Ligand Bridging Mediates Integrin α_{IIb}β_{3} (Platelet GPIIb-IIIa) Dependent Homotypic and Heterotypic Cell–Cell Interactions

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Abstract

The aggregation of cells bearing recombinant integrin α_{IIb}β_{3} (platelet GPIIb-IIIa) has been analyzed by two-color flow cytometry. As in normal platelets, aggregation requires functional α_{IIb}β_{3}, “activation” of α_{IIb}β_{3}, and fibrinogen (fg) binding to α_{IIb}β_{3}. Cellular aggregation required that both interacting cells express functional α_{IIb}β_{3}, because a binding defective mutant, α_{IIb}β_{3} (D119 → Y), failed to support interaction with wild type α_{IIb}β_{3}-bearing cells. In addition, cells bearing resting α_{IIb}β_{3} were incorporated into aggregates formed by cells bearing a constitutively active mutant, α_{IIb}β_{3} (β_{3}-2), indicating that only one of the cells in an interacting pair must be activated. Finally, heterotypic interactions occurred between cells bearing activated α_{IIb}β_{3} and cells bearing α_{IIb}β_{3}, a fg-binding integrin present on endothelial and tumor cells. Thus, ligand bridging between fg-binding integrins represents a mechanism of cell–cell interaction, cells bearing resting α_{IIb}β_{3} (e.g., resting platelets) may be incorporated into aggregates formed by cells bearing active α_{IIb}β_{3}, and α_{IIb}β_{3} mediates heterotypic interactions with cells bearing other fg receptors. (J. Clin. Invest. 1991. 88:1128–1134.) Key words: thrombosis • hemostasis • platelet aggregation • fibrinogen • RGD

Introduction

The integrins comprise a family of heterodimeric cell surface receptors composed of α and β subunits which mediate cell–cell and cell-extracellular matrix interactions (1, 2). The integrin α_{IIb}β_{3} (GPIIb-IIIa) is obligatory for physiologic platelet aggregation (3, 4). The binding of fibrinogen (fg) to α_{IIb}β_{3} is necessary for normal aggregation, and fg binding requires cellular activation by agonists such as ADP, epinephrine, or thrombin (3, 4). A closely related but more widely distributed integrin, α_{V}β_{3} (vitronectin receptor), is found on platelets (5) but is also present on a variety of other cell types such as endothelial, smooth muscle, and tumor cells (1, 6, 7). Because α_{V}β_{3} also binds adhesive proteins such as fg (7–9), the presence of this integrin on endothelial and tumor cells may mediate interactions involved in events such as tumor attachment and invasion through the endothelium.

Several integrins mediate cell–cell interactions by binding to integral membrane protein counter-receptors on other cells. In the case of leukocyte integrins, at least three such counter-receptors (ICAM-1 [10], ICAM-2 [11], and VCAM-1 [12]) are members of the immunoglobulin supergene family. Members of the immunoglobulin family are present on the platelet surface as well (13). Moreover, the binding of fg to α_{IIb}β_{3} provokes changes in the conformation of both the α_{IIb}β_{3} (14) and the fg (15). Thus, it is possible that the conformational changes in α_{IIb}β_{3} which follow fg binding enable it to mediate aggregation by binding to a cellular counter-receptor. In the present work, we have evaluated the requirement for an α_{IIb}β_{3} counter-receptor by analysis of fg-mediated aggregation of Chinese hamster ovary (CHO) cells bearing recombinant α_{IIb}β_{3}. The α_{IIb}β_{3} in these cells, once activated, binds fg at 1:1 stoichiometry with an affinity similar to the affinity of platelet α_{IIb}β_{3} (16). Furthermore, the α_{IIb}β_{3}-expressing transfectants undergo fg-mediated aggregation which mimics features of physiologic platelet aggregation (17). In the present study, the molecular requirements for β_{3}-mediated cell–cell interaction have been analyzed using two-color flow cytometry. We found that (a) aggregation requires functional β_{3} receptors on both apposing cells, but activation of α_{IIb}β_{3} is not absolutely required for cellular incorporation into existing aggregates, and (b) α_{IIb}β_{3}-bearing cells undergo fg-mediated binding to cells bearing activated α_{IIb}β_{3}, indicating a potential mechanism of platelet interaction with tumor or endothelial cells.

Methods

Stable cell lines expressing recombinant human β_{3} integrins. Stable CHO cell lines, cotransfected with human β_{3} and α_{IIb} or α, cDNAs were prepared and characterized as described (16, 18). Cell lines bearing either α_{IIb} or α, together with β_{3} (D119 → Y), which fail to bind ligands were also employed (18). A six amino acid substitution mutant, β_{3} (β_{3}-2), was cotransfected with α_{IIb} to establish stable cell lines which spontaneously bind fg as described (19). All transfectants expressed approximately equal quantities of recombinant integrins on the cell surface as judged by flow cytometric immunofluorescence with α_{IIb}, β_{3}, and α_{IIb} monoclonal antibodies as described (16–18).

Preparation of cell suspensions and fluorescent labeling of cells. Monolayer cultures of wild-type and the transfectant cells were removed from culture flasks using 3.5 mM EDTA and 0.01% TPCK-trypsin ( Worthington Biochemicals, St. Louis, MO) in PBS, pH 7.4. This dissociates cells into a single cell suspension without affecting gross α_{IIb}β_{3} structure (17). Cells were washed twice in the presence of 0.05% soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO) resuspended in Tyrode's solution (137.5 mM NaCl, 12 mM NaHCO_{3}, 2.6 mM KCl, and 1 mM MgCl_{2}, pH 7.4) containing 0.1% BSA and 0.1% dextrose. The cell count was adjusted to 10^{6} cells/ml. Stock solutions of sulfo-fluorescein diacetate (SFDA) ( Molecular Probes, Inc., Junction City, OR) and hydroethidine (HE) (Polysciences, Inc., Warrington, PA) were prepared by dissolving the fluorochromes in DMSO at a concentration of 10 mM or 80 mg/ml, respectively. These stock solutions were stored at −20°C for a maximum of 4 wk and thawed just before use. Working solutions were prepared by diluting.

Abbreviations used in this paper: CHO, Chinese hamster ovary; fg, fibrinogen; HE, hydroethidine; SFDA, sulfo-fluorescein diacetate.

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stock with Tyrode's buffer to 200 μM SFDA and 20 μg/ml HE and filtering through a 0.22-μm filter. Homogenous cell fluorescence was achieved by adding equal volumes of dyes to the cell suspensions and incubation for 45 min at 22°C under gentle agitation. Labeled cells were washed thrice in cation-free Tyrode's buffer and resuspended in divalent cation-containing Tyrode's buffer (2 mM CaCl₂, 1 mM MgCl₂), pH 7.4. The assay mixture contained 4 × 10⁶ cells/ml. The viability of labeled cells was always >96% based on Trypan Blue exclusion.

**Aggregation assay.** Aggregation experiments were performed on a gyrorotary shaker (American Rotator Y; American Dade, Miami, FL) in 1% BSA precoated 24-well tissue culture plates (NUUNC, Denmark) at room temperature, as described previously (17). Equal volumes of labeled cells were mixed and 100 μl were added to the wells. When indicated, cells were incubated with monoclonal antibodies (20 nM of 4F10, 1 μM LM609, 8 μM anti-LIBS2) or with GRGDSP or H12 peptides (1 mM) for 20 min. Aggregation was initiated by addition of 300 μg/ml fg (10 μl) and incubation for 15 min at 100 rpm. The cells were fixed by addition of 100 μl of 0.5% paraformaldehyde and samples were held on ice for 30 min before subsequent FACS analysis. The concentrations of SFDA or HE used did not reduce cell viability or aggregation.

**Two-color flow cytometry and fluorescence microscopy.** Data was obtained in a FACStar 440 and analyzed by Consort 30 software (Becton Dickinson Co., Mountain View, CA). The optics were configured to excite fluorescence of both dyes using the 488-nm peak of an Argon laser, filtered through band pass 488±5 nm filter. Emissions were split and filtered to include 515–545 nm as the green SFDA signal (BP 530±15 nm), and 607–643 nm (BP 625±17 nm) as the red HE signal. A dichroic mirror (DM 570) was used for side scatter adjustment. Particles were passed with a flow rate of 300–700 particles/s through a 70-μm orifice, which was found in preliminary experiments not to dissociate aggregates (data not shown). Aggregates were detected as two-color particles in the green (FL1) versus red fluorescence (FL2) contour plots using 5 log scales. The stained cells or aggregates, were also observed through a Zeiss universal epifluorescence microscope and photographed on Ektachrome 400 film. To determine the exact composition of aggregates, incorporated red (HE) and green (SFDA) cells were counted visually.

**Monoclonal antibodies and reagents.** Monoclonal antibody mAb62 recognizes an epitope on β₃ and stimulates fg binding and aggregation as either intact antibody or Fab fragments (16, 17). 4F10 (20) and LM609 (7) are inhibitory anti-α₅β₃ and anti-α₃β₃ antibodies, respectively, which were generously provided by Dr. Virgil Woods (University of California San Diego) and Dr. David Cheresh (Research Institute of Scripps Clinic, La Jolla, CA). The peptides GRGDSP and HHLGGAQADGV (H12) were prepared as described (21, 22). Fibronectin-depleted fg was purified according to described methods (21). All other reagents or chemicals used were of the highest grade available.

**Results**

**Validation of two color FACS coaggregation assay.** CHO cells expressing human α₅β₃ undergo activation and fg-dependent aggregation as assessed microscopically or by particle counting (17). To analyze heterotypic aggregation, we labeled cells with either sulfofluorescein (green fluorescence) or hydroethidium (red fluorescence), and then coaggregation was examined by use of two-color flow cytometry. With this method, aggregation

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**Figure 1.** FACS analysis of cellular coaggregation. Contour plots illustrate the two-color FACS analysis of aggregation of SFDA-green and HE-red-labeled α₅β₃-bearing cells. Cells (10⁷ cells/ml) were mixed and incubated in the presence of an activating monoclonal antibody, mAb62 (8 μM). After 20 min, fg was added (1 μM) and the mixture was rotated at 100 rpm at room temperature for 30 min. Aggregates were identified as two-color particles (top left). The effect of omission of MAB 62 (top right) or addition of an anti-α₃β₃ monoclonal antibody, mAb4F10 (20 nM), which inhibits fg binding is shown (bottom left). Aggregation experiments were also performed using a transfectant expressing a point mutation of β₃, α₅β₃(D119→Y) which lacks fg binding function (bottom right). Each panel represents the analysis of 10,000 particles. The contour lines are 3, 9, 27, and 81. The percentage of total events lying in the aggregate (two-color particle) area are 22.4% (top left), 2.8% (top right), 1.3% (bottom left), and 1.9% (bottom right).

**Figure 2.** Coaggregation requires functional fg receptors on both cell types. Equal amounts of SFDA-labeled α₅β₃-bearing cells and HE-labeled CHO (top left), α₅β₃(D119→Y) (top right) or α₅β₃ (bottom)—expressing cells were mixed and incubated with activating antibody mAb62 for 20 min. fg (1 μM) was added and aggregation was performed in gyration at 100 rpm. The percentage of particles lying within the aggregate (two-color particle) region were: 1.1% (left), 0.9% (right), and 21.6% (bottom).
of activated $\alpha_{\text{m}}\beta_{3}$ bearing cells was readily observed (Fig. 1), as indicated by the detection of particles bearing both fluorochromes. This aggregation required the activating mAb 62 and was blocked by inhibitors of $\alpha_{\text{m}}\beta_{3}$ binding as mAb 4F10 (Fig. 1). Aggregation did not occur with untransfected cells (not shown), or cells transfected with nonfunctional $\alpha_{\text{m}}\beta_{3}$, or $\alpha_{\text{m}}\beta_{3}$ (D119 $\rightarrow$ Y) (Fig. 1). The two-color FACS analysis thus corresponded well with previous results obtained by direct microscopic evaluation.

Coaggregation requires functional $\alpha_{\text{m}}\beta_{3}$ on both cells. The interaction of cells with other cells bearing occupied $\alpha_{\text{m}}\beta_{3}$ receptors required the presence of $\alpha_{\text{m}}\beta_{3}$, because wild-type CHO cells did not form coaggregates with $\alpha_{\text{m}}\beta_{3}$-bearing cells in the presence of activating antibody and $\alpha_{\text{m}}\beta_{3}$ (Fig. 2). Moreover, functional $\alpha_{\text{m}}\beta_{3}$ was necessary for cells to coaggregate, because the $\alpha_{\text{m}}\beta_{3}$ (D119 $\rightarrow$ Y) mutant, which lacks $\alpha_{\text{m}}\beta_{3}$ binding function, also failed to coaggregate with $\alpha_{\text{m}}\beta_{3}$-expressing cells (Fig. 2). The results of these two-color FACS analyses were confirmed by direct observation of mixed cell aggregates by fluorescence microscopy (Fig. 3). Thus, when two populations of $\alpha_{\text{m}}\beta_{3}$-bearing cells were each labeled with a different fluorochrome, mixed aggregates between these two were readily observed (Fig. 3 A). In contrast, when $\alpha_{\text{m}}\beta_{3}$-bearing cells, labeled with hydroethidine (red), were coaggregated with sulfofluorescein (green) labeled $\alpha_{\text{m}}\beta_{3}$ (D119 $\rightarrow$ Y)-bearing or wild-type CHO cells, red aggregates were observed surrounded by green single cells (Fig. 3 B). Indeed, direct counting of cells incorporated in aggregates revealed that these aggregates were comprised almost exclusively of red cells (not shown, but cf. Fig. 6). In reverse experiments, when the $\alpha_{\text{m}}\beta_{3}$-expressing cells were labeled with sulfofluorescein, green aggregates excluding red single cells were observed when the mutant $\alpha_{\text{m}}\beta_{3}$ (D119 $\rightarrow$ Y) or CHO cells were labeled with hydroethidine (data not shown). Thus, in order for $\alpha_{\text{m}}\beta_{3}$-mediated cell–cell interaction to proceed, functional receptors must be present on both cell types.

Cells bearing “resting” $\alpha_{\text{m}}\beta_{3}$ coaggregate with cells bearing “activated” $\alpha_{\text{IIb}}\beta_{3}$. Because $\alpha_{\text{m}}\beta_{3}$ must be activated to bind $\alpha_{\text{IIb}}\beta_{3}$ (3, 4) and to initiate cell–cell interaction, we asked whether both partners in a cellular aggregate must bear activated receptors. To do this, we made use of a mutant, $\alpha_{\text{m}}\beta_{3}$ (Y$\rightarrow$F), which results in $\alpha_{\text{m}}\beta_{3}$ which constitutively binds fg (19). When these cells were labeled with both green and red dyes, they formed coaggregates in the absence or presence (Fig. 4) of the activating antibody. This aggregation was completely inhabitable by a complex specific anti-$\alpha_{\text{m}}\beta_{3}$ monoclonal antibody. When resting $\alpha_{\text{m}}\beta_{3}$-bearing cells were mixed with those bearing this spontaneously active mutant in the presence of fg, coaggregates were observed (Fig. 5) which were increased further by the presence of the activating monoclonal antibody mAb62. These coaggregates were completely inhabitable with the $\alpha_{\text{m}}\beta_{3}$ specific monoclonal antibody mAb4F10 (Fig. 5) or GRGDSP peptide (not shown). The appearance of two-color particles in the FACS analysis suggested that the resting $\alpha_{\text{m}}\beta_{3}$-bearing cells could be incorporated into aggregates formed by cells expressing constitutively active $\alpha_{\text{m}}\beta_{3}$ (Y$\rightarrow$F), although they appear to do less efficiently than cells bearing activated $\alpha_{\text{m}}\beta_{3}$. To assess this more directly, microscopic analysis was undertaken (Fig. 6). When both cells in a red/green pair were activated, aggregates were formed of equal numbers of red and green cells (Figs. 3 A and 6). In contrast, when only one of the cells in a pair were activated > 80% of the cells in each aggregate were of the activated phenotype (Fig. 6) and a lesser number of cells

![Figure 3. Evaluation of coaggregation using fluorescence microscopy. A representative two-color aggregate is shown composed of $\alpha_{\text{m}}\beta_{3}$-bearing cells, labeled separately with SFDA (green) and HE (red) (A). In B, $\alpha_{\text{m}}\beta_{3}$-expressing cells (red) were mixed with transfectants bearing the nonfunctional $\alpha_{\text{m}}\beta_{3}$ (D119 $\rightarrow$ Y) (green). Aggregation experiments were performed in presence of 8 mM mAb62 and 1 mM fg. Note that aggregation occurred between $\alpha_{\text{m}}\beta_{3}$-bearing cells (red), whereas $\alpha_{\text{m}}\beta_{3}$ (D119 $\rightarrow$ Y)-expressing cells (green) were not incorporated into aggregates.](image-url)
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ture with the spontaneously active mutants (Fig. 5). Finally, when an activating antibody was added to the mixture of "resting" (αmβ2) and "activated" (αmβ2β3-2) cells, equal quantities of both cells were incorporated into the aggregates (Fig. 6). Thus, activation of αmβ2 is not an absolute requirement for cellular incorporation into aggregates as long as other cells in the mixture contain activated receptors.

Activated αmβ2 mediates coaggregation with cells bearing αβ3. Cells bearing other fg binding integrins (e.g., the αβ4 vitronectin receptor [23] or CD11b/CD18 [24]) may readily come into contact with activated platelets. Thus, we asked whether cells bearing αβ3 receptor could coaggregate with cells bearing activated αmβ2 in the presence of fg. To do this, we examined CHO cells expressing recombinant αβ3 for their capacity to coaggregate with cells bearing recombinant αmβ2. When αmβ2-bearing cells were mixed with αβ3-expressing cells in the presence of an activating antibody and fg, both cell types were incorporated into aggregates (Fig. 7). Coaggregation was specific, because it was inhibitable by a complex-specific anti-αβ3 monoclonal antibody, LM609. (Fig. 7). Moreover, the mutant form αβ3 (D119 → Y) did not coaggregate with activated αmβ2-bearing cells (data not shown). Microscopic analysis of the composition of mixed aggregates formed with αmβ2-bearing cells revealed that 14.0±5.7% of cells in aggregates bore αβ3. In contrast, cells carrying αβ3 (D119 → Y) failed to coaggregate with αmβ2-bearing cells (3.9±4.6% of cells in aggregates bore αβ3 [D119 → Y]).

It is likely that activated platelets might come into contact with endothelial cells or tumor cells which might not be in an activated state. Therefore, we asked whether the "resting" αβ3-bearing cells could coaggregate with cells bearing active αmβ2 in the absence of activating agents. When cells expressing αmβ3 (β3-2), were mixed with the αβ3-bearing cells, coaggregation was observed which was inhibitable with LM609 (Fig. 8). This indicates that αβ3-bearing cells do not have to be activated to interact with cells bearing activated αmβ2.

Discussion
The major findings of this work are: (a) fg-dependent cellular aggregation may occur when functional β3 receptors are present on both apposing cells. Thus, αmβ2 appears to require no counter-receptor and fg bridging between two functional β3 receptors is a likely mechanism of aggregation. (b) Although activation of αmβ2 is required for fg binding and platelet aggregation, cells bearing resting αmβ2 may be incorporated into aggregates as long as other cells in the mixture contain activated fg receptors. (c) Cells bearing other fg receptors, e.g.,

\[ αmβ2 \text{-dependent Cell-Cell Interactions} \]
bridging.

Figure 6. Coaggregation of cells bearing fg receptors in a resting and activated state. The SFDA-labeled, constitutively active mutant, αmβ3(β1-2), was mixed with HE-labeled CHO cells or transfectants bearing αmβ1 or αmβ3(β1-2). The cells were incubated with gyration in the absence or presence of the activating antibody mAb62. The percentage of cells incorporated in aggregates was determined by counting the numbers of “red” (HE) and “green” (SFDA) cells in aggregates. The HE-labeled CHO cells bore (solid) αmβ3, (hatched) αmβ3(β1-2), or (stippled) no transfected β3 receptor. Results are expressed as mean percentage of HE-labeled cells in 20 aggregates±SD.

αβ3 may interact with cells bearing activated αmβ3 via fg bridging. Thus, activated αmβ3 may mediate heterotypic interactions between platelets and cells bearing other fg receptors, e.g., endothelial cells, leukocytes, tumor cells. These findings, which are summarized in Fig. 9, indicate that ligand bridging is a mechanism of integrin-mediated cell–cell interaction, provide insight into the mechanism of platelet aggregation, and suggest molecular mechanisms for platelet interactions with tumor cells and other fg receptor-bearing cells.

Physiologic platelet aggregation requires the presence of platelet αmβ3, cellular activation to expose fg binding sites, and fg binding (3, 4). In the present study, we have exploited unique properties of recombinant αmβ3 in CHO cells to specifically analyze the αmβ3-dependent element of platelet aggregation. (a) Because agonist-mediated activation has not been observed in the CHO cell system (16) the potential contribution of numerous “positive feedback” loops in platelet aggregation (25) can be discounted. (b) The requirement for maintenance of an active fg receptor through signal transduction pathways was

Figure 7. Coaggregation of αmβ3 and αβ3 transfectants. SFDA-labeled αmβ3-bearing cells were mixed with HE-labeled vitronectin-receptor (αβ3)-expressing transfectants in the presence or absence of the activating antibody, mAb62. Aggregation was initiated by addition of fg. The effect of anti-αmβ3 (mAb4F10) and anti-αβ3 (mAbLM609) which inhibit ligand binding is shown in lower panels. The percentage of particles in the aggregate region are 9.2% (top left), 2.4% (top right), 1.8% (bottom left), 3.8% (bottom right).

Figure 8. Coaggregation of αmβ3(β1-2) and αβ3 transfectants. SFDA-labeled constitutively active mutant αmβ3(β1-2) were mixed with HE-labeled αβ3-bearing cells. Aggregation was initiated in the absence of activating antibody mAb62 by addition of fg. The effect of antibodies which inhibit fg binding to αmβ3 (top right) or to αβ3 (bottom left) are shown. (Bottom right) The effect of omission of fg. Percentage of particles in aggregate quadrants are 8.2% (top left), 2% (top right), 2.2% (bottom left), and 1.8% (bottom right).

Figure 9. Fg receptors and cell–cell interaction. Illustrated is a summary of the requirements for fg-mediated coaggregation of cells. As shown in the left panel, two cells bearing functional fg receptors coaggregate when at least one of the cells’ receptors are “activated.” As illustrated in the right panels, coaggregation does not occur when one of the cells lacks functional fg receptors.
obviated by use of monoclonal antibodies which conformationally alter the αMβ2 to produce a stable activated fg receptor (16). (c) Because the parent CHO cells lacks the capacity to undergo fg-mediated aggregation, the transfected receptor is the single unique platelet component required for this response. Moreover, because the αMβ2 was recombinant, its ligand-binding state and state of activation could be controlled by introduction of selected mutations. With these tools it was possible, by use of two-color flow cytometry, to directly investigate the possibility of cellular counter-receptors for αMβ2. The aggregation response required that αMβ2 be present on both cell types suggesting that no cellular counter-receptor sufficient to mediate the aggregation response was present. fg undergoes conformational changes (15) after binding to αMβ2 and monoclonal antibodies reactive with neoeptopes on receptor-bound fg inhibit platelet aggregation (26). The epitope for one of these antibodies is distinct from known αMβ2 recognition sequences in fg (26), suggesting the possibility that conformationally altered fg might interact with αMβ2 at a site distinct from its known (18, 27-29) ligand binding pocket. This seems unlikely, because cells transfected with αMβ2 (D119 → Y), which lacks interaction with known αMβ2 binding sequences in fg (18), failed to undergo coaggregation. Thus, because monovalent peptides (30) or fragment D (31) bind to αMβ2 but fail to support aggregation, the simplest explanation of these results is that a single fg molecule interacts with the ligand binding pockets of αMβ2 molecules on adjacent cells thus bridging the two cells. It is clear that platelets must be activated to initiate the aggregation response (3, 4). In the present work we have employed a spontaneously active mutant of αMβ2 (19) and have found that cells bearing "resting" αMβ2 may be incorporated into aggregates with cells bearing activated αMβ2. Considering the lability of platelet agonists such as ADP and thrombin within the vasculature, these results suggest that activated platelets may nucleate an aggregate, which may contain both activated and resting cells. Because one of the cells in the interacting pair must be activated, it also suggests that platelet aggregates may be "capped" by a layer of resting platelets. It is superficially surprising that "resting" αMβ2 should mediate coaggregation with cells bearing activated αMβ2, because there is an activation requirement for soluble fg binding. Nevertheless, Coller (32) found that "resting" platelets adhered to insolubilized fg, and recombinant αMβ2, which could not be "physiologically" activated in CHO cells, also supported adherence to insolubilized fg (16). One potential explanation of this would be that fg binds to resting αMβ2 with a low affinity, and receptor bound or surface insolubilized fg, being multivalent with respect to platelets, binds with a higher effective affinity. An alternative possibility is that receptor bound fg undergoes a conformational change (26) resulting in accessibility of the RGDS in the α chain of fg to αMβ2. Because peptides containing the RGDS sequence also induce a conformational change in αMβ2 associated with high-affinity fg binding (33), the receptor-bound fg may carry its own internal activator for platelet αMβ2. Evidence in support of this model is provided by the finding that adhesion of resting platelets to fg, in contrast to fg binding to activated platelets, is dependent on the region of the fg α chain containing an RGDS sequence (34).

Since the pioneering studies of Gasic (35-37), it has become clear that platelet-tumor cell interaction may modulate the metastatic behavior of some tumors. In the case of certain tumors, platelet αMβ2 interaction with adhesive ligands plays a role (38, 39). It is also clear that certain tumors have fg receptors such as αLβ1 (40) and these receptors may be involved in interaction with platelets. The present studies, by use of recombinant receptors, have reconstructed a potential molecular mechanism of platelet-tumor cell interaction, i.e., a heterophilic bridge interaction involving αMβ2 on the platelet and a resting or activated fg receptor on the tumor cell. αMβ2 is also expressed on endothelial cells, thus it seems probable that the ability of αMβ2 to support fg-mediated aggregation with cells bearing activated αMβ2 provides a mechanism for platelet-endothelial cell interaction as well.

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