Two Synergistic Activation Mechanisms of α2β1 Integrin-mediated Collagen Binding

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Activation of protein kinase C by 12-O-tetradecanoylphorbol-13-acetate (TPA) induces ligand-independent aggregation of a cell surface collagen receptor, α2β1 integrin. Concomitantly, TPA increases the avidity of α2β1 for collagen and the number of conformationally activated α2β1 integrins. The structural change was shown using a monoclonal antibody 12F1 that recognizes the “open” (active) conformation of the inserted structural change was shown using a monoclonal antibody 12F1 that recognizes the “open” (active) conformation of the inserted domain in the α2 subunit (α2I). Amino acid residue Glu-336 in α2 subunit is proposed to mediate the interaction between α2I domain and β1 subunit. Glu-336 seems to regulate a switch between open and “closed” conformations, since the mutation α2E336A inhibited the TPA-related increase in the number of 12F1 positive integrins. E336A also reduced cell adhesion to collagen. However, E336A did not prevent the TPA-related increase in adhesion to collagen or α2B1 aggregation. Thus, α2B1 integrin avidity is regulated by two synergistic mechanisms, first an α2E336-dependent switch to the open α2I conformation, and second an α2E336-independent mechanism temporally associated with receptor aggregation.

Integrin-type cell adhesion receptors undergo functional regulation via different conformational states (1). In an inactive integrin α/β heterodimer, the ligand binding headpiece is bent toward the cell surface (2), while an active integrin stands tall but may still have multiple activation states. Nine α subunits out of 24 human integrin heterodimers have an inserted αI (or αA) ligand binding domain, which can exist in multiple configurations of differing affinity. The conformation of αI is regulated by a spring-like mechanism formed by a conserved glutamate residue in the α7 helix acting as an intrinsic ligand of the I-like domain of the β subunit (βI; Refs. 3 and 4).

Avidity, the overall strength of integrin mediated interactions between cells and extracellular ligands, is multistate involving the affinity of individual receptors and variations in the spatial arrangement of the integrins. The total number of receptors participating in the interaction, or valency, is expected to be larger if integrins form clusters. The relationship between conformational regulation and cluster formation has been thoroughly studied in αLβ2 integrin, a leukocyte receptor for intercellular adhesion molecules (5–7). Recent studies suggest that αLβ2 clustering takes place only after binding a multivalent ligand and that clustering strengthens adhesion after ligand binding (8). The requirement of a ligand for receptor clustering has also been reported for αVβ3 (9) and αIIbβ3 integrins (10).

Four collagen receptors, α1β1, α2β1, α10β1, and α11β1, form a subgroup of αI domain containing integrins. Integrin α2β1 is expressed on platelets, epithelial cells, and many mesenchymal cell types (11). The α2I domain, like other integrin I domains, can exist in both an “open” and a “closed” conformation (12), as well as a less frequent, intermediate form (13). Thus, α2β1 can also be found in more than one conformation (14), likely regulated similarly to other αI domain integrins. The unique functions of collagen receptor integrins may also be governed by the regulation of the receptor distribution and local density. For example, α2β1 and α11β1 participate in the formation and rearrangement of collagen fibrils (15, 16), and echovirus-1-dependent clustering leads to rapid relocation and internalization of α2β1. In this process, α2β1 behaves differently from other integrin family members, such as αV integrins (17).

The phorbol ester TPA can increase the avidity of α2β1 binding to collagen (18). We report here that while TPA induces rapid (5–15 min) clustering of cell surface α2β1 in the absence of ligand and increases α2β1 avidity for collagen, it also activates the integrin via a conformational mechanism involving α2E336 and intermolecular cross-talk between the α2I and β1I domains. Aggregation of α2β1 is independent of E336 and temporally associated with increased avidity of adhesion. We conclude that the avidity of α2β1 for collagen is regulated by two synergistic mechanisms.

EXPERIMENTAL PROCEDURES

Cell Culture—Chinese hamster ovary (CHO) and Saos (ATCC) cells were stably transfected with human integrin α2 (19, 20), α10 subunit (21) in pAWneo2 vector, or vector only as

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described. The CHO-α2/α1 cell line has been described by Ivaska et al. (22). Integrin α2 mutants Y410A, E309A, and E336A were constructed by a modified QuikChange method (Stratagene). Stable CHO-α2E309A, CHO-α2Y410A, and CHO-α2E336A cell lines were created by using FuGENE6 transfection reagent (Roche Applied Science). α2 positive cells were first selected with G418 (0.5 mg/ml; Invitrogen). Additionally, positive cells were stained with integrin α2 mAb 12F1 (BD Biosciences) and anti-mouse FITC-IgG (Dako) and isolated by flow cytometry (FACSCalibur, BD Biosciences). Cell lines GD25-α2B1 and GD25-α2B1mut (called also GD25-α2B1A and GD25-α2B1Amut; kind gifts from Professor Kristofer Rubin from Uppsala University, Sweden and Professor Deane F. Mosher, University of Wisconsin) stably expressing wild type human integrin α2 subunit and either wild type mouse β1 or β1 carrying Y783/795F mutations in its cytoplasmic tail have been described earlier (23–25).

Cell Spreading Assay—Costar 96-well plates were coated with collagen I (col I, 5 µg/cm²; Vitrogen) or 0.1% BSA. Cells were allowed to spread in serum-free medium with 50 µM cycloheximide (Sigma) for 2 h at 37 °C. Fixed cells were analyzed by phase-contrast microscopy as described earlier (26).

Receptor Staining—100 nM TPA (Calbiochem) was added to subconfluent cells in serum free Dulbecco’s modified Eagle’s medium. Released cells were fixed (4% formaldehyde) and suspended in phosphate-buffered saline with 3% BSA and 0.6% rat IgG at time points. Cells were stained with mAb 12F1 or left untreated. Fixed cells (4% paraformaldehyde) were permeabilized (0.2% Triton X-100) and stained with mAb 12F1, kind gifts from Dr. Fedor Berditchevski, University of Birmingham, Birmingham, UK) and anti-mouse Alexa-488-IgG (Molecular Probes) and treated with 1 nM BIS or 1 µM TPA. Fixed cells were analyzed by laser scanning confocal microscopy (LSM510, Carl Zeiss), and all images and volume and side view renderings were produced with BioImageXD software.

Structural Modeling—Structural models of human α2β1 integrin headpiece domains were based on the crystal structures of the αβ3 integrin (Ref. 2; Protein Data Bank ID: 1JW2) and the α2 integrin-inserted domain (Ref. 12; Protein Data Bank ID: 1DZI). Structural coordinates were obtained from the RCSB Protein Data Bank (28) and sequence data from the UniProt online data base (29). Sequences were aligned in Bodil v0.8 (30) and structures modeled using Modeler v7.7 (31). Molecular graphics were created using PyMOL v0.98 (32).

On-line Supplemental Material—Four-dimensional microscopy shows TPA-induced clustering and internalization of α2β1 integrin in Saos-α2 cells. The movie (see supplemental material) was created using BioImageXD.

RESULTS

TPA induces a Ligand-independent but PKCα-dependent Aggregation and Internalization of α2β1 Integrins—Virus and antibody-mediated α2β1 clustering are known to activate PKCα (17). Here, the effect of the phorbol ester TPA, a PKC activator, on integrin clustering and internalization was investigated using Saos osteosarcoma cells transfected to express α2β1 integrin (22). Cells were stimulated with TPA, and the experiments were done in the absence of collagen ligands. In four dimensional confocal microscopy studies, both fast macroclustering and internalization of α2β1 integrin induced by TPA were observed (Fig. 1A and supplemental movie). The effect of TPA was comparable with, but not as extensive as, that obtained by secondary antibody-induced clustering, which was used as a positive control (Fig. 1A). As shown in Fig. 1B, the average time for the appearance of clear clusters after TPA addition was about 10 min, and the average time for their internalization was about 14 min. For secondary antibody-induced clustering, these times were about 3.5 and 7 min, respectively. No clustering or internalization was observed in negative control cells that were not stimulated in any way. Furthermore, BIS, a chemical inhibitor of PKCs, prevented the formation of TPA-induced integrin aggregates and their internalization (Fig. 1C). Thus, we propose bidirectional talk between PKCα activation and integrin aggregation: receptor clustering activates PKCα (17) and PKCα further promotes the aggregation.

TPA Increases the Avidity of α2β1-mediated Adhesion to Collagen in a PKC-dependent Manner—We have previously reported a fast and sensitive cell adhesion assay based on the use of collagen-coated polystyrene beads and flow cytometry (27). This sophisticated method allows accurate studies of the effect of TPA on the integrin avidity for collagen. CHO cells transfected to express either α2β1, α10β1, or mutant α2β1 integrin (α2/α1 chimera) as their only collagen receptor (CHO-α2WT, CHO-α2/α1, CHO-α10WT; Refs. 20–22 and 33) were used. The binding of CHO-α2WT cells to collagen I coated beads was detected by flow cytometry at successive time points (Fig. 2A). Cell adhesion to collagen I appeared to increase nearly linearly for up to 50 min before equilibrating. However, when CHO-α2WT cells were treated with TPA, a significantly faster adhesion, which reached its equilibrium in 40 min, was obvious. At every time point, TPA caused a 50% increase in cell adhesion.
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cells (Fig. 2D). Furthermore at the 10-min time point, TPA treatment increased CHO-a10WT cell adhesion to collagen I more than seen in CHO-a2WT or CHO-a2/a1 cells indicating an earlier overall response. Altogether, the results show that the intracellular domains of integrin α subunits may not directly participate in regulation by TPA and that the effect is not limited to the α2β1 heterodimer.

To verify that the observed increases in adhesion were mediated by α2β1 integrin, a series of control experiments were performed using CHO-a2WT, CHO-a2/a1, and CHO-pAW cells. All cell types showed an invariably low level of interaction with negative control beads, which were either left uncoated or coated with BSA (Fig. 2E). Additionally, vector-transfected control cells, CHO-pAW cells, did not interact with collagen I-coated beads (Fig. 2E). However, CHO-a2WT and CHO-a2/a1 cells adhered strongly to collagen I-coated beads, revealing a specific interaction. This adhesion, both basal and TPA-mediated, was significantly decreased by pretreating cells with the α2 integrin-specific function blocking mAb P1H5, thereby verifying that adhesion occurred in all conditions via the α2β1 integrin (Fig. 2E).

To confirm that the TPA-mediated increase in α2β1 integrin avidity to collagen I uses a PKC-related signaling pathway, the PKC inhibitor, BIS, was tested in the presence and absence of TPA. When CHO-a2WT and CHO-a2/a1 cells were pretreated with BIS, the TPA-mediated cell adhesion to collagen decreased about 35%. However, BIS did not significantly alter the basal adhesion of CHO-a2WT cells to collagen I. In comparison, the PKC inhibitor slightly reduced basal adhesion in CHO-a2/a1 cells (Fig. 2F).

**Integrin β1 Subunit Is Essential for TPA-related Increase in α2-mediated Cell Adhesion to Collagen I**—Talin is a cytoskeletal protein and a well known substrate for PKC (34). Talin binding to the cytosolic part of the integrin β subunit is typically required for the initiation of integrin activation (35). To analyze the importance of the β1 subunit in TPA-mediated integrin avidity regulation, we used GD25 cells expressing similar levels of either wild type α2β1 or α2β1 with Y783/795F mutations in the cytoplasmic tail of the β1 subunit (25). Substituting these tyrosine residues in conserved NPXY motifs of the β tail has previously shown to be sufficient to inhibit talin binding (35,

**FIGURE 1. TPA induces ligand-independent clustering of α2β1 integrins.** A, Saos-α2 cells were treated with TPA, cluster-inducing antibodies (positive control), or left unstimulated (negative control) and imaged with four-dimensional live cell confocal microscopy. Three-dimensional volume renderings made with BiomegaX3D show that TPA induced internalization of α2β1 integrin clusters. The white rectangle indicates a cell section rendered from the side and shown at top. See also the on-line supplemental movie. B, average time for the appearance and internalization of at least three clear integrin clusters was determined by visual inspection of optical slices and volume renderings. Clustering was not seen in untreated cells. C, Saos-α2 cells were treated with TPA or BIS ± TPA for 40 min, fixed, and imaged by confocal microscopy. Internalized α2β1 integrin aggregates were determined from the images.

Compared with untreated CHO-a2WT cells (Fig. 2A). Increased adhesion was already clear at the 10 min time point, indicating the rapidity of TPA-induced activation (Fig. 2B).

Previously, it has been shown that the cytoplasmic tail of the α2 subunit is required for outside-in signaling mediated by α2β1 integrin (22, 33). In these studies, a chimeric α2 integrin subunit bearing an α1 cytoplasmic tail (α2/α1) appeared to be unable to mediate heterodimer-specific signals. To investigate the role of the α2 cytoplasmic tail in TPA-activated adhesion, CHO-a2/a1 cells were tested in time series. Temporal profiles of both untreated and TPA-treated CHO-a2/a1 cell adhesion to collagen I resembled the adhesion of CHO-a2WT cells. However, the TPA mediated increase in cell adhesion was substantially higher in CHO-a2/a1 cells than in CHO-a2WT cells (Fig. 2C). Thus, the swap mutation did not affect the ability of the receptor to become activated. CHO cells bearing α10β1 integrin were analyzed similarly (CHO-a10WT; Fig. 2D).

In this case, the adhesion in both TPA treated and untreated cells reached saturation at earlier time points than in CHO-a2WT
Here, in agreement with previous studies (25), both wild type \( \alpha_2 \beta_1 \) and \( \alpha_2\beta_1 \text{mut} \) integrin heterodimers could mediate cell attachment to collagen I-coated beads when analyzed by flow cytometry (Fig. 3). Additionally, TPA dramatically increased the collagen binding in GD25-\( \alpha_2\beta_1 \) cells as expected. In sharp contrast, TPA did not affect the activation of GD25-\( \alpha_2\beta_1 \text{mut} \) cells (Fig. 3). The results suggest that integrin \( \beta_1 \) subunit is required for appropriate activation of \( \alpha_2\beta_1 \) heterodimer by TPA.

The expression of integrin \( \alpha_2 \) on the cell surface decreased in the presence of TPA between the first two measured time points (0 and 10 min) in all three independent experiments as shown in Fig. 4. This was not surprising given that some internalization of \( \alpha_2\beta_1 \) integrin was seen by confocal microscopy in TPA treated Saos cells (Fig. 1). Notwithstanding the initial decrease, the amount of \( \alpha_2 \) integrin on the cell surface remained stable after 10 min in all three experiments. In contrast, a significant increase in the number of 12F1 epitopes corresponding to the active conformation of \( \alpha_2 \) integrin was apparent in each experiment beginning at between 20 and 40 min after stimulation with TPA. The increase was transient in each case, lasting from 10 to 20 min before declining to near baseline levels (Fig. 4). Interestingly, the transient conformational activation of the \( \alpha_2 \) integrin seemed to occur later than the increase in avidity (within 10 min).

CHO-\( \alpha_2E336A \) Cells Retain Adhesion to Collagen, but the Mutation Inhibits TPA-related Conformational Change—In leukocyte \( \beta_2 \) integrins, e.g. in \( \alpha M\beta 2 \) and \( \alpha L\beta 2 \) integrins, ligand binding is shown first to cause a change in the conformation of the \( \alpha 7 \) domain, including a downward movement of the \( \alpha 7 \) helix. This is followed by a similar change in the \( \beta 1 \) domain, leading to separation of the leg domains of the \( \alpha \) and \( \beta \) subunits...
A conserved glutamate residue on the α7 helix of the αI domain was suggested to act as an intrinsic ligand for the βI1 domain. The same structural link between the αI and βI domains seems to be important in integrin inside-out signaling and regulation of affinity. The corresponding glutamate in the α2I domain, the conserved Glu-336, may interact in a similar manner with the βI1 domain and participate in the switch between open and closed α2I domain conformations (Fig. 5A). We wanted, however, also to test the hypothesis that the conformational regulation of α2I takes place in a different manner than the regulation of αLβ2 and αMβ2. Thus, we mutated amino acid residues Glu-336, Glu-309, and Tyr-410. Glu-309 is located close to the C terminus of the α7 or terminal helix of the α2I domain and potentially could interact with the βI1 domain metal binding sites (Fig. 5A). This residue was mutated to alanine to remove the negative charge of the side group. The mutation Y410A was constructed to probe the importance of the interaction between the α2 β-propeller and the βI1 domain. A large surface area is buried between these two subunits, and there appears to be a specific interaction at the center of the α2 β-propeller (Fig. 5A). From our molecular models of this specific interaction, based on the αVβ3 crystal structures (2), Tyr-410 could be one of the three aromatic residues involved in a cation-π interaction with β1K269. Disruption of this interaction might lead to defects in signaling and/or conformational activation.

CHO cells transfected to express integrin α2 subunits carrying either E309A, Y410A, or E336A mutations (CHO-α2E309A, CHO-α2Y410A, and CHO-α2E336A) were tested by flow cytometry for their ability to adhere to collagen I-coated polystyrene beads. It appeared that the base level adhesion of CHO cells transfected to express integrin α2 subunits carrying either E309A, Y410A, or E336A mutations (CHO-α2E309A, CHO-α2Y410A, and CHO-α2E336A) were tested by flow cytometry for their ability to adhere to collagen I-coated polystyrene beads. It appeared that the base level adhesion of

**FIGURE 3.** β1 subunit is required for TPA-related increase in α2β1 adhesion to collagen I. GD25-α2β1 cells and GD25-α2β1mut cells, with β1 subunit carrying Y783/795F mutations in its cytoplasmic tail, were incubated for 50 min with either col I- or BSA-coated beads ± 100 nM TPA. Cell binding to coated beads was assayed by flow cytometry (mean ± S.D., n = 3).

**FIGURE 4.** TPA increases conformationally activated integrins on cell surface. In three independent experiments, TPA-induced (100 nM) conformational activation over time of CHO-α2WT cells was determined using mAbs recognizing either total (○; 16B4) or active, open (●; 12F1) α2 integrin.
CHO-α2E336A to collagen I was clearly affected compared with CHO-α2WT cells (Fig. 5B). Mutation Y410A also decreased α2 integrin binding to collagen I, whereas mutation E309A had no effect (Fig. 5B). Reduced CHO-α2E336A cell binding to collagen I is in agreement with previous studies indicating that αL and αM integrins lose their ability to bind their ligands after the mutation of the residue corresponding to E336 in α2 (E310A in αL and E320A in αM; Refs. 3 and 39). Notably, CHO-α2E336A cells retained a low level of avidity to collagen and were able to spread on collagen I-coated surfaces in 2 h (Fig. 5C). However, CHO-α2WT cells, as well as CHO-α2Y410A and CHO-α2E309A cells, spread significantly more quickly on collagen (Fig. 5C).

The TPA-induced conformational activation of the α2 integrin with the E336A mutation was studied in a flow cytometry based assay by using CHO-α2E336A cells and antibodies recognizing either total or open α2 integrins on the cell surface. In CHO-α2E336A cells, the level of total α2 expression was stable over the course of the experiment (Fig. 5D). Interestingly, in these cells, as opposed to cells bearing the wild type α2 subunit (Fig. 2A), the TPA treatment did not induce an increase in the amount of the active α2 integrin conformation recognized by mAb 12F1 (Fig. 5D).

**DISCUSSION**

The interaction of α2β1 integrin and collagenous matrix has been implicated in a number of biological and pathological processes such as thrombosis, inflammation, angiogenesis, and wound healing. The precise regulation of integrin α2β1 activation is very important for example for platelet function (40). In general, integrin activation is an essential mechanism for the formation of matrix adhesion sites. Thus, understanding the mechanism of α2β1 activation thoroughly will provide insight into a cellular event of broad biological significance. Here, we report that α2β1 integrin activation is regulated by two synergistic mechanisms: receptor clustering and conformational activation.

The structural basis of outside-in signaling in leukocyte β2 integrins has been investigated in depth (1, 38). The integrins are considered to have at least three different conformations with distinct abilities to bind their ligands. In inactive integrins,
the ligand binding head part is bent toward the plasma membrane in a manner that makes the binding of large ligands difficult. However, inside-out signaling mechanisms such as talin binding to the cytoplasmic domains of the α2 subunit (35) can induce a switchblade-like movement in the integrin raising the integrin head part up. The avidity of standing integrin for its ligand can still be increased by further conformational modifications. In αI domain integrins the high avidity conformation has been recognized as the open conformation of the αI domain. Here, we have studied a model in which α2I2/α2I1 integrins were activated by TPA. It was clear that the TPA effect on integrin avidity could, at least partially, be explained by the ability of TPA to switch α2I domains from the closed to the open conformation. This conformational change was studied using 12F1 anti-α2 mAb binding to the cell surface. In a recent study, the monoclonal antibody 12F1 was shown to favor the activated, open conformation of the α2 subunit on platelets (14). Monoclonal antibody 16B4, which recognizes an epitope located outside the ligand binding region of the α2 subunit, was used to determine the total α2 on the cell surface. Previous studies on αLβ2 and αMβ2 integrins have proposed that a glutamate residue in the α7 helix of the αI domain (E320 in αL and E310 in αM) can act as an intrinsic ligand for the β subunit and mediate conformational regulation between the αI domain and the β subunit in two directions (3, 4). Our molecular model of α2β1 integrin supports the idea that Glu-336 in the α2 subunit could have a similar role. This hypothesis was tested using CHO cells transfected with the α2 subunit harboring the E336A mutation. E336A prevented TPA-dependent increases in the number of 12F1 epitopes on the cell surface indicating the essential role of Glu-336 in conformational regulation.

Despite the fact that integrin conformation was shown to participate in the regulation of avidity, it seemed clear that additional mechanisms are likely to exist. This conclusion was based on two observations: first, the conformational change in α2β1 integrins was observed only after 10 min, while the avidity started to increase earlier. Typically, a transient increase in the number of 12F1 epitopes occurred 20–40 min after stimulation with TPA. Second, E336A mutation could not block the effect of TPA. In contrast, the E310A mutation in αL, which is an equivalent to E336A in α2, prevents the effect of TPA on avidity (8). In our experiments, base-line cell adhesion was lower in CHO-α2E336A than in CHO-α2WT cells, which is in agreement with previous papers that have described the loss of ligand binding by αL and αM integrins after mutation in the corresponding residue (3).

The clustering of αLβ2 is seen after ligand binding, and it is an important mechanism in the strengthening of adhesion (8). Ligand binding to α2β1 integrin is known to induce clustering and concomitant activation of specific signaling pathways (17). Thus, ligand-dependent receptor clustering is a well known phenomenon. However, the role of ligand-independent integrin clustering in the regulation of avidity has been a controversial issue (41, 42). Here, using confocal microscopy, TPA was...
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documented to induce the macroclustering of α2β1 integrins in α2β1 overexpressing Saos-α2β1 osteosarcoma cells and CHO-α2β1 cells. The role of integrin aggregation was supported by two facts: clustering took place very quickly, in less than 10 min after TPA treatment similarly to the increase in avidity. Additionally, the E336A mutation could not prevent integrin clustering, and it could not prevent the increase in avidity.

The effects of TPA on α2β1 avidity and clustering are likely mediated by activation of PKC, since a chemical inhibitor, bisindolylmaleimide, could block the effect of TPA. Interestingly, we have previously shown that α2β1 clustering can activate the α isoform of PKC, and specific inhibitors of PKCα prevent clustering-dependent integrin internalization (17).

Thus, our results propose that α2β1 integrin clustering and PKC may regulate each other in both directions. Here, the structural requirements of TPA-mediated α2β1 integrin activations were also studied. Our earlier studies have shown that an integrin chimera having the α2 extracellular domain, and the cytoplasmic domain of α1 the subunit (α2/α1) cannot mediate heterodimer-specific outside-in signals (22). In this study, however, this swap mutation neither destroyed the ability of the receptor to become activated by TPA nor affected the time course of activation. In both α2- and mutant α2/α1-mediated collagen I binding, the TPA-dependent increase was already clear at the 10-min time point. These results suggest that the intracellular domains of integrin α subunits may not directly participate in regulation by TPA. In fact, collagen binding activity by CHO cells transfected to express α10β1 integrin was also activated by TPA. One previous study suggests that PKC might interact directly with β1 subunit (43) or through talin-containing integrin activation complex (44). For example αIIbβ3 integrin has previously shown to be activated by TPA only when PKCα and talin are present (44). In agreement, cells expressing wild type α2 and talin-binding defective β1 subunit (GD25-α2β1mut) were not stimulated by TPA. The results suggest that instead of the α subunit, the β subunit is required for TPA-mediated activation.

We conclude that α2β1 integrin forms clusters upon TPA induced activation and that receptor aggregates may contribute to the increase in the avidity of adhesion. Similarly, a recent paper has suggested that ligand free clusters of αLβ2 integrin may occur (45), while in other experimental models, receptor clustering has only followed ligand binding (8, 9). In addition, some earlier studies have suggested that clustering would regulate integrin avidity even without conformational activation of individual receptors (46, 47). This report and our previous results suggest that the same signaling proteins that are activated by ligand mediated clustering may also initiate ligand-free clustering. Furthermore, structural activation seems to be required for the high avidity configuration of the α2β1 integrin. Thus, we propose a model in which α2β1 clustering and conformational activation take place in a synergistic manner.

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