Specific Binding of Integrin αvβ3 to the Fibrinogen γ and α_E Chain C-Terminal Domains†

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ABSTRACT: Integrin αvβ3, a widely distributed fibrinogen receptor, recognizes the RGD$^{572-574}$ motif in the α chain of human fibrinogen. However, this motif is not conserved in other species, nor is it required for αvβ3-mediated fibrin clot retraction, suggesting that fibrinogen may have other αvβ3 binding sites. Fibrinogen has conserved C-terminal domains in its α (E variant), β, and γ chains (designated α_EC, βC, and γC, respectively), but their function in cell adhesion is not known, except that αIIbβ3, a platelet fibrinogen receptor, binds to the γC HHLGGAKQAGDV$^{400-411}$ sequence. Here we used mammalian cells expressing recombinant αvβ3 to show that recombinant α_EC and γC domains expressed in bacteria specifically bind to αvβ3. Interaction between αvβ3 and γC or α_EC is blocked by LM609, a function-blocking anti-αvβ3 mAb, and by RGD peptides. αvβ3 does not require the HHLGGAKQAGDV$^{400-411}$ sequence of γC for binding, and α_EC does not have such a sequence, indicating that the αvβ3 binding sites are distinct from those of αIIbβ3. A small fragment of γC (residues 148−226) supports αvβ3 adhesion, suggesting that an αvβ3 binding site is located within the γ chain 148−226 region. We have reported that the CYDMKTTC sequence of β3 is responsible for the ligand specificity of αvβ3. γC and α_EC do not bind to wild-type αvβ1, but do bind to the αvβ1 mutant (αvβ1-3-1), in which the CYDMKTTC sequence of β3 is substituted for the corresponding β1 sequence CTSEQNC. This suggests that γC and α_EC contain determinants for fibrinogen's specificity to αvβ3. These results suggest that fibrinogen has potentially significant novel αvβ3 binding sites in γC and α_EC.

Fibrin interaction with integrin αvβ3 expressed on endothelial cells is important for angiogenesis associated with wound healing and tumorigenicity (*1*, *2*). αvβ3 expression and fibrin generation increase in microvessels after focal cerebral ischemia (*3*). Thus, αvβ3-fibrin(ogen) interaction is of biological significance and is a potential target for modulating these processes. Fibrinogen is a 340 kDa glycoprotein that consists of two identical disulfide-linked subunits. Each subunit is composed of three nonidentical polypeptide chains: α, β, and γ (*4*). The β and γ chains have conserved C-terminal domains (designated βC[1] and γC, respectively). The crystal structure of the isolated γC domain (*5*), as well as that of the γC and βC domains in the fibrinogen D fragment (*6*), revealed that both domains are similarly folded. A minor 420 kD form of fibrinogen (fibrinogen-420) has an alternative extended form of the α chain (α_E), which has a C-terminal domain (designated α_EC) whose sequence and fold are highly homologous to those of the βC and γC domains (*7*, *8*). Although several functions of the βC and γC domains have been well defined, the function of the α_EC domain in fibrinogen-420 remains unknown.

Integrin αvβ3 is a fibrinogen receptor that is found in a wide range of cell types including endothelial cells, fibroblasts, leukocytes, and tumor cells. It is widely accepted that αvβ3 recognizes the RGD$^{572-574}$ sequence of the fibrinogen α chain in αvβ3-mediated cell adhesion (*9*, *10*). However, the α chain RGD$^{572-574}$ sequence is not conserved or is absent in fibrinogen from other species including primate. Rat, mouse, or bovine fibrinogen has RGD$^{252-254}$ instead of RGD$^{572-574}$ (*11*, *12*), and rhesus monkey or pig fibrinogen has no RGD sequence in the α chain (*11*). Therefore, fibrinogen that has no α chain RGD sequence should have another αvβ3 binding motif. αvβ3 has been shown to be critically involved in endothelial (or tumor) cell-mediated fibrin clot retraction (*13*, *14*); interestingly, the fibrinogen α chain RGD$^{95-97}$ and RGD$^{572-574}$ sequences and the γ chain AGDV$^{408-411}$ sequence are not required for this process (*14*, *15*). These results suggest that other unknown αvβ3 binding sites are involved in fibrin clot retraction and cell adhesion.

In the present study, we discovered that the fibrinogen C-terminal domains (γC and α_EC) support αvβ3-mediated adhesion, using recombinant C-terminal domains. Since the C-terminal dodecapeptide sequence of γC (HHLGGAKQAGDV$^{400-411}$) is not required for γC binding to αvβ3, the

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[1] Abbreviations: CHO, Chinese hamster ovary; α_EC, fibrinogen α_E chain variant C-terminal domain; βC, fibrinogen β chain C-terminal domain; γC, fibrinogen γ chain C-terminal domain; FITC, fluorescent isothiocyanate; mAb, monoclonal antibody; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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αvβ3 binding site in γC is distinct from that of integrin αIIbβ3, a platelet fibrinogen receptor that requires the HHLGGAKQAGDV$^{400-411}$ sequence (ref *16*, and references therein). These results suggest that fibrinogen has novel αvβ3 binding sites in the γC and α_EC domains that may be biologically significant.

EXPERIMENTAL PROCEDURES

Antibodies. LM609 (to αvβ3) (*9*) was provided by D. Cheresh (The Scripps Research Institute, La Jolla, CA). PT25-2 (to αIIbβ3) (*17*) was provided by M. Handa and Y. Ikeda (Keio University, Tokyo, Japan). MAb 13 (to human β1) (*18*) was a kind gift from K. Yamada (NIH, Bethesda, MD).

CHO Cells Expressing Different Human Integrins. Untransfected CHO cells express endogenous α5, αv, and β1, but not β2 or β3. αIIbβ3-CHO cells express human αIIbβ3 and hamster αv/human β3 hybrid (*19*). β1-CHO cells express hamster αv (or α5)/human β1 hybrid (*20*). β3-CHO cells express hamster αv/human β3 hybrid (*20*). β1-3-1-CHO cells express the αvβ1-3-1 mutant described in ref *20*.

Recombinant Fibrinogen C-Terminal Domains. The fibrinogen C-terminal domains, α_EC, γC, and its truncated mutant, γC/399t, were produced in *Escherichia coli* using the pET-21a+ expression vector. The cDNA fragments encoding the fibrinogen C-terminal domains (α_EC, residues 612−847 of the αE chain; γC, residues 151−411 of the γ chain; and γC/399t, residues 151−399 of the γ chain) were amplified by polymerase chain reaction. Fibrinogen γ chain cDNA (ATCC59710) was used as a template for γC, and a human liver cDNA library (Clontech) was used as templates for α_EC. The primers used were 5′-GGGGATCCAAAGATTGTCAAGACATTGCC-3′ and 5′-CGGAATTCTTAAACGTCTCCAGCCTG-3′ for γC, and 5′-GGAATTCCATATGGACTGTGATGATGTCCTCC-3′ and 5′-ACCGCTCGAGCTATTGGGTCACAAGGGGCC-3′ for α_EC. The cDNA fragments were subcloned into the *Bam*HI/*Eco*RI site (γC) and the *Nde*I/*Xho*I site (α_EC) of PET21a+ vector. The cDNA fragments were completely sequenced to confirm that no errors occurred during amplification. A stop codon was introduced by mutagenesis at position 400 for the γC/399t mutant. Synthesis of γC, the γC/399t mutant, and α_EC, and purification and refolding of recombinant proteins, were performed as previously described (*21*). To confirm the refolding of the proteins, fluorescence measurements of thermal unfolding were performed as described (*21*).

Preparation of the Recombinant γ148−226 Fragment. We used SDS−PAGE to analyze the lysate of the bacterial cells that produce a mutant γC (148−411) in which Asp199-Gly200 is mutated to Gly-Ala (*22*), and found that the pellet contained full-length γC (148−411) and a substantial amount of a 9 kDa fragment. This fragment was immunopositive with antifibrinogen polyclonal antibody, suggesting that it represents a proteolytically truncated γC mutant. The fragment was separated from the full-length γC mutant by size-exclusion chromatography on Superdex 75 in 8 M urea and then refolded according to the protocol described earlier for wild-type γC (148−411) (*21*). NH_2-terminal sequence analysis of the full-length 9-kDa fragment and its tryptic fragments separated by HPLC revealed that it corresponds to the γ148−226 region containing the Asp199-Gly200 to Gly-Ala mutation.

Fluorescence measurements of thermal unfolding were performed in an SLM 8000-C fluorometer by monitoring either intrinsic fluorescence intensity at 370 nm, or the ratio of the intensity at 370 nm to that at 330 nm, with excitation at 280 nm as described earlier (*21*).

SDS−PAGE was carried out as previously described (*23*).

Adhesion Assay. Adhesion assays were performed as previously described (*24*). Hepes-Tyrode buffer (10 mM HEPES, 150 mM NaCl, 12 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, 2.5 mM KCl, 0.1% glucose, 0.02% BSA, and 1 mM Mg^{2+}, pH 7.4) was used instead of Dulbecco's modified Eagle medium. In some experiments, cells were preincubated with 250 μM RGD peptide or anti-integrin monoclonal antibodies.

Ligand Binding Assay. Fibrinogen and recombinant C-terminal domains were labeled with fluorescein-isothiocyanate (FITC) as described (*25*). Cells ($1-1.5 \times 10^6$ cells) were incubated with soluble FITC-labeled fibrinogen, recombinant γC, or recombinant α_EC, in 100 μL of Dulbecco's modified Eagle medium for 30 min at room temperature. The activating anti-αIIbβ3 mAb PT25-2 was used to activate αIIbβ3. Cells were washed with ice-cold medium and analyzed by cell sorter.

RESULTS

Preparation and Characterization of the Recombinant Fibrinogen C-Terminal Domains. To study the involvement of the fibrinogen C-terminal domains in αvβ3/fibrinogen interaction, we produced in *E. coli* recombinant C-terminal domains. These include wild-type γC (residues 151−411 of the γ chain), the truncated mutants γC/399t (residues 151−399 of the γ chain), and α_EC (residues 612−847 of the α_E chain). All of the recombinant fibrinogen C-terminal domains used were more than 90% homogeneous as revealed by SDS−PAGE (Figure 1A and data not shown). Since all of the recombinant domains were expressed in a bacterial system, contained disulfides, and were subjected to the refolding procedure, it was necessary to verify their structural integrity. Like the well-characterized recombinant γC (148−411) (*21*), both wild-type γC and mutant γC/399t exhibited fluorescence spectra with maximum at 344 nm (Figure 1B), which is consistent with the presence of a compact structure. When heated in the fluorometer while the fluorescence intensity at 370 nm was monitored they both exhibited a sigmoidal transition (Figure 1C) that was very similar to that of γC (148−411). The refolded α_EC domain exhibited fluorescence spectra at an even shorter wavelength with a maximum at 338 nm (Figure 1B). The sigmoidal transition in α_EC monitored by the fluorescence intensity was less pronounced, but it became obvious when the ratio of fluorescence intensity at 370 nm to that at 330 nm was monitored upon heating (Figure 1D). These results indicate that the recombinant proteins used in this study are properly folded.

Interaction between β3 Integrins and the Recombinant C-Terminal Domains. It has been reported that the platelet fibrinogen receptor αIIbβ3 binds to γC (148−411) (*21*). We examined the ability of our recombinant γC (151−411) and α_EC to bind to αIIbβ3. We tested FITC-labeled recombinant γC and α_EC to see if they would bind to αIIbβ3-CHO cells in the presence of mAb PT25-2, which specifically activates

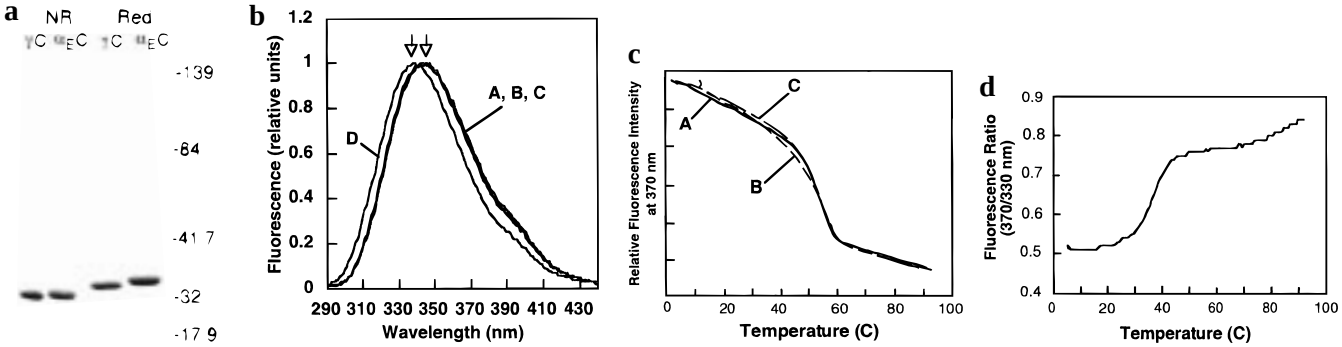

FIGURE 1: Characteristics of recombinant C-terminal domains. (a) SDS−PAGE of the recombinant fibrinogen C-terminal domains γC and α_EC on 10% gel under reducing (Red) and nonreducing (NR) conditions. Gel was stained with Coomassie brilliant blue and destained. (b) The fluorescent spectra of recombinant γC (148−411) (A), recombinant γC (residues 151−411) (B), its truncated mutant, γC/399t (C), and recombinant α_EC (residues 612−847) (D). Arrows indicate fluorescence intensity maximum at 344 nm (A, B, and C) or at 338 nm (D). (c) Fluorescence-detected thermal denaturation of recombinant γC (148−411) (A), recombinant γC (residues 151−411) (B), and its truncated mutant, γC/399t (C). The experiments were performed in 100 mM Gly, pH 8.6, containing 0.5 mM Ca^{2+}. (d) Fluorescence-detected thermal denaturation of recombinant α_EC under the same conditions as described above. These results suggest that the recombinant proteins are properly folded.

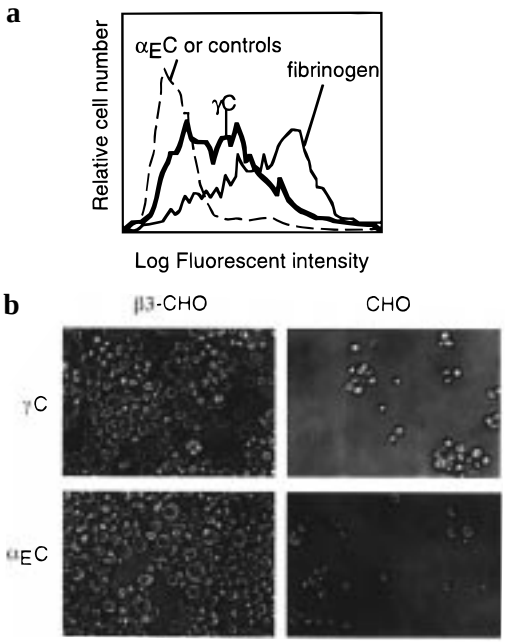

FIGURE 2: Interaction of γC and α_EC domains with different β3 integrins. (a) Binding of FITC-labeled γC to αIIbβ3-CHO cells. Cells were incubated with soluble FITC-labeled recombinant γC or fibrinogen in the presence of the activating anti-αIIbβ3 mAb PT25-2 and analyzed by cell sorter. Binding of γC and fibrinogen was not detected in the absence of PT25-2. Binding of α_EC was not detected, even in the presence of PT25-2. (b) Recombinant γC and α_EC adhere to β3-CHO cells that express αvβ3, but do not adhere to CHO cells that do not express αvβ3. Wells were coated with recombinant γC and α_EC (at 50 μg/mL coating concentration). After gently rinsing the wells once with buffer to remove unbound cells, we observed bound cells by phase contrast microscope (200×). Note that β3-CHO cells do not spread on γC or α_EC. Untransfected CHO cells did not attach to γC or α_EC.

αIIbβ3 (but not αvβ3). As shown in Figure 2A, recombinant γC binds to αIIbβ3-CHO cells, but α_EC does not. We did not detect any significant binding of FITC-labeled soluble γC or α_EC to β3-CHO cells (data not shown).

To determine whether recombinant γC and α_EC interact with αvβ3, we used β3-CHO cells that express αvβ3 (but not αIIbβ3). Parent CHO cells do not express αvβ3. As shown in Figure 2B, β3-CHO cells adhered to recombinant γC and α_EC, but parent CHO cells did not. β3-CHO cells

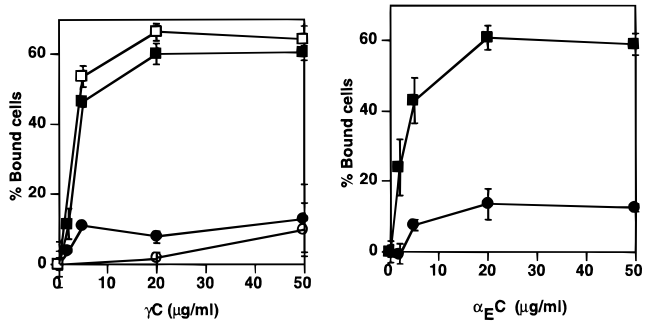

FIGURE 3: Adhesion of CHO cells and β3-CHO cells to recombinant γC and α_EC as a function of ligand concentration. Wells of 96-well microtiter plates were coated with increasing concentrations of γC, γC (148−411), or α_EC (up to 50 μg/mL). CHO cells (circles) and β3-CHO cells (squares) on γC (151−411, closed circles or squares) or γC (148−411, open circles or squares). The data are expressed as mean ± sd of three experiments. The data suggest that adhesion of β3-CHO cells to these recombinant proteins saturates at a 20 μg/mL coating concentration.

did not spread on the recombinant C-terminal domains. Adhesion of β3-CHO cells to recombinant γC and α_EC was saturated at a coating concentration of 20 μg/mL (Figure 3), indicating that recombinant γC and α_EC are equivalent in supporting αvβ3 adhesion. Similarly, β3-CHO cells adhered to a well-characterized recombinant γC (148−411) (Figure 3) (*21*). Adhesion of β3-CHO cells to these proteins was completely inhibited by RGD peptides, EDTA, and LM609, a function-blocking anti-αvβ3 mAb (Figure 4A). We also found that M21 human melanoma cells that express natural αvβ3 adhere to these domains, and that this adhesion is completely blocked by LM609 (Figure 4B). These results indicate that αvβ3 (recombinant or natural) specifically interacts with these C-terminal domains.

αvβ3 Does Not Require the HHLGGAKQAGDV$^{400-411}$ Sequence for Adhesion to γC. The fact that the α_E chain does not have the dodecapeptide-like sequence, but still supports αvβ3 adhesion, suggests that αvβ3 adhesion to γC does not depend on the dodecapeptide sequence. To define the role of the γC C-terminal region in αvβ3 binding, we examined the effects of truncating the C-terminal portion of γC (Figure 5). We found that the γC mutant with truncation

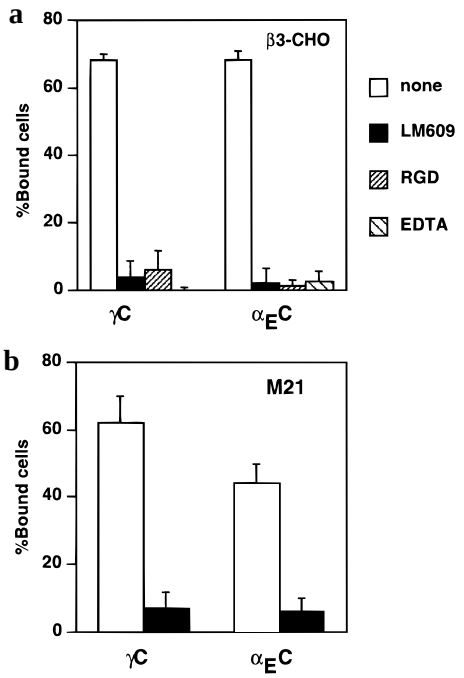

FIGURE 4: Specificity of adhesion of β3-CHO cells (a) and M21 melanoma cells (b) to γC and α_EC. The adhesion assay was performed as described in the legend to Figure 2. Wells were coated with 50 μg/mL γC or α_EC. LM609 at 250× dilution of ascites, 250 μM RGD peptide, or 2mM EDTA (instead of 1 mM $MgCl_2$) was used. The data are expressed as mean ± sd of three experiments. The data suggest that adhesion of β3-CHO and M21 cells to γC and α_EC is specifically mediated by integrin αvβ3.

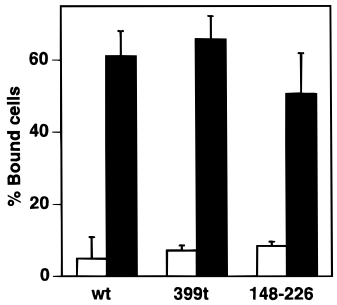

FIGURE 5: Localization of αvβ3 binding sites within γC. Wild-type recombinant γC, the γC/399t truncation mutant, and a small γC fragment (residues 148−226) were used for coating wells at 50 μg/mL: CHO cells (open bar); β3−CHO cells (solid bar). Adhesion assays were performed as described in the legend to Figure 3. The data are expressed as mean ± sd of three experiments. The data suggest that the HHLGGAKQAGDV$^{400-411}$ sequence of γC is not required for adhesion to αvβ3 and that an αvβ3 binding site is located within a region spanning residues 148−226.

of the complete dodecapeptide sequence HHLGGAKQ-AGDV$^{400-411}$ (399t) still supported adhesion of β3-CHO cells. These results suggest that αvβ3-mediated adhesion to γC is not dependent on the HHLGGAKQAGDV$^{400-411}$ sequence. This is in contrast to previous reports that αIIbβ3-mediated platelet aggregation requires the AGDV$^{408-411}$ sequence of γC (ref *16* and references therein). We also tested the recombinant fibrinogen γ chain 148−226 fragment (Asp199-Gly200 is mutated to Gly-Ala) and found that β3-CHO cells bound to this fragment. These results suggest that an αvβ3 binding site is located within the γ chain 148−226 region.

The γC and α_EC Domains Direct Fibrinogen's αvβ3 Specificity. We recently identified a region of the β subunit

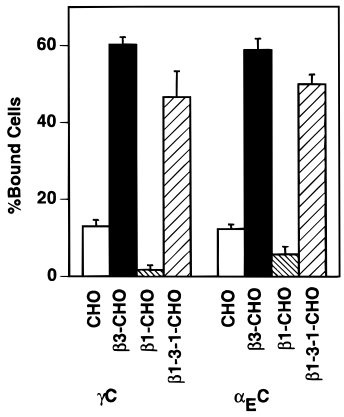

FIGURE 6: γC and α_EC are recognized by the αvβ1-3-1 mutant, but not by αvβ1. β1-CHO cells express αvβ1, but not αvβ3. β3-CHO cells express both αvβ1 and αvβ3. β1-3-1-CHO cells express αvβ1-3-1. αvβ3 recognizes fibrinogen, but αvβ1 does not. The αvβ1-3-1 mutant recognizes fibrinogen, although the anti-β1 function-blocking antibody blocks this interaction. Adhesion assays were performed as described in the legend of Figure 3. The data are expressed as mean ± sd of three experiments. The data suggest that recombinant γC and α_EC (which have no RGD sequence) support αvβ1-3-1 adhesion, suggesting that these domains contain determinants for the αvβ3 specificity of fibrinogen.

that is critical for the ligand specificity of integrins αvβ1 and αvβ3 (*20*). Integrin αvβ3 recognizes fibrinogen, von Willebrand factor, vitronectin, and fibronectin, while αvβ1 recognizes only fibronectin. When the sequence CTSEQNC (residues 187−193) of β1 is replaced with the corresponding CYDMKTTC sequence of β3, the ligand specificity of αvβ1 is altered; the mutant (αvβ1-3-1) recognizes the same ligands as αvβ3. We studied whether the αvβ1-3-1 mutant adheres to γC and α_EC. We found that β1-3-1-CHO cells do adhere to recombinant γC and α_EC, but control β1-CHO cells do not (Figure 6). Adhesion of αvβ1-3-1 to the C-terminal domains was blocked by mAb 13, a function blocking anti-β1 mAb. These results suggest that fibrinogen C-terminal domains have determinants for binding to αvβ3, even though these domains do not contain the RGD motif.

DISCUSSION

The present study provides direct evidence that αvβ3 specifically recognizes the fibrinogen C-terminal domains, γC and α_EC, even though these fragments lack RGD motifs. Unlike αIIbβ3, αvβ3-mediated adhesion to γC does not require the dodecapeptide HHLGGAKQAGDV$^{400-411}$ sequence; this is consistent with the fact that α_EC, which does not have the dodecapeptide-like sequence, still supports αvβ3 adhesion. These findings indicate that the γC and α_EC domains each have at least one major αvβ3 binding site, and that the γC sequence required for αvβ3 binding is distinct from the sequence required by αIIbβ3. Adhesion of β3-CHO cells to the C-terminal domains is blocked by RGD peptides, even though the domains do not have RGD motifs. This suggests that the C-terminal domains may use binding sites in αvβ3 that are common to other RGD-containing αvβ3 ligands (e.g., vitronectin).

This study also provides the first demonstration of a possible biological function of α_EC, which is unique to fibrinogen-420. Fibrinogen-420 represents only 1−2% of the fibrinogen in an adult human, and its physiological role is unknown. However, its levels in fetal human plasma are

about 3 times higher than in adult human plasma (*26*). Additionally, α_EC is conserved among vertebrates (*27*, *28*). The present study suggests that fibrinogen-420 has two additional $\alpha v\beta 3$-binding sites situated in the α_EC domains. It is possible that fibrinogen-420 may play a significant role in cell adhesion during development.

$\alpha v\beta 3$ is critically involved in endothelial (or tumor) cell-mediated fibrin clot retraction (*13*, *14*), and interestingly this process does not require the α chain RGD^{95-97} and $RGD^{572-574}$ sequences or the γ chain $AGDV^{408-411}$ sequence (*14*, *15*). These observations are consistent with the findings of the present study on the fibrinogen recognition and specificity of $\alpha v\beta 3$, which specifically binds to the fibrinogen C-terminal domains that lack RGD motifs. $\alpha v\beta 3$-γC interaction is retained when the dodecapeptide sequence of γC is truncated. Taken together, these findings suggest that γC and α_EC are the most likely locations for the $\alpha v\beta 3$ binding sites in $\alpha v\beta 3$-mediated clot retraction. The fact that these domains are well conserved among species, unlike the α chain RGD^{95-97} and $RGD^{572-574}$ sequences, supports the idea that these domains may mediate the primary function of fibrinogen, clot retraction. It should be noted that in our preliminary experiments, partially purified recombinant βC (residues 209−461 of the β chain) supported $\alpha v\beta 3$-mediated cell adhesion, which is blocked by LM609 and RGD peptides (data not shown). These results suggest that βC might also be involved in $\alpha v\beta 3$-mediated cell adhesion.

It has been established that deletion of the γ chain AGDV sequence blocks $\alpha IIb\beta 3$−fibrinogen interaction (*29*, *30*). In transgenic mice that have no QAGDV sequence in their fibrinogen γ chain, binding of fibrinogen to $\alpha IIb\beta 3$ and platelet aggregation are highly defective, but clot retraction is normal (*30*). The retained clot retraction in these transgenic mice has not been fully explained. The present study suggests that the interaction between $\alpha v\beta 3$ and γC is retained when the $HHLGGAKQAGDV^{400-411}$ sequence of the γ chain is truncated, even though this truncation eliminates $\alpha IIb\beta 3$-γC interaction. It is likely that $\alpha v\beta 3$-mediated clot retraction partly compensates for the lack of $\alpha IIb\beta 3$-mediated clot retraction in QAGDV-motif-deleted mice. The retained $\alpha v\beta 3$−fibrinogen interaction possibly explains the normal fibrin clot retraction in the QAGDV-deficient mice.

It has been reported that a proteolytic fibrinogen fragment that lacks $\alpha RGD^{572-574}$ (fragment X) cannot support $\alpha v\beta 3$-mediated adhesion of endothelial cells (*9*) and melanoma cells (*31*). Mutation of the $\alpha RGD^{572-574}$ sequence to RGE has been reported to markedly reduce adhesion of endothelial cells to fibrinogen (*12*). The present study does not contradict these previous reports. One possible explanation is that the $\alpha RGD^{572-574}$ sequence of human fibrinogen may play a dominant role, and possibly overshadow the contribution of the C-terminal domains, in $\alpha v\beta 3$-mediated cell adhesion. Consistent with this explanation, mutation of the $\alpha RGD^{572-574}$ sequence to RGE significantly reduces, but does not completely eliminate, $\alpha v\beta 3$-mediated adhesion (*12*). However, the $\alpha RGD^{572-574}$ sequence of human fibrinogen may not be critical for $\alpha v\beta 3$-mediated clot retraction.

We have shown that γC and α_EC contain determinants for fibrinogen's specificity to $\alpha v\beta 3$. We have previously reported that $\alpha v\beta 1$ does not recognize whole fibrinogen, but $\alpha v\beta 3$ and the $\alpha v\beta 1$-3-1 mutant do (*20*). These results suggest that the YDMKTT sequence of $\beta 3$ is critical for fibrinogen specificity. We have shown in this paper that fibrinogen's determinants for $\alpha v\beta 3$ specificity are located in its γC and α_EC domains. This is consistent with the finding that $\alpha v\beta 3$ specifically recognizes the fibrinogen C-terminal globular domains. There is one $\alpha v\beta 3$ binding site in γC that is probably located within residues 148−226 of γC. The γ chain 148−226 fragment contains domain A (residues 148−191) and part of domain B (residues 192−226). According to its 3-dimensional structure, the latter portion is stabilized by several internal hydrogen bonds and side chain−side chain contacts and also forms hydrogen bonds and side chain contacts with domain A. Thus it may be that the ordered conformation of the fragment accounts for its resistance to proteolysis. In addition the fragment contains the region 190−202 (P1), which has been reported to interact with $\alpha M\beta 2$ (*32*). The Asp199-Gly200 to Gly-Ala mutation did not interfere with that interaction (*22*). The putative $\alpha v\beta 3$ binding sites in the C-terminal domains might be major targets for modulating this interaction.

The present study does not address whether $\alpha IIb\beta 3$ recognizes α_EC or γC/399t. We found that $\alpha IIb\beta 3$-CHO cells adhered to γC, α_EC, and γC/399t in preliminary experiments (data not shown). $\alpha IIb\beta 3$-CHO cells express both human $\alpha IIb\beta 3$ and hamster αv/human $\beta 3$ hybrid. $\alpha IIb\beta 3$-CHO cell adhesion to γC and α_EC is not completely inhibited by the anti-$\alpha v\beta 3$ antibody LM609, but is completely inhibited by adding both LM609 and the anti-$\alpha IIb\beta 3$ antibody OPG2. $\alpha IIb\beta 3$-CHO cell adhesion to the γC/399t mutant is completely blocked by LM609. On the basis of these preliminary results, we suspect that $\alpha IIb\beta 3$ binds to γC and binds only weakly to α_EC, but does not bind to γC/399t. Further studies must be performed using the cells that express $\alpha IIb\beta 3$ but not $\alpha v\beta 3$ to clarify this point.

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