Identical or Overlapping Sequences in the Primary Structure of Human $\alpha_2$-Macroglobulin Are Responsible for the Binding of Nerve Growth Factor-\(\beta\), Platelet-derived Growth Factor-BB, and Transforming Growth Factor-\(\beta\)\(^*\)

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$\alpha_2$-Macroglobulin (\(\alpha_2\)M) functions as a proteinase inhibitor and as a carrier of diverse growth factors. In this study, we localized binding sites for platelet-derived growth factor-BB (PDGF-BB) and nerve growth factor-\(\beta\) (NGF-\(\beta\)) to a linear sequence in the 180-kDa human \(\alpha_2\)M subunit which includes amino acids 591–774. A glutathione S-transferase fusion protein containing amino acids 591–774 (FP3) bound PDGF-BB and NGF-\(\beta\) in ligand blotting assays whereas five other fusion proteins, which collectively include amino acids 89–590 and 775–1451 did not. The \(K_D\) values for PDGF-BB and NGF-\(\beta\) binding to immobilized FP3 were 300 ± 40 and 180 ± 30 nM, respectively; these values were comparable with those determined using methylamine-modified \(\alpha_2\)M, suggesting that higher-order \(\alpha_2\)M structure is not necessary for PDGF-BB and NGF-\(\beta\) binding. PDGF-BB and NGF-\(\beta\) blocked the binding of transforming growth factor-\(\beta 1\) (TGF-\(\beta 1\)) to FP3. Furthermore, murinoglogulin, which is the only known member of the \(\alpha\)-macroglobulin family that does not bind TGF-\(\beta\), also failed to bind PDGF-BB and NGF-\(\beta\). These results support the hypothesis that either a single linear sequence in human \(\alpha_2\)M or overlapping sequences are responsible for the binding of TGF-\(\beta\), PDGF-BB, and NGF-\(\beta\), even though there is minimal sequence identity between these three growth factors. FP3 blocked the binding of PDGF-BB to a purified chimeric protein, in which the extracellular domain of the PDGF \(\beta\) receptor was fused to the IgG\(_1\)Fc domain, and to PDGF receptors on NIH 3T3 cells. Thus, FP3 may inhibit the activity of PDGF-BB.

Human \(\alpha_2\)-macroglobulin (\(\alpha_2\)M)\(^1\) is a member of a gene family that includes structurally related proteinase inhibitors and the complement components, C3, C4, and C5 (1). Although homologues of human \(\alpha_2\)M have been identified in numerous species, including primitive invertebrates (1–3), the function of this protein remains incompletely defined. \(\alpha_2\)M is found at high concentration in the plasma and has the capacity to inhibit diverse proteinases that differ in specificity and mechanism of action (4). However, in the presence of physiologic concentrations of other naturally occurring plasma proteinase inhibitors, the role of \(\alpha_2\)M in the regulation of most plasma proteinases is secondary. An interesting exception arises when foreign proteinases, such as snake venoms, are introduced into the circulation since \(\alpha_2\)M may be essential for neutralizing these proteinases (5).

The mechanism by which \(\alpha_2\)M inhibits proteinases is unique and depends on the organization of its four identical 180-kDa subunits into a hollow cylinder-like structure (1, 4). Unlike other proteinase inhibitors, \(\alpha_2\)M presents multiple target peptide bonds to attacking proteinases, thus accounting for its broad inhibitory specificity (6). The target peptide bonds are located within a small region in the center of the \(\alpha_2\)M subunit called the “bait domain” (4, 6). Proteinase-induced bait domain cleavage causes conformational change in \(\alpha_2\)M and a change in the size and shape of the central cavity (7–9). As a result, reacting proteinases are irreversibly trapped within the \(\alpha_2\)M cylinder (1, 4).

In native \(\alpha_2\)M, each subunit has a single \(\beta\)-cysteinyl-\(\gamma\)-glutamyl thiol ester bond that is formed by the side chains of Cys\(^{340}\) and Gln\(^{352}\) (10, 11). Bait domain cleavage exposes the thiol esters, rendering these bonds reactive with various nucleophiles, including lysine side chains in the attacking proteinase (12). Nucleophiles that react with the thiol esters become covalently linked to Gln\(^{352}\). In the absence of proteinases, \(\alpha_2\)M thiol esters still react with small primary amines, such as methylamine, triggering a conformational change which is equivalent to that caused by trypsin or chymotrypsin (13, 14).

In addition to proteinases, \(\alpha_2\)M binds a variety of functionally dissimilar proteins, including cytokines and growth factors, \(\beta\)-amyloid peptide, apolipoprotein E, myelin basic protein, and leptin (15–20). Although it is possible that some of these interactions depend on intact \(\alpha_2\)M quaternary structure, we recently identified a linear sequence in \(\alpha_2\)M that serves as a binding site for transforming growth factor-\(\beta 1\) (TGF-\(\beta 1\)) and TGF-\(\beta 2\) (21). The TGF-\(\beta\)-binding site was located in the center of the \(\alpha_2\)M subunit (aa 591–774) and overlapped with the bait domain. Binding of TGF-\(\beta\) to \(\alpha_2\)M may be particularly important since \(\alpha_2\)M regulates the activity of TGF-\(\beta\) that is produced endogenously by cells in culture (22–24). Furthermore, the \(\alpha_2\)M

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§ The abbreviations used are: \(\alpha_2\)M, \(\alpha_2\)-macroglobulin; TGF-\(\beta\), transforming growth factor-\(\beta\); NGF-\(\beta\), nerve growth factor-\(\beta\); PDGF-BB, platelet-derived growth factor; TNP-\(\alpha\), tumor necrosis factor-\(\alpha\); VEGF, vascular endothelial growth factor; MUG, murinoglogulin; \(\beta\)-emithione, serum albumin; MA, methylamine-modified \(\alpha_2\)M; GST, glutathione S-transferase; FP, fusion protein; R\&Fc, the extracellular domain of the PDGF \(\beta\) receptor fused to human IgG\(_1\)Fc; PAVE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; aa, amino acids.
gene knock-out mouse demonstrates abnormal responses to various forms of exogenous challenge that may be attributed to deficient TGF-β regulation (25, 26).

Like TGF-β, nerve growth factor-β (NGF-β) and platelet-derived growth factor-BB (PDGF-BB) bind noncovalently and reversibly to αM (16, 27). In all three growth factors, a conserved set of disulfide bonds yields a unique three-dimensional structure called the cystine knot (28); however, TGF-β, PDGF-BB, and NGF-β demonstrate almost no sequence identity. At least one protein that binds to αM, β-amyloid peptide, recognizes a sequence outside of FP3, near the COOH terminus of the αM subunit (17). Thus, multiple regions of αM may be responsible for different binding interactions.

The goal of the present investigation was to identify the binding sites for NGF-β and PDGF-BB in the structure of human αM. Our results indicate that these sites are located within FP3 and are identical or overlapping with the binding site for TGF-β. Other cytokines that have been reported to associate with αM, including tumor necrosis factor-α (TNF-α) (29) and vascular endothelial growth factor (VEGF) (30), did not bind to FP3, suggesting that the growth factor binding activity of FP3 is selective. Furthermore, murinoglobulin (MUG), which is the only α-macroglobulin identified to date that does not bind TGF-β (26), also failed to bind NGF-β and PDGF-BB. Thus, FP3 may serve as a novel region for growth factor interaction within the structure of human αM.

MATERIALS AND METHODS

Reagents and Proteins—TGF-β1, NGF-β, PDGF-BB, TNF-α, and VEGFα5 has, were from R&D Systems (Minneapolis, MN). Methylamine HCl, diethiothreitol, iodoacetamide, and bovine serum albumin (BSA) were from Sigma. pGEX-2T and prepackaged glutathione-Sepharose-4B columns were from Amersham Pharmacia Biotech. Immunol 2 microtiter plates were from Dynatech Laboratories (Chantilly, VA). 125I-Labeled PDGF-BB was from NEN Life Science Products Inc. TGF-β1 was radiiodinated with chloramine T to a specific activity of 0.2–1.0 μCi/μg as described previously (31). NGF-β and TNF-α were radiolabeled with IODO-GEN to a specific activity of 100–200 μCi/μg as described previously (31). NGF-β and TNF-α were radiolabeled with IODO-GEN to a specific activity of 1–5 μCi/μg according to the instructions from the manufacturer (Pierce). VEGF was radiolabeled with chloramine T to a specific activity of 0.2–1.0 μCi/μg.

αM and Related Derivatives—αM was purified from human plasma by the method of Imber and Pizzo (32). The mature αM subunit, in which the signal peptide has been removed, contains 1451 amino acids (33). MUG was purified from CD-1 female mice as described previously (26). SDS-PAGE analysis of purified MUG revealed a single band with an apparent mol wt of 200,000. αM-MMA was prepared by dialyzing αM against 200 mM methylamine HCl in 50 mM Tris-HCl, pH 8.2, for 12 h at 22 °C followed by extensive dialysis against 20 mM sodium phosphate, 150 mM NaCl, pH 7.4 (PBS), at 4 °C. Reaction of αM with methylamine was confirmed by demonstrating the characteristic shift in mobility by non-denaturing PAGE (34). An 18-kDa proteolytic fragment of the αM subunit, which includes the carboxyl terminus (aa 1514–1451) was prepared by digesting αM-MMA with papain at pH 4.5 (35) and purified as described previously (36). The 18-kDa fragment contains the binding site for the αM receptor at low density lipoprotein receptor-related protein (35).

Preparation of αM-Peptide-Glutathione S-Transferase (GST) Fusion Proteins—Fusion proteins that contain fragments of the αM sequence, including FP1, FP2, FP3, and FP6 (aa 1341–1451), were prepared by polymerase chain reaction, using the intact αM cDNA in pBluescript as a template. The oligonucleotides encoded restriction sites for BamHI (5′) and EcoRI (3′), allowing direct cloning of the polymerase chain reaction product into pGEX-2T. The final construct was subjected to sequence analysis to verify its orientation and integrity and then transformed into BL21 cells. FP6 was expressed and purified as described previously (21).

Ligand Blotting—Ligand blotting has been previously used to demonstrate specific and saturable binding of TGF-β2 to FP3 (21). In preparation for ligand blotting experiments, native αM, αM-MMA, MUG, the 18-kDa fragment, and the fusion proteins (FP1–FP6) were treated with 1 μl dithiothreitol in 2% SDS for 30 min at 37 °C. Free sulfhydryls were then blocked with 5 mM iodoacetamide for 2 h at 22 °C. Equivalent amounts of each intact α-macroglobulin (1 μg) or fusion protein (0.5 μg) were subjected to SDS-PAGE. The proteins were electrotransferred to PVDF membranes that were subsequently blocked with 5% milk in PBS and 0.1% Tween 20 (PBS-T). Membranes were probed with 125I-TGF-β1, 125I-NGF-β1, 125I-PDGF-BB, 125I-FP3, or 125I-VEGF for 1 h at 22 °C, washed with PBS-T, and analyzed by PhosphorImager analysis (Molecular Diagnostics).

Binding of Growth Factors to Immobilized Fusion Proteins—Purified FP3 (2 μg in 100 μl), FP6 (2 μg in 100 μl), and αM-MMA (10 μg in 100 μl) were immobilized in 96-well microtiter plates by incubation for 2 h at 37 °C. The wells were then washed three times with PBS-T and blocked with PBS-T for 2 h at 37 °C. Control wells were blocked with PBS-T without prior protein immobilization.

125I-TGF-β1 (0.2 μl) was incubated in wells with immobilized FP3, FP6, or αM-MMA, in the presence of 200 nM unlabelled TGF-β1 or increasing concentrations of NGF-β or PDGF-BB for 1 h at 22 °C. The wells were then washed three times with PBS-T. 125I-TGF-β1 that was associated with the immobilized phase was recovered in 0.1 M NaOH, 2% SDS and quantitated in a γ-counter. Specific binding of 125I-TGF-β1 was determined as the fraction of binding that was inhibited by unlabelled TGF-β1 and plotted against the log of the concentration of PDGF-BB or NGF-β. Each reported value represents the mean ± S.E. of at least three separate experiments with internal duplicate replicates. Assuming that PDGF-BB and NGF-β completely block 125I-TGF-β1 binding to the immobilized protein, then the IC50 is related to the Kd for the binding of PDGF-BB or NGF-β to FP3 or αM-MMA by the equation:

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K_d = IC_50/(1 + ([TGF-β1]/K_d)) \quad (\text{Eq. 1})
\]

Kd is the equilibrium dissociation constant for 125I-TGF-β1-binding to FP3 or αM-MMA, which is about 30 or 80 nM, respectively (21, 37).

Inhibition of PDGF-BB-binding to the PDGF β-Receptor—A chimeric protein in which the extracellular domain of the PDGF β-receptor is fused to the human IgG1 Fc domain was obtained from R&D Systems. The chimera (Rg/Fc) (1 μg/ml) was immobilized in Immulon-2 plates by incubation for 12 h at 22 °C. The wells were then washed three times with PBS-T and blocked with 10 μg/ml BSA for 2 h at 37 °C. As a control, some wells were blocked with BSA without first immobilizing the Rg/Fc. 125I-PDGF-BB (0.1 nM) was incubated with immobilized Rg/Fc in the presence of FP3, FP6, or αM-MMA (each at 1.0 μl) for 1 h at 37 °C. In some experiments, a 100-fold excess of unlabelled PDGF-BB was added to determine specific binding. The wells were then washed three times with PBS-T. 125I-PDGF-BB that was associated with the immobilized phase was recovered in 1.0 M NaOH, 2% SDS and quantitated in a γ-counter.

Inhibition of PDGF-BB Binding to NIH 3T3 Cells—NIH 3T3 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). The cells were passaged when subconfluent using enzyme-free dissociation buffer (Life Technologies, Inc.) and maintained in culture for at least 48 h. The cells were then washed with Earle’s balanced salts solution supplemented with 5 mM HEPES and 5 mg/ml BSA, pH 7.4, and incubated with 125I-PDGF-BB (0.1 μM) in the presence of FP3, FP6, or αM-MMA (1.0 μl) for vehicle or 4 h. Specific binding was determined by including a 100-fold molar excess of unlabelled PDGF-BB. After washing the cultures, cell-associated radioactivity was recovered in 1 M NaOH and quantitated in a γ-counter. Total cellular protein was determined with fluoroadhesive reagent (Pierce).

RESULTS

Ligand Blot Analysis of Growth Factor Binding to αM-Peptide Fusion Proteins—We previously demonstrated that TGF-β2 binds to FP3 and not to FP1, FP2, FP4, or FP5 (21). The COOH terminus of αM was not examined in our original study since LaMarre et al. (38) had previously demonstrated that TGF-β does not bind to the 18-kDa peptide, a proteolytic fragment of αM which includes the COOH terminus. More recently, Hughes et al. (17) demonstrated that the αM COOH terminus contains a significant binding site for another non-
proteinase, β-amyloid peptide. Thus, we generated a sixth construct (FP6) which encodes the α₂M COOH terminus (aa 1242–1451). As shown in Fig. 1, 125I-TGF-β1 bound to FP3, but not to any of the other fusion proteins, including FP6. In separate studies, TGF-β1 also failed to bind to PVDF-immobilized 18-kDa fragment and purified GST (results not shown).

PDGF-BB binds noncovalently to intact α₂M whereas PDGF-AA does not (16, 39). To determine whether a linear sequence in the structure of α₂M is responsible for PDGF-BB-binding, 125I-PDGF-BB (15 pM) was incubated with the six PVDF-immobilized fusion proteins. As shown in Fig. 2, 125I-PDGF-BB bound to FP3, but not to the other five fusion proteins or purified GST. Identical results were obtained when these experiments were repeated using 125I-PDGF-BB at different concentrations (30 and 60 pM). The 18-kDa fragment also failed to bind PDGF-BB, confirming the results obtained with FP6 (results not shown).

The neurotrophins, NGF-β, NT-3, NT-4, and brain-derived neurotrophic factor, cross-compete for binding to intact α₂M, suggesting interaction with the identical or overlapping binding sites (27). As shown in Fig. 2, 125I-NGF-β (20 pM) bound to FP3, but not to the other fusion proteins or purified GST. Equivalent results were obtained in experiments with 40 pM 125I-NGF-β. Thus the binding sites for three structurally and functionally diverse growth factors (TGF-β, NGF-β, and PDGF-BB) are contained within a small region of the α₂M subunit (amino acids 591–774). 125I-VEGF and 125I-TNF-α did not demonstrate specific binding to any of the α₂M fusion proteins, including FP3. Thus, the growth factor/cytokine binding activity of FP3 is selective within the context of proteins which have been previously reported to interact with intact α₂M.

**PDGF-BB Inhibits TGF-β Binding to FP3**—125I-TGF-β1 (0.2 nM) was incubated with FP3 and α₂M-MA that were immobilized in microtiter plates. In the absence of competing agents, 6.9 ± 0.5 and 3.6 ± 0.4 fmol of 125I-TGF-β1 bound to FP3 and α₂M-MA, respectively. 125I-TGF-β1 binding to FP3 and α₂M-MA was decreased by greater than 85% when 200 nM unlabeled TGF-β1 was added, indicating that the observed binding was specific. Furthermore, less than 0.1 fmol of 125I-TGF-β1 bound to immobilized FP6 and to PBS-T-blocked control wells.

PDGF-BB inhibited the binding of 125I-TGF-β1 to immobilized α₂M-MA and FP3 (Fig. 3). The highest concentration of PDGF-BB (0.8 μM) decreased TGF-β1 binding to FP3 by greater than 95%, suggesting that PDGF-BB and TGF-β binding to FP3 are mutually exclusive. The IC₅₀ for inhibition of TGF-β1 binding to FP3 was 300 ± 40 nM, which represents an accurate estimate of the KD for PDGF-BB binding to FP3, since the concentration of 125I-TGF-β1 was low (21). The IC₅₀ for inhibition of 125I-TGF-β1 binding to immobilized α₂M-MA was 270 ± 20 nM. This value compares favorably with the previously reported KD (370 nM) for PDGF-BB binding to α₂M-MA in solution (16) and suggests that α₂M-MA immobilization does not adversely affect the PDGF-BB/α₂M interaction.

**NGF-β Inhibits TGF-β Binding to FP3**—NGF-β inhibited the specific binding of 125I-TGF-β1 to immobilized FP3 and α₂M-MA by up to 85% (Fig. 4), suggesting that NGF-β binds to a sequence in FP3 that is either identical to or overlapping with the TGF-β1-binding site. The IC₅₀ values for inhibition of TGF-β1 binding to immobilized FP3 and α₂M-MA were 180 ± 30 and 150 ± 30 nM, respectively. The IC₅₀ determined with immobilized α₂M-MA compares favorably with the previously
reported $K_D$ (110 nM) for NGF-$\beta$ binding to $\alpha_2$M-MA in solution (16). These results and our previously reported studies (27) demonstrating competition between various neurotrophins for binding to intact $\alpha_2$M suggest that the neurotrophins bind to a linear sequence in the structure of $\alpha_2$M that is identical or overlapping with the TGF-$\beta$-binding site.

Murinoglobulin Fails to Bind PDGF-BB and NGF-$\beta$—MUG is a monomeric $\alpha$-macroglobulin from mouse and the only $\alpha$-macroglobulin described thus far that fails to bind TGF-$\beta$ (26). Having co-localized the binding sites for TGF-$\beta$, PDGF-BB, and NGF-$\beta$ in FP3, we undertook studies to determine whether PDGF-BB and NGF-$\beta$ bind to MUG. Since ligand blotting was utilized, growth factor binding to isolated $\alpha$-macroglobulin subunits was assessed. TGF-$\beta$2 binds equivalently to the subunits of native $\alpha_2$M and $\alpha_2$M-MA, probably reflecting the absence of higher-order structure following exposure to SDS (21). Fig. 5 shows that $^{125}$I-PDGF-BB also bound equivalently to native $\alpha_2$M and $\alpha_2$M-MA but not MUG. Identical results were obtained with $^{125}$I-NGF-$\beta$. Thus, TGF-$\beta$, PDGF-BB, and NGF-$\beta$ recognize sequence(s) that are present in human $\alpha_2$M but not MUG.

**FP3 Inhibits Binding of PDGF-BB to the PDGF $\beta$ Receptor**—To determine whether the interaction of FP3 with PDGF-BB blocks binding of PDGF-BB to its signaling receptor, purified R$\beta$/Fc was immobilized in microtiter plates. R$\beta$/Fc contains the growth factor-binding domain of the PDGF $\beta$ receptor. In the absence of inhibitors, 0.57 ± 0.04 fmol of $^{125}$I-PDGF-BB bound to R$\beta$/Fc and 87 ± 6% of the binding was specific. FP3 and $\alpha_2$M-MA inhibited specific binding by 84 and 85%, respectively, whereas FP6, at the same concentration, had no effect (Fig. 6A). In these experiments, inhibition of PDGF-BB binding to R$\beta$/Fc is based on the ability of FP3 or $\alpha_2$M-MA to bind $^{125}$I-PDGF-BB in solution and thereby decrease the concentration of free $^{125}$I-PDGF-BB that is available to interact with the immobilized receptor. The extent of inhibition is related to the concentration of competing agent, as described previously (21).

**FP3 Inhibits PDGF-BB Binding to NIH 3T3 Cells**—To con-
firm the results of our experiments with purified Rβ/Fc, we studied the binding of 125I-PDGF-BB to NIH 3T3 cells in culture. When 0.1 nM 125I-PDGF-BB was incubated with the cells at 4 °C, in the absence of competing agents, specific binding was 28 ± 6 fmol/mg of cell protein. FP3 and αM-MA reduced specific binding by greater than 95%, whereas FP6, at the identical concentration, had no effect (Fig. 6B). These results provide further evidence that FP3 and αM-MA bind 125I-PDGF-BB in solution, forming complexes that are no longer recognized by PDGF receptors.

**DISCUSSION**

The αM subunit includes well defined regions that are responsible for specific functions, including the bait domain (aa 666–706) (6), the thiol ester loop region (aa 949–952) (10, 11), and the αM receptor/LRP recognition sequence (including aa 1370–1374) (40, 41). A binding site for TGF-β has been identified in FP3 (aa 591–774) (21) and a distinct binding region has been identified for αM-MA (21). Thus, higher-order structure in αM, which is probably lost in FP3, is not necessary for interaction with PDGF-BB and NGF-β.

The binding sites for PDGF-BB and NGF-β in FP3 are overlapping or identical with the TGF-β-binding site since the three growth factors cross-competed for FP3 binding. FP3 inhibited the binding of PDGF-BB to a chimeric protein containing the extracellular domain of the PDGF β receptor, suggesting that FP3 and the β receptor recognize identical or adjacent sequences in PDGF-BB. Furthermore, MUG, which lacks TGF-β binding activity (21), also failed to bind PDGF-BB and NGF-β. These results support the hypothesis that there is a conserved sequence in human αM and multiple other α-macroglobulins, but not MUG, that is required for the binding of TGF-β, PDGF-BB, and NGF-β.

The list of α-macroglobulins reported to bind TGF-β includes human αM, murine αM, rat αM, rat αM, bovine αM, and rabbit αM (26, 42, 43). In these α-macroglobulins, the bait domain, which is contained within FP3, is an area of extreme sequence diversity (6, 44). Thus, it is reasonable to assume that the binding sites for TGF-β, PDGF-BB, and NGF-β may be located adjacent to the bait domain in an area of increased sequence conservation. In the three-dimensional structure of intact tetrameric αM, the bait domain is apparently part of a flexible loop located inside the hollow cylinder structure (45, 46). Previous studies have demonstrated that growth factors, that bind to intact αM, are not recognized by growth factor-specific antibodies (47). Furthermore, in αM-proteinase complexes, the number of growth factor-binding sites is limited by the number of proteinases that are bound (one or two) (48), although the results presented here suggest that each subunit expresses an independent growth factor-binding site. These results suggest that the growth factor-binding site in FP3, like the bait domain, is located inside the αM cylinder and that steric constraints limit the number of growth factors that may be bound by each intact αM.

The gene for αM is located on chromosome 16 in a region that has been implicated in late-onset Alzheimer's disease (49). Some but not all studies suggest that polymorphisms in the αM gene may be associated with Alzheimer's disease (50–53). Reactive microglial cells that border neuritic plaques produce αM which could regulate disease progression (54); however, De Strooper et al. (55) has shown that αM does not affect processing of amyloid precursor protein and formation of β-amyloid peptide. Other mechanisms by which αM may influence Alzheimer's disease progression include regulation of proteinases involved in processes other than amyloid precursor protein processing, binding of β-amyloid peptide, or binding of growth factors such as neurotrophins and TGF-β. One αM gene polymorphism that has been associated with Alzheimer's disease is a 5′ splice site deletion preceding exon 18, which encodes part of the bait domain (50, 56). Exon 18 is a major contributor to FP3, which we have now identified as the neurotrophin-binding site. If the 5′ splice site deletion causes aberrant splicing, then neurotrophin binding to αM may be affected in addition to proteinase inhibition.

Despite previous studies demonstrating TNF-α binding to intact αM (29, 57), we were not able to localize a TNF-α-binding site within the six fusion proteins. These results suggest that the TNF-α/αM interaction may be qualitatively different than the other interactions described here. For example, TNF-α binding may depend on intact quaternary αM structure. Interestingly, Wu et al. (58) reported that oxidation of intact αM decreases binding of growth factors involved in tissue repair, including TGF-β1, TGF-β2, PDGF-BB, and NGF-β, while increasing the binding affinity of αM for inflammatory cytokines, including TNF-α. The tissue repair growth factors referred to by Wu et al. (58) are in fact the family of growth factors that bind to FP3. Thus, it is reasonable to suggest that oxidative modification of a single amino acid in FP3 may be responsible for the decrease in binding affinity of intact αM for TGF-β1, PDGF-BB, and NGF-β. The paradoxical increase in binding affinity for TNF-α, which is observed when αM is oxidized, is consistent with this interaction being independent of the growth factor-binding site in FP3.

The availability of a recombinant peptide with the capacity to bind and neutralize the activity of functionally diverse growth factors is novel. We have already demonstrated the ability of FP3 to promote endothelial cell growth in vitro in an assay configured to detect TGF-β activity (21). Our studies with Rβ/Fc and NIH 3T3 cells suggest that FP3 may counteract the biological activities of PDGF-BB as well. The activities expressed by FP3 in different experimental systems will probably reflect the spectrum of growth factors that are functional in each system.

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