}I-TGF-β1 to α_2M was determined by autoradiography. Free ^{125}I-TGF-β1 was not recovered in the gel.

Plasma clearance experiments. Plasma clearance studies were performed in 20-wk old CD-1 mice (Charles River Breeding Laboratories, Inc., Wilmington, MA) as previously described (18). Briefly, ^{125}I-TGF-β1, ^{125}I-α_2M, or various FPLC-purified ^{125}I-TGF-β1-α_2M complexes were injected into the lateral tail veins of anesthetized mice. The total injection volume was 400 μl. Blood samples (25 μl) were taken, beginning at 10 s, from the retroorbital venous plexus using heparinized hematocrit tubes. Radioactivity in each blood sample was determined in a gamma counter and plotted as a percentage of the radioactivity present in the 10-s sample. Each experiment was performed at least in triplicate.

Clearance competition experiments were performed by coinjecting FPLC-purified ^{125}I-TGF-β1-α_2M-methylamine and 1.0 mg of α_2M-methylamine (40-fold molar excess over α_2M present in FPLC preparation) or 0.5 mg of α_2M-trypsin (20-fold molar excess over α_2M present in FPLC preparation). α_2M-methylamine and α_2M-trypsin compete comparably for receptor binding sites in mice (15–18). Unlike α_2M-methylamine, the TGF-β1 binding activity of α_2M-trypsin is not increased compared with native α_2M (11).

Plasma clearance of a radioligand may result from reversible processes such as noncovalent binding to cell-surface receptors or irreversible processes such as endocytosis (15). To distinguish between these two possibilities, delayed competition experiments were performed. ^{125}I-TGF-β1-α_2M-methylamine complex was injected intravenously in mice. After 15 min, 1.0 mg of α_2M-methylamine was administered in a second intravenous injection. Blood sampling was conducted throughout. If the radioligand is reversibly bound to cellular receptors, the second injection causes redistribution of radioactivity back into the plasma (15). This result has been observed with asialotransferrin (27).

Organ distribution studies. ^{125}I-TGF-β1 or FPLC-purified ^{125}I-TGF-β1-α_2M-methylamine complex was injected intravenously in mice. After 45 min, the animals were killed. The major body organs were recovered intact, rinsed in normal saline, blotted to remove surface moisture, and weighed. The radioactivity was then determined in each organ and expressed as a percentage of the total recovered radioactivity. The results were normalized for organ size by dividing the percentage of recovered radioactivity in each organ by the organ mass.

Results

TGF-β1 binding to α_2M. Superose-6 FPLC was used to study the binding of ^{125}I-TGF-β1 to α_2M. In this chromatography system, recovery of free ^{125}I-TGF-β1 was poor. Most of the radioactivity eluted as a broad peak after the total accessible volume of the column. It is important that no free ^{125}I-TGF-β1 was recovered in the early elution volumes characteristic of α_2M.

^{125}I-TGF-β1 was incubated with α_2M-methylamine for 1.5 h at 37°C. When the preparation was subjected to chromatography on Superose-6, 50% of the radioactivity coeluted with the α_2M-methylamine, suggesting complex formation (Table I). The TGF-β1-α_2M-methylamine complex was separated from free TGF-β1 by FPLC on Superose-6, stored at 4°C for 48 h, and then subjected to FPLC again. Greater than 90% of the radioactivity was recovered and 98% of the recovered radioactivity eluted in the high molecular weight α_2M peak. This result

Table I. Analysis of TGF-β1 Binding to α_2M by Chromatography on Superose-6

α_2M Species	Temperature	Incubation time	^{125}I-TGF-β1 bound
	°C	h	%
Native	22	1.0	3
α_2M-methylamine	22	1.0	35
Native	37	1.5	12
α_2M-methylamine	37	1.5	50
α_2M-M_{Pt}	37	1.5	50
600-kD fragment	37	1.5	33

demonstrates that recovery of ^{125}I-TGF-β1-α_2M-methylamine complex from Superose-6 is nearly quantitative and that the purified complex is relatively stable.

The Superose-6 chromatography system was used to compare the binding of ^{125}I-TGF-β1 to different forms of α_2M. Both native α_2M and α_2M-methylamine bound TGF-β1 without the aid of stabilizing cross-linking agents; however, the binding was much more extensive with α_2M-methylamine (Table I) confirming the work of other investigators (11).

To determine whether the TGF-β1 binding site overlaps the α_2M receptor binding site, ^{125}I-TGF-β1 was incubated with α_2M-M_{Pt} and the 600-kD fragment. In α_2M-M_{Pt}, the receptor binding site is chemically modified (23). In the 600-kD fragment, the receptor binding domain is enzymatically removed (25). Both preparations of modified α_2M-methylamine bound TGF-β1 and the extent of binding was significantly greater than that observed with native α_2M.

Analysis of TGF-β1 binding to α_2M by PAGE and autoradiography. To confirm the results of the chromatography experiments, binding of ^{125}I-TGF-β1 to the various α_2M preparations was studied by PAGE and autoradiography (Fig. 1). As shown in the Coomassie blue-stained gel, α_2M-methylamine demonstrated increased mobility relative to native α_2M. This change in electrophoretic migration is a well characterized consequence of α_2M conformational change (14). The 600-kD fragment and α_2M-M_{Pt} also demonstrated increased mobility relative to native α_2M. A faint low mobility band present in lane *c* may indicate that the α_2M-M_{Pt} preparation included a small percentage of α_2M dimers, consistent with the bifunctional nature of *cis*-DDP.

As demonstrated by autoradiography, each of the three modified α_2M preparations bound significantly greater amounts of ^{125}I-TGF-β1 than native α_2M. These results are consistent with those obtained by chromatography. After incubation with native α_2M, most of the bound ^{125}I-TGF-β1 migrated more rapidly than the major Coomassie blue-stained band. This result suggests that the TGF-β1 may selectively bind to trace amounts of conformationally transformed α_2M species in the native α_2M preparation, even though these species are not detected by native or SDS-PAGE. Assuming a one:one stoichiometry for TGF-β1-α_2M complex, < 0.2% conformationally transformed species in the native α_2M preparation might be sufficient to account for the TGF-β1 binding observed in the PAGE and chromatography experiments.

Plasma clearance of α_2M. The plasma clearance of human ^{125}I-α_2M has been reported previously (15–18). Equivalent studies are presented here in order to facilitate interpretation of the

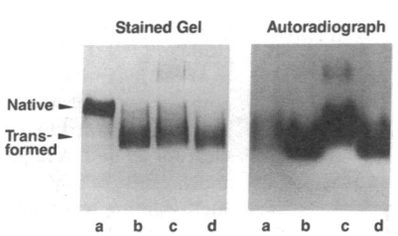

Figure 1. Binding of ^{125}I-TGF-β1 to various forms of α_2M analyzed by PAGE and autoradiography. ^{125}I-TGF-β1 was incubated with native α_2M (lane *a*), α_2M-methylamine (lane *b*), α_2M-M_{Pt} (lane *c*), or the 600-kD fragment (lane *d*). An equal amount of α_2M was loaded in each lane. Electrophoresis was conducted for 0.5 h at 75 V followed by 3.5 h at 150 V. The Coomassie stained gel and the autoradiograph have been aligned so that mobilities of the bands may be directly compared.

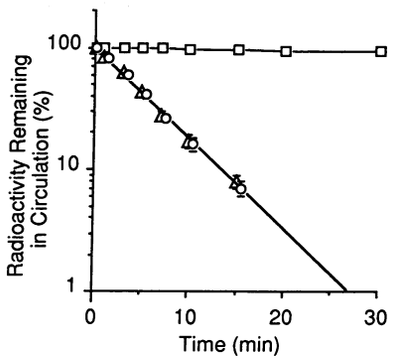

Figure 2. The plasma clearance of native ^{125}I-α_2M (□), ^{125}I-α_2M-methylamine (○), and ^{125}I-α_2M-trypsin (△). The error bars represent 1 SD.

^{125}I-TGF-β1 clearance experiments (Fig. 2). ^{125}I-native α_2M was injected intravenously. Blood sampling was performed for 30 min. During this time, the circulating level of radioligand remained stable. By contrast, both ^{125}I-α_2M-trypsin and ^{125}I-α_2M-methylamine cleared rapidly after intravenous injection. In both cases, elimination from the vascular compartment was described by a first-order process with a half-life of 3–5 min. The saturability of the α_2M-proteinase clearance pathway can be easily demonstrated in vivo (15). A large molar excess of α_2M-proteinase or α_2M-methylamine causes a concentration-dependent decrease in the clearance rate of ^{125}I-α_2M-trypsin or ^{125}I-α_2M-methylamine (15–18).

Plasma clearance of ^{125}I-TGF-β1 and ^{125}I-TGF-β1-α_2M-methylamine complex. The plasma clearance of ^{125}I-TGF-β1 was complex (Fig. 3). More than 50% of the initial radioligand (that present at 10 s) was eliminated within 2 min. Subsequently, the rate of ^{125}I-TGF-β1 clearance decreased until a stable level of intravascular radioactivity was attained at 7–10 min. The plasma clearance of TGF-β1 in mice, reported here, is comparable to that determined in rats by Coffey et al. (28).

The plasma elimination of ^{125}I-TGF-β1-α_2M-methylamine complex differed significantly from the clearance of the free growth factor. The clearance curve was monophasic and adequately described by a first-order process with a half-life of 4 min. By 30 min, residual radioligand in the blood samples reached background levels (< 2%).

Organ distribution studies. The distribution of ^{125}I-TGF-β1 was studied 45 min after intravenous injection (Table II). Radioactivity was recovered predominantly in the liver; however, significant levels were also detected in the kidneys and lungs. When the data were standardized for organ mass, the highest density of ^{125}I-TGF-β1 was present in the lungs.

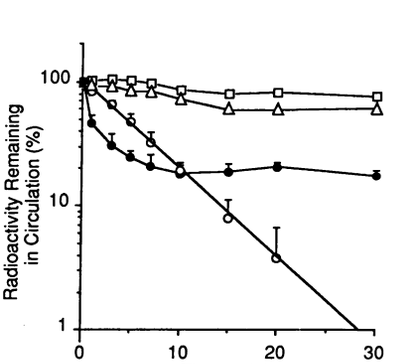

Figure 3. The plasma clearance of free ^{125}I-TGF-β1 (●) and ^{125}I-TGF-β1 bound to various forms of α_2M including α_2M-methylamine (○), α_2M-M_{Pt} (□), and the 600-kD fragment (△). In each study, TGF-β1–α_2M complex was resolved from free TGF-β1 by chromatography on Superose-6. The error bars represent 1 SD.

Table II. Organ Distribution Studies

Organ	TGF-β1		TGF-β1–α_2M-methylamine	
	Radioactivity recovered	Normalized radioactivity	Radioactivity recovered	Normalized radioactivity
	%	%/g	%	%/g
Liver	65±6	43±8	93±2	58±9
Spleen	4±1	24±4	2±0	17±4
Kidneys	17±2	43±5	3±0	7±2
Lungs	13±4	72±5	2±1	10±4
Heart	1±1	14±5	1±0	3±1

Normalized radioactivities were determined by dividing the radioactivity recovered in each organ (percentage of total recovered radioactivity) by the organ mass (g). Values represent the mean±SEM (n = 3 for TGF-β1; n = 4 for α_2M-methylamine–TGF-β1).

The organ distribution of ^{125}I-TGF-β1-α_2M-methylamine complex was significantly different. Over 90% of the radioactivity was recovered in the liver. The nearly exclusive role of the liver was evident even after correcting the data for organ mass. The percentage of recovered radioactivity in each organ was within 2% of that reported for ^{125}I-α_2M-methylamine elsewhere (15).

Plasma clearance of TGF-β1 bound to modified forms of α_2M. ^{125}I-TGF-β1-α_2M-M_{Pt} complex and the complex formed by ^{125}I-TGF-β1 with the 600-kD fragment did not clear from the circulation (Fig. 3). The plasma clearance profiles for these complexes were equivalent to those reported previously for ^{125}I-α_2M-M_{Pt} (23) and ^{125}I-600-kD fragment (29). This result strongly suggests that an intact α_2M receptor recognition site is necessary for the plasma clearance of TGF-β1–α_2M-methylamine in mice.

Clearance competition experiments. FPLC-purified ^{125}I-TGF-β1–α_2M-methylamine was injected in the presence of excess α_2M-methylamine or α_2M-trypsin (Fig. 4). The competing ligands significantly decreased the rate of radioligand clearance. By contrast, a 40-fold molar excess of native α_2M had no effect (data not shown). These data indicate that ^{125}I-TGF-β1–α_2M-methylamine clearance requires available α_2M receptors in vivo. The TGF-β1 binding activity of α_2M-trypsin is not greater than that of native α_2M (11). Therefore, the competing ligands did not affect the clearance of ^{125}I-TGF-β1-α_2M-methylamine complex by interacting with the radioligand in vivo. Since α_2M-methylamine and α_2M-trypsin almost completely arrested ^{125}I-TGF-β1–α_2M-methylamine clearance, pathways for the plasma elimination of the radiolabeled complex other than the α_2M receptor probably do not exist in mice.

Delayed competition experiments were performed to determine if ^{125}I-TGF-β1-α_2M-methylamine can be displaced back into the vascular compartment after clearance occurs (Fig. 5). The radioligand was injected alone and allowed to clear until < 20% remained. A large molar excess of unlabeled α_2M-methylamine was then injected. The competing ligand inhibited the clearance of the remaining ^{125}I-TGF-β1–α_2M-methylamine; however, no significant increase in plasma radioactivity was observed. This study demonstrates that ^{125}I-TGF-β1–α_2M-methylamine that clears from the circulation is not in reversible equilibrium with α_2M-methylamine in the plasma. While we cannot rule out irreversible (covalent) radioligand binding to cellular receptors, the results suggest that ^{125}I-TGF-β1-α_2M-methylamine undergoes endocytosis, as has been described for α_2M-methylamine after receptor binding in vivo (15).

In separate experiments, the plasma clearance of free ^{125}I-TGF-β1 was studied after injecting α_2M-trypsin (0.5 mg) in order to block the α_2M receptor-dependent clearance pathway. The clearance of the TGF-β1 was not affected by the α_2M-trypsin (data not shown). This result suggests that the α_2M receptor does not contribute to the plasma elimination of TGF-β1 unless the TGF-β1 is bound to a receptor-recognized form of α_2M.

Discussion

The binding of TGF-β1 to various forms of α_2M has been previously described (10–12, 30). α_2M is the most important carrier of TGF-β1 in the circulation (10); however, the functional consequences of complex formation between TGF-β1 and α_2M remain unclear.

The studies presented in this investigation demonstrate that ^{125}I-TGF-β1 can be cleared from the circulation as a complex with α_2M-methylamine via the α_2M receptor. The growth factor-carrier complex is sequestered almost exclusively in the liver, mirroring the organ distribution of ^{125}I-α_2M-methylamine or ^{125}I-α_2M-proteinase. When the function of the α_2M receptor was abrogated by altering the α_2M receptor recognition site (α_2M-M_{Pt}, 600-kD fragment) or by coadministering a

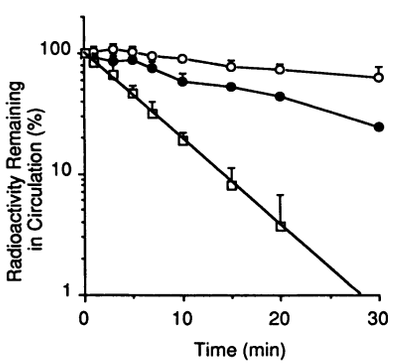

Figure 4. Clearance competition studies. ^{125}I-TGF-β1–α_2M-methylamine complex was purified by chromatography on Superose-6 and injected simultaneously with a large dose of α_2M-methylamine (○) or α_2M-trypsin (●). The plasma clearance of ^{125}I-TGF-β1–α_2M-methylamine in the absence of competing ligands is shown for comparison (□).

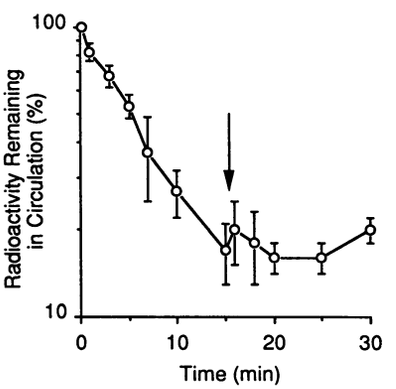

Figure 5. Delayed clearance competition studies. ^{125}I-TGF-β1–α_2M-methylamine complex was injected alone and allowed to clear for 15 min. A large molar excess of α_2M-methylamine was then administered intravenously in a second injection. The time of the second injection is shown by the arrow.

competing dose of receptor-recognized α_2M (α_2M-methylamine, α_2M-trypsin), the complexed ^{125}I-TGF-β1 persisted in the circulation. These experiments suggest that α_2M and the α_2M receptor may function as a system to limit the dispersal of TGF-β1 released from platelets and stromal cells in vivo.

The available evidence indicates that active forms of TGF-β1 must be able to bind to specific TGF-β receptors (2–6). In our plasma clearance studies, free TGF-β1 was rapidly eliminated from the vascular compartment, most likely due to interaction with TGF-β receptors located diffusely in multiple organs. By contrast, clearance of TGF-β1–α_2M-methylamine complex was dependent on the α_2M receptor exclusively. When interaction with the α_2M receptor was prevented, alternate pathways for the plasma clearance of TGF-β1–α_2M-methylamine were not available. The inability of TGF-β receptors to recognize TGF-β1–α_2M complex has been demonstrated previously in cell culture experiments (10, 11). This study confirms this result for the first time in an in vivo model system.

α_2M-methylamine provides a useful model to study the structure and function of α_2M after conformational change. Based on numerous physico-chemical studies (31), differences in the structure of α_2M-methylamine and α_2M-proteinase are minimal, perhaps no greater than the variability reported for different α_2M-proteinase complexes. From the standpoint of the current investigation, α_2M-methylamine provides an excellent model to study α_2M receptor interactions (15–18); however, this particular form of α_2M is not physiologically significant. Therefore, in order to hypothesize that the α_2M receptor mediates TGF-β1 clearance in vivo, one must identify an α_2M species that binds TGF-β1 with high affinity and is α_2M receptor-recognized. Unpublished studies from our laboratories have identified a number of complexes that demonstrate these properties, including α_2M-plasmin. At this time, it is not clear why all α_2M-proteinase complexes do not uniformly demonstrate increased TGF-β1 binding activity. Since plasmin activates the TGF-β1 precursor molecule and reacts readily with α_2M, α_2M-plasmin complexes may play an important role in removing excess active growth factor from a site of TGF-β1 precursor activation.

Native α_2M in the plasma originates primarily from the liver (32); however, macrophages can also secrete α_2M at sites of inflammation or an immune response (33). Therefore, complexes of α_2M with TGF-β1 may form both inside and outside the vascular compartment. Since the hepatocyte is the primary cell responsible for clearing α_2M-methylamine and α_2M-proteinase from the circulation (19), it is likely that hepatocytes bind TGF-β1–α_2M-methylamine complex as well. At this time, it is not clear whether TGF-β1 retains any activity when presented to hepatocytes as a TGF-β1–α_2M-methylamine complex by the α_2M receptor.

Huang et al. (11) proposed that conformationally transformed α_2M species (receptor recognized) may be responsible for most of the TGF-β1 binding activity in the plasma. The studies presented here and elsewhere (30) support this hypothesis by demonstrating selective binding of ^{125}I-TGF-β1 to conformationally transformed α_2M, even when the transformed α_2M is present as a minor fraction in purified native α_2M preparations. If the previously described latent form of TGF-β1 (10, 11) is TGF-β1–α_2M-proteinase complex, then our studies suggest that the plasma half-life of this latent form is very short.

The selective binding of TGF-β1 to conformationally transformed α_2M does not necessarily mean that TGF-β1–α_2M-proteinase complexes are more abundant than TGF-β1–native α_2M complex in the circulation. Plasma concentration is a function not only of complex formation but of plasma survival (plasma clearance). Due to the rapid clearance of TGF-β1–α_2M-proteinase complex, the fraction of TGF-β1 bound to native α_2M in samples of plasma may be significantly higher than that predicted by in vitro binding studies.

In cell culture systems, TGF-β1 is a long-acting inhibitor of hepatocyte proliferation under serum free conditions (30, 34, 35). The mitoinhibitory activity of TGF-βs towards hepatocytes in culture is decreased by serum or by purified α_2M (30). In vivo, TGF-β1 reversibly inhibits the proliferative response of hepatocytes following partial hepatectomy; however, the effect is transient and limited compared with the responses obtained in vitro (36). The role of α_2M and α_2M receptors in the in vivo response to TGF-β1 is a topic for future studies.

Finally, it should be noted that TGF-β1 is a single member of a family of growth factors that interact with α_2M. Some factors such as IL-1β and TGF-β1 bind preferentially to α_2M-proteinase complexes and α_2M-methylamine (11, 37) while others such as platelet-derived growth factor bind preferentially to the native form of the inhibitor (11). The studies presented here demonstrate for the first time clearance of a growth factor via a receptor system for a different protein. It is intriguing to speculate that the α_2M receptor may provide a clearance pathway for other growth factors and cytokines as well.

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