TA205, an anti-talin monoclonal antibody, inhibits integrin–talin interaction

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Abstract  Talin mediates integrin signaling by binding to integrin cytoplasmic tails through its FERM domain which consists of F1, F2 and F3 subdomains. TA205, an anti-talin monoclonal antibody, disrupts actin stress fibers and focal adhesion when microinjected into fibroblasts. Here, we showed that TA205 caused an allosteric inhibition of integrin $\alpha_{IIb}\beta_3$ binding to the talin FERM domain and mapped the TA205 epitope to residues 131–150 in talin F1. Furthermore, binding of a talin rod fragment to talin head was partially inhibited by TA205. These findings suggest that talin F1 may be important in regulation of integrin binding and talin head–rod interaction.

Keywords: Integrin; Talin; Monoclonal antibody

1. Introduction

Integrins are $\alpha\beta$ heterodimeric adhesion receptors that mediate bidirectional inside-out and outside-in signaling [1]. Talin, a cytoskeletal protein consisting of a globular head and a flexible rod domains, is a prominent component of focal adhesion linking integrins with actin filaments [2,3]. Recently, talin–integrin interaction has been implicated in inside-out signaling processes that activate integrins to increase their ligand binding affinity. Thus, overexpression of talin head fragments into Chinese hamster ovary (CHO) cells expressing integrin $\alpha_{IIb}\beta_3$ results in receptor activation [4,5]. Conversely, talin knockdown by transfection of small interfering RNAs into cells inhibits cellular activation of $\beta_3$ and $\beta_1$ integrins [6].

There are two integrin binding sites per talin molecule, one in the talin head and another near the C-terminus of the talin rod [4,7–10]. The talin head contains an ~300 amino acid FERM (band four-point-one, ezrin, radixin, and moesin) domain comprising of three subdomain folds (F1, F2 and F3) that form a clover-shaped structure [3,11]. Talin F3 contains a phosphotyrosine-binding domain that interacts with the NPXY/F reverse turn in integrin $\beta$ tails [11]. In the $\alpha_{IIb}\beta_3$ expressing CHO cell system, integrin activation can be achieved by expression of talin F3 alone [5]. In contrast, talin F2 also binds to the $\beta_3$ tail with a 4-fold lower affinity but cannot activate $\alpha_{IIb}\beta_3$. At present, the functional role of talin F1 is not known.

TA205, an anti-talin monoclonal antibody, disrupts focal adhesion, stress fiber formation and cell migration when microinjected into human fibroblasts [12]. In this study, we showed that TA205 causes an allosteric inhibition on integrin binding to the talin FERM domain, providing a possible mechanism of its inhibitory action on integrin-mediated cell function. Furthermore, we mapped the TA205 epitope to talin F1, and showed that TA205 modulates talin head–rod interaction.

2. Materials and methods

2.1. Reagents

The anti-talin monoclonal antibodies TA205, TD77 and 8d4 were purchased from Upstate (Charlottesville, VA), Research Diagnostic Inc. (Concord, MA), and Sigma (St. Louis, MO), respectively. AP-3, an anti-$\beta_3$ monoclonal antibody, was produced as ascites fluid in mice and purified by Protein A chromatography. Streptavidin was obtained from Sigma (St. Louis, MO). TA205, 8d4, AP-3 and streptavidin were labeled with carrier-free Na$^{125}$I (ICN Biomedicals, Inc., Irvine, CA) using the Iodobeads iodination reagent (Pierce, Rockford, IL) to a specific activity of approximately 2 $\mu$Ci/µg for antibodies and 0.5 $\mu$Ci/µg for streptavidin. Peptides corresponding to residues 131–150 of mouse talin (RELMEEKKDEGTGTLRKDKT) or human talin (RELMEEKKDEGTGTLRKDKT) and the biotin-$\beta_3$-(716–733) peptide were synthesized by Invitrogen (Carlsbad, CA).

2.2. Talin cDNA constructs

Generation of human talin head and rod constructs has been described previously [8,9]. Site-directed mutation of talin head was performed using the QuickChange mutagenesis kit (Stratagene, La Jolla, CA). To create fragments of the talin FERM subdomains, polymerase chain reactions (PCR) were performed on the talin head construct using Pfu and oligonucleotide primer pairs derived from the human talin cDNA sequence (GenBank accession number AF078828). The PCR products were cloned into the pET-30a(+) vector (Novagen, EMD Biosciences, Inc., Madison, WI), and all constructs were verified by DNA sequencing. The amino acid residues of the talin fragments were: F1, 88–202; F2, 200–311; F3, 303–410; F1–F2, 88–311; F1–F2–F3, 88–410.

2.3. Protein isolation

Integrin $\alpha_{IIb}\beta_3$ was purified from octylglucoside extracts of outdated human platelets by RGD affinity chromatography followed by gel filtration on a Sephacryl S-300 HR column [7]. Talin was purified from Triton X-100 extracts of outdated human platelets as described [13], and dialyzed into phosphate-buffered saline (PBS: 10 mM sodium phosphate, pH 7.3, 0.15 M NaCl).
Recombinant talin fragments were produced and purified as hexahistidine-tagged proteins. Briefly, cDNA constructs of talin fragments were transformed into *Escherichia coli* BL21(DE3)-competent cells, and protein expression was induced with 1 mM isopropylthio-β-D-galactoside for 4 h at 37 °C. The hexahistidine-tagged proteins were purified by chromatography on Ni²⁺ resin (Novagen, EMD Biosciences, Inc., Madison, WI), followed by desalting on Sephadex G-15 columns. The wild type and mutant talin head fragments, containing the hexahistidine tag at their C-termini downstream of the endogenous calpain cleavage at Gin⁴³³Gln⁴³⁴ in talin, were digested with m-calpain (Sigma, St. Louis, MO) and the hexahistidine tags were removed by gel filtration on a Sephacryl S-100 column (16×60 cm). All other talin head fragments with the hexahistidine tags fused to their N-termini were digested with enterokinase (Novagen, EMD Biosciences, Inc., Madison, WI) and the histidine tags were absorbed with Ni²⁺ resin. The talin-(434–2541) rod fragment was produced and purified as described [9].

2.4. ELISA and solid-phase binding assay

Intact talin purified from platelet extracts or recombinant talin fragments were coated onto Immulon 4 HBX microtiter strips (Thermo Labsystems, Franklin, MA), followed by blocking with 3% bovine serum albumin (BSA). For enzyme-linked immunosorbent assay (ELISA), TA205 (0.5 μg/ml) was added and incubated for 2 h at 22 °C. Bound TA205 was detected with horseradish peroxi-
dase-conjugated secondary antibodies using o-phenylenediamine hydrochloride as the substrate and A₄₅₀ was measured after 15-30 min incubation at 22 °C. Alternatively, binding of 125I-labeled TA205 was performed and bound antibody was quantified by γ-counting. For solid-phase binding assay, purified αIIbβ₃ or biotin-β₃-(716–733) peptide was added to protein-coated wells and incubated for 3 h at 37 °C. After washing, bound αIIbβ₃ was detected with 50 nM 125I-AP-3, and bound biotin-β₃-(716–733) peptide was detected with 0.5 μM 125I-streptavidin [8]. In the immunocapture binding assay, microtiter wells were coated with 8d4 (40 μg/ml, 50 μl/well) and blocked with BSA. Talin (200 μg/ml, 50 μl/well) was allowed to bind to 8d4-coated wells by overnight incubation at 4 °C. Purified αIIbβ₃ was then added with or without TA205; and bound receptor was detected as described above.

3. Results

3.1. TA205 inhibits integrin αIIbβ₃ binding to talin head

TA205 disrupts integrin–talin interaction, we examined the effect of TA205 on αIIbβ₃ binding to immobilized full-length talin and the talin head domain. Fig. 1A shows that TA205, but not an isotype-matched mouse IgG, dose-dependently inhibited the binding of αIIbβ₃ to both talin and the talin head fragment. Additionally, 8d4 and TD77, two other anti-talin monoclonal antibodies that react with the talin rod domain [12,14], had no effect on αIIbβ₃ binding to full-length talin (results not shown). At high concentrations, TA205 inhibited αIIbβ₃ binding to full-length talin by ~50%, whereas complete inhibition of αIIbβ₃ binding to the talin head was observed. Because coating of talin onto microtiter wells may change its conformation, we examined the inhibitory effect of TA205 on αIIbβ₃ binding to talin immunocaptured by 8d4. Again, Fig. 1B shows that TA205, but not mouse IgG, inhibited αIIbβ₃ binding to 8d4-captured full-length talin by ~50%. Thus, results of these inhibition studies are consistent with the observations that two integrin binding sites are present per talin molecule, one in the talin head and another in the talin rod. TA205 blocks integrin binding to the high affinity site in the talin head but not to the low affinity site in the talin rod. Furthermore, TA205 is likely to inhibit stress fiber and focal adhesion formation by blocking integrin interaction with the talin head domain.

Next, we determined whether TA205 acts as an allosteric or competitive inhibitor of αIIbβ₃ binding to the talin head. As an initial approach, we performed αIIbβ₃ binding isotherms to immobilized talin head in the presence and absence of 10 nM TA205 which caused sub-maximal inhibition. As shown in Fig. 2A, TA205 had no effect on the apparent Kᵢ value of αIIbβ₃-talin head interaction, which was estimated by half saturation binding to be ~12 nM input αIIbβ₃. Rather, in the presence of TA205, maximal αIIbβ₃ binding to the talin head was significantly decreased. These results indicate a non-competitive mode of inhibition. To substantiate these observations, we examined the inhibitory effect of TA205 over a 5-fold difference of αIIbβ₃ concentration. If TA205 behaves as an allosteric inhibitor, the inhibitor concentration required for half-maximal inhibition would be unchanged [15]. Indeed, Fig. 2B shows that the concentration of TA205 required for half-maximal inhibition (~20 nM) was not significantly different at high or low αIIbβ₃ concentration. Together, these results suggest that TA205 causes an allosteric rather than competitive inhibition on αIIbβ₃ binding to the talin head.

The juxtaposing membrane-proximal helices of integrin αIIb and β₃ tails interact with each other to lock the receptor in an inactive state [16,17]. The talin head binds to the NPLY reverse turn and the membrane-proximal helix (residue 716–733) of the β₃ tail [8,11,16]. It has been suggested that talin...
binding to the β3 membrane-proximal helix initiates α–β tail separation resulting in receptor activation [18]. Since TA205 inhibits αIIbβ3–talin head interaction, we tested its effect on the binding of a biotin-β3-(716–733) peptide to the talin head. As shown in Fig. 3, TA205 also inhibited the binding of the β3 membrane-proximal peptide to talin head, whereas mouse IgG had no effect.

3.2. Epitope-mapping of TA205 in the talin FERM domain

To gain further insight into TA205 inhibition of talin–integrin interaction, we performed experiments to map the TA205 epitope in the talin FERM domain. Initially, we tested the ability of TA205 to immunoblot talin head fragments. Fig. 4A shows that recombinant talin fragments migrated at the expected molecular masses on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. On immunoblot, TA205 reacted with talin F1–F2 (lane 1), F1–F2–F3 (lane 2), and F1 (lane 3), but not with F2 (lane 4) and F3 (lane 5) (Fig. 4B). These results indicate that the TA205 epitope resides within talin F1.

It has been reported that TA205 immunoblots human and chicken talin, but not the mouse isoform [12]. Within talin F1, there are two amino acid changes (i.e., E139D and I141G) from human to mouse sequence [8]. Therefore, we synthesized peptides corresponding to residues 131–150 of human and mouse talin, and tested their ability to block TA205 binding to talin head. As expected, the human talin-(131–150) peptide effectively blocked TA205 binding to talin head whereas the mouse talin-(131–150) peptide had no effect (Fig. 5A). These findings indicate that the TA205 epitope is present within the human talin-(131–150) sequence. We then performed site-directed mutagenesis on the human talin head and examined the effect of E139D or I141G single substitution on TA205 reactivity by immunoblot analysis. Fig. 5B shows that TA205 immunoblotted wild type and the E139D mutant of talin head. In contrast, the I141G mutant failed to bind TA205. Thus, a single I141G substitution in the human talin head abolished its ability to bind TA205.

3.3. Talin head–rod interaction and its effect on TA205 binding to talin

In other FERM family proteins, an intramolecular interaction between their head and tail domains regulate the exposure of their binding sites for cell surface receptors and actin [19,20]. However, direct interaction of the talin head and rod domains has not been demonstrated to date. Thus,
we performed solid phase binding studies to examine whether a soluble talin rod fragment binds to immobilized talin head, and whether such interaction affects TA205 binding. Fig. 6A shows that the talin-(434–2541) fragment corresponding the entire talin rod binds to talin head-coated microtiter wells but not to control wells coated with BSA. Furthermore, we consistently observed that pre-incubation of talin head-coated wells with a saturating concentration of TA205 (0.2 μM) significantly inhibited talin rod binding by ~30% whereas mouse IgG had no effect. Nevertheless, complete inhibition was not observed with TA205 even though an anti-talin head polyclonal antibody completely blocked talin head–rod interaction (results not shown). It should be noted that the concentration of TA205 used in these experiments caused complete inhibition of αIIbβ3 binding to the talin head (see Fig. 1A). We next examined whether the talin rod fragment blocks TA205 binding to the talin head. As shown in Fig. 6B, pre-incubation of talin head-coated wells with the talin rod fragment caused a dose-dependent inhibition of TA205 binding. Interestingly, similar to the inhibitory effect of TA205 on talin head–rod binding, only partial inhibition of ~36% was observed at the highest concentration of the talin rod fragment tested.

4. Discussion

Interaction of talin with integrin cytoplasmic domains plays a central role in integrin inside-out and outside-in signaling. In the present study, we showed TA205, a function-blocking anti-talin monoclonal antibody [12], inhibits integrin αIIbβ3 binding to the talin head domain. In biochemical and mutational studies, we mapped the TA205 epitope to residues 131–150 in the human talin F1 sequence with Ile 141 being a critical residue for antibody binding. While the crystal structure of talin F1 has not been resolved, both F1 domains of radixin and moesin have a typical ubiquitin fold consisting of a long α-helix packed against a 5-stranded mixed β-sheet [19,20]. If talin F1 folds similarly as these ERM proteins, the TA205 epitope would reside near the N-terminal region of the α-helix according to their sequence alignment [5]. Inasmuch as the integrin binding site(s) is present in talin F2 and F3, but not in talin F1 [5], the ability of TA205 to inhibit αIIbβ3 binding to the talin head indicates that its epitope is not directly involved in integrin binding. Indeed, we found that TA205 does not compete directly with αIIbβ3 for binding to the talin head, but instead causes an allosteric inhibitory effect which may be due to conformational changes of the talin FERM domain upon antibody binding.

Integrin–talin interaction has been implicated as a final step of inside-out signaling responses that lead to integrin activation [5,6]. Increasing evidence suggest that integrins are main-
tained in the low affinity inactive state by interaction of the α and β transmembrane helices extending through the membrane-proximal cytoplasmic regions [16,17,21]. Talin binding to the β3 tail, particularly to its membrane-proximal α-helix, is thought to disrupt intersubunit interaction thereby resulting in integrin activation [18]. Since TA205 blocks integrin binding to the talin head, it may affect cellular function by inhibition of talin-induced integrin activation. In support of this postulation, we found that TA205 also inhibits the binding of a peptide corresponding to the β3(716–733) membrane-proximal sequence to the talin head. It is noteworthy that the membrane-proximal region of the β3 tail also interacts with talin F3 [22], but not with talin F1 where the TA205 epitope resides.

If talin is involved in integrin activation, it follows that integrin–talin interaction must be tightly regulated through inside-out signaling. In other ERM proteins, an intramolecular association of their FERM domains with the C-terminal tails masks their functional binding sites for actin and adhesion receptors [19,20]. Disruption of head–tail association in ERM proteins is regulated by the binding of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) or by PKC-mediated phosphorylation [19,20,23]. Talin exists either as a globular monomer or an anti-parallel homodimer [24,25]. In both structures, the talin head and rod domains are likely in close contact with each other. Nevertheless, direct interaction of the talin head and rod domains has not been reported. In the present study, we demonstrated that a talin rod fragment binds to the talin head (see Fig. 6A), and we also observed that such interaction modulates integrin binding to talin (manuscript in preparation). Consistent with this notion, Yan et al. [26] reported that proteolysis of talin with calpain, which separates its head and rod domains, increases the binding affinity of the integrin β3 tail to the talin head.

Finally, we found that TA205 inhibits talin head–rod interaction by approximately 30%, and conversely, the talin rod fragment also causes similar inhibition of TA205 binding to the talin head. These results suggest that TA205 binding to talin F1 also modulates the binding affinity of the talin rod to the talin head. In view that TA205 causes an allosteric inhibition on integrin binding to the talin head, it is tempting to speculate that such allosteric effect also persists for talin head–rod interaction. However, at present, we cannot exclude the possibility that the talin rod fragment binds close to the TA205 epitope in talin F1, thus causing partial blockage of each other’s binding to the talin head.

In sum, TA205 recognizes a species-specific epitope distinct from the high affinity integrin binding site in the talin FERM domain, and yet it causes an allosteric inhibition of integrin–talin binding and partially blocks talin head–rod interaction. Thus, it represents a useful tool for examining the role of talin in cellular function and the signaling mechanism regulating integrin–talin interaction.

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