RGD-independent Binding of Integrin $\alpha_9\beta_1$ to the ADAM-12 and -15 Disintegrin Domains Mediates Cell-Cell Interaction*  

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ADAMs (a disintegrin and metallopeptases) mediate several important processes (e.g. tumor necrosis factor-α release, fertilization, and myoblast fusion). The ADAM disintegrin domains generally lack RGD motifs, and their receptors are virtually unknown. Here we show that integrin $\alpha_9\beta_1$ specifically interacts with the recombinant ADAMs-12 and -15 disintegrin domains in an RGD-independent manner. We also show that interaction between ADAM-12 or -15 and $\alpha_9\beta_1$ supports cell-cell interaction. Interestingly, the cation requirement and integrin activation status required for $\alpha_9\beta_1$/ADAM-mediated cell adhesion and cell-cell interaction is similar to those required for known integrin-extracellular matrix interaction. These results are quite different from recent reports that ADAM-2/ $\alpha_9\beta_1$ interaction during sperm/egg fusion requires an integrin activation status distinct from that for extracellular matrix interaction. These results suggest that $\alpha_9\beta_1$ may be a major receptor for ADAMs that lack RGD motifs, and that, considering a wide distribution of ADAMs and $\alpha_9\beta_1$, this interaction may be of potential biological and pathological significance.

ADAM1 (for a disintegrin and metallopeptase), or MDC (metallopeptase/disintegrin/cysteine-rich), proteins are a family of membrane-anchored glycoproteins. More than 30 ADAMs have been identified. ADAMs are involved in fertilization (ADAM-1, -2, and -3) (1–4), muscle fusion (meltrin-α, ADAM-12) (5, 6), release of tumor necrosis factor-α from the plasma membrane (TACE, ADAM-17) (7, 8) for review), and in some of these critical biological processes.

The disintegrin domain of ADAMs is a potential integrin ligand, but generally lacks the RGD motif (unlike RGD-containing snake venom disintegrins): Human ADAM-15 is the only ADAM that has an RGD motif in its disintegrin-like domain (14). Integrin $\alpha_9\beta_1$ has been reported to interact with the disintegrin domains of fertilin α and β (ADAM-1 and -2) complex that has no RGD motif during fertilization (2). Integrin $\alpha_9\beta_1$ has been reported to specifically bind to the disintegrin domain of human ADAM-15 in an RGD-dependent manner (15, 16). Interestingly, mouse ADAM-15 (mADAM-15) has a TDD sequence instead of RGD (17). This raised doubts about the role of ADAM-15 as a genuine integrin ligand. ADAM-15 (metargindin) is widely expressed in various tissues and cell types (14, 17) and is implicated in atherosclerosis, since ADAM-15 is over-expressed in atherosclerotic lesions (18). ADAM-12 has been implicated in myoblast fusion during myogenesis (5, 6), and has a catalytically active metallopeptase domain and a non-RGD disintegrin domain. The cysteine-rich domain of ADAM-12 has a putative fusion peptide and a short hydrophobic stretch (19, 20). The truncated mouse ADAM-12, which lacks the metallopeptase domain, enhances fusion of C2C12 myoblastic cells in vitro (5). These findings suggest that the disintegrin and/or cysteine-rich domains of ADAM-12 should be involved in cell-cell interaction during myoblast fusion. Since the ADAM-12 gene is activated in condensed mesenchymal cells that give rise to skeletal muscle, bones, and visceral organs (21), ADAM-12 may be involved in development of other organs as well.

A major question is whether the non-RGD disintegrin domains of ADAMs interact with integrins. In the present study, we designed experiments to address this question using recombinant disintegrin domain fragment and cells expressing recombinant ADAMs. We demonstrated a novel interaction between integrins and ADAMs that is RGD-independent and may play crucial roles in cell-cell interaction during development and in pathological conditions.

MATERIALS AND METHODS

Monoclonal Antibodies and Cell Lines—Hybridomas for antibodies T82/16 (anti-β1, activating) and AIIB2 (anti-β1, function-blocking) were obtained from American Type Culture Collection. Chinese hamster ovary (CHO) cells expressing different integrins have been described (15). Ntera-2 human embryonal carcinoma cells were provided by Amos Baruch (Scripps Research Institute, La Jolla, CA). G361 human melanoma cells were obtained from American Type Culture Collection.

Preparation of K562 Cells Expressing Recombinant $\alpha_9\beta_1$—cDNA

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in expression vector (22) was transfected into K562 cells. After selection with G-418 (1 mg/ml of medium), cells stably expressing human integrins were cloned by limited dilution and designated a4K562. a4K562 cells express a4 (as a4b1) as detected by flow cytometry in FACScan (Becton Dickinson, San Jose, CA) with the anti-a4 antibody Y9A2 (23) (Fig. 1A).

Preparation of the GST Fusion Protein of the Disintegrin-like Domain of Mouse ADAM-15, and Human and Mouse ADAM-12—A cDNA fragment of about 270 nucleotides that encodes the disintegrin-like domain of mouse ADAM-15 (Met-420 to Glu-510) (17) was amplified by polymerase chain reaction (PCR) with a mouse expressed sequence tag clone (AI57315) as a template using 5′-GCGGAATTC-TACTCGCCATCCCCTAGGCTG and 3′-GCGGCCCATATGAGTGGAGAACAG and 5′-GCGGAGTAC-TTATGCCATGCTGGAAGTACAC as primers. A cDNA fragment of 264 nucleotides that encodes the disintegrin-like domain of human ADAM-12 (Cly-423 to His-513) was amplified by PCR with a human placenta cDNA library (Invitrogen) as a template using 5′-GCGGATCCGCGGCGGACAAGATTGGAGAACAG and 5′-GCGGATCC-TATGGCCATATGAGTGGAGAACAG and 5′-GCGGATCCTATTATGCCATGCTGGAAGTACAC as primers. A cDNA fragment of 270 nucleotides that encodes the disintegrin-like domain of mouse ADAM-12 (Cly-423 to His-510) was amplified by PCR with a full-length mouse ADAM-12 cDNA (5) as a template using 5′-GCGGATCCCGGCGGACAAGATTGGAGAACAG and 5′-GCGGATCC-TATGGCCATATGAGTGGAGAACAG and 5′-GCGGATCCTATTATGCCATGCTGGAAGTACAC as primers. These cDNA fragments were subcloned into the BamHI site of the modified pGEX-2T vector (Amersham Pharmacia Biotech), in which a 6-His sequence was inserted between the thrombin cleavage site and the BamHI site. Synthesis of the GST fusion protein of the ADAM-15 disintegrin-like domain was induced in Escherichia coli DH5a by adding 0.1 mM isopropyl-

1-thio-β-D-galactopyranoside in culture medium as described previously (24). Protein was extracted from the bacterial suspension by sonication and purified using glutathione-agarose (Sigma) affinity chromatography. The recombinant wild type fusion proteins used in this study are shown in Fig. 1A.

Protein concentration was calculated from absorbance at 280 nm. We calculated the extinction coefficients based on the amino acid sequence using the Expasy Protparam tool available from the Expasy web site (25).

Removal of GST from GST Fusion Protein—The GST fusion protein has a 6-His sequence inserted between its thrombin cleavage site and disintegrin domain. The GST fusion protein was digested with thrombin (Sigma; 1 unit/mg of GST fusion protein) at room temperature for 5 h. The GST portion was removed by passing the digested materials through a G-418 buffer containing 2 mM CaCl2, 2 mM MgCl2, or 1 mM MnCl2 was used instead of RPMI 1640 to determine the ion dependence of the cell to cell interaction.

Other Methods—Site-directed mutagenesis was carried out using the unique site elimination method (31). The presence of mutations was verified by DNA sequencing. Flow cytometry was performed as described previously (27).

RESULTS

Adhesion of Integrin a4b1 to the Mouse ADAM-15 Disintegrin Domain in an RGD-independent Manner—We have previously shown that the recombinant disintegrin-like domain of human ADAM-15 binds to a4b1 in an RGD-dependent manner (15). However, a mouse ADAM-15 homologue has recently been reported to have a TDD sequence instead of an RGD sequence in the putative integrin-binding site of its disintegrin domain (17). We generated a recombinant mouse ADAM-15 disintegrin fragment (Fig. 1A) and studied whether it supports a4b1-mediated cell adhesion. We did not detect significant adhesion of β1-CHO cells that express hamster α1/human β1 hybrid to the mouse ADAM-15 disintegrin domain (Fig. 1B).

We studied whether other integrin receptors bind to the mouse ADAM-15 disintegrin domain using CHO cells expressing different recombinant integrins. Parent CHO cells express a4b1 and a2b1, but do not express β2 or β3 (27). wt-GST (GST alone, a negative control) does not support adhesion to any integrins tested. GST-FN (a positive control: the rat fibronectin 8–11th type III repeats) supported adhesion to all of the cell lines tested. We found that the mouse ADAM-15 disintegrin domain supported adhesion of α1-CHO cells (which express human α1/hamster β1 hybrid), but did not support adhesion of parent CHO cells and CHO cells expressing other integrins, including a4b1, a2b1, a5b1, a6b1, and a9b1, except for a4b1, which weakly bound to mouse ADAM-15 (Fig. 1B). These results indicate that a4b1 recognizes the mouse ADAM-15 disintegrin domain.

Adhesion to the human and mouse ADAM-15 disintegrin domains was determined as a function of substrate concentration (Fig. 1C). a4b1-CHO cells showed maximum adhesion to both human and mouse ADAM-15 disintegrin domains at the coating concentration of 20 μg/ml protein. β1-CHO cells showed maximal adhesion to human ADAM-15 disintegrin domain at 5 μg/ml coating concentration, but never showed significant adhesion to mouse ADAM-15 disintegrin domain at the highest coating concentration used (50 μg/ml). These results are consistent with the previous results that a4b1 recognizes ADAM-15 in an RGD-dependent manner (15). We studied...
whether mutation of the RGD motif in the putative integrin-binding site of the ADAM-15 disintegrin domain affects adhesion to α9β1. α9-CHO cells adhered to the two human ADAM-15 disintegrin domain mutants, human/SQA (in which the RGD sequence is mutated to SQA (Ref. 15)) and human/TDD (in which the RGD sequence is mutated to TDD), as well as to wild-type human and mouse ADAM-15 disintegrin domains. These results suggest that α9β1 recognizes ADAM-15 in an RGD-independent manner. Adhesion of α9-CHO cells to these recombinant proteins was completely blocked by Y9A2, an anti-human α9 integrin monoclonal antibody (23) (Fig. 1D), suggesting that this adhesion is specific to α9β1.

These results suggest that α9β1 may bind to other non-RGD ADAMs. We studied whether another non-RGD ADAM, human and mouse ADAM-12, or meltrin-α, which lacks the RGD motif, binds to α9β1 using CHO cells expressing different recombinant integrins. We found that the human and mouse ADAM-12 disintegrin domains supported adhesion of α9-CHO cells, but not of parent CHO cells or cells expressing the other integrins we tested, except for α9β1 and α9β2, which weakly bound to this protein (Fig. 2A). The α9-CHO cells showed maximum adhesion to both the human and mouse ADAM-12 disintegrin domains at a coating concentration of 20 μg/ml (Fig. 2B). This interaction is also blocked by the anti-α9 mAb Y9A2 (Fig. 2C), suggesting that ADAM-12 also specifically interacts with α9β1.

We studied whether non-recombinant α9β1 binds to ADAM-12 and -15 using Ntera-2 human embryonic carcinoma cells that express α9 and β1 on the surface (Fig. 3A). Ntera-2 cells adhered to ADAM-12 and -15 in an α9β1-dependent manner, but required activation by activating anti-β1 mAb TS2/16 (Fig. 3B). We obtained very similar results with G361 human melanoma cells that also express α9β1 (32); G361 cells adhere to ADAM-12 and -15 in an α9-dependent and activation-dependent manner (data not shown). These results suggest that...
RGD-independent \( \alpha_9 \beta_1 \) Integrin/ADAM Interaction

Non-recombinant \( \alpha_9 \beta_1 \) also mediates specific adhesion to ADAM-12 and -15. ADAMs have been implicated in cell-cell interaction during fertilization and myoblast fusion. We studied whether \( \alpha_9 \beta_1 \) interaction with ADAM-15 or ADAM-12 actually supports cell-cell interaction in vitro. We used CHO cells transiently expressing ADAM-15, ADAM-15/PM \( \alpha_9 \beta_1 \), and K562 cells homogeneously expressing \( \alpha_9 \beta_1 \) (K562 cells) (Fig. 4) to detect \( \alpha_9 \beta_1 \)-ADAM protein interaction. Since monoclonal antibodies against ADAM-15 or ADAM-12 are not readily available, we used the IRES2-EGFP bicistronic vector (29, 30), in which both EGFP and ADAMs are encoded in a single mRNA with two separate translation starting sites. Transient EGFP expression was used as a marker of ADAM expression (Fig. 4B), and typically more than 30% of transfected cells are EGFP-positive.

Binding of fluorescence-labeled \( \alpha_9 \)-K562 and parent K562 cells to CHO cells transiently expressing ADAM-15, ADAM-15/PM \( \alpha_9 \beta_1 \), or ADAM-12 was determined. \( \alpha_9 \beta_1 \) in K562 and parent K562 cells bound at low levels (typically 5% of added cells) to control
**FIG. 4. αβ₁ and ADAMs mediate cell-cell interaction.**

**A.** Expression of αβ₁ on K562 cells. Parent K562 cells or αβ-K562 cells were stained with control mouse IgG (dotted line) or Y9A2 (anti-αβ₁ mAb) (solid line), followed by fluorescein isothiocyanate-labeled goat anti-mouse IgG. Stained cells were analyzed by flow cytometry. The data suggest that αβ-K562 cells homogeneously express αβ₁.**

**B.** Expression of EGFP or EGFP containing ADAMs on CHO cells. The IRES2-EGFP bicistronic vector, in which both EGFP and ADAMs are encoded in a single mRNA with two separate translation starting sites, was used. EGFP vector alone, EGFP vector containing the full-length human ADAM-15 cDNA, the truncated human ADAM-15 cDNA fragment encoding residues 420–814 lacking pro- and metalloprotease domains (ADAM-15/PM−), or the full-length mouse ADAM-12 cDNA (solid line) was transfected into CHO cells. These cells and mock-transfected (thin line) CHO cells were analyzed 48 h after transfection. Expression was analyzed by flow cytometry (excitation at 488 nm and emission at 507 nm). Transient EGFP expression was used as a marker of ADAM expression, and typically approximately 30% of transfected cells are EGFP-positive.**

**C.** αβ₁ and ADAM-15 or ADAM-12 mediate cell-cell interaction. CHO cells transiently expressing ADAMs were plated in wells of 96-well plastic culture plates, and grown overnight to near confluence. Fluorescence-labeled αβ-K562 or parent K562 cells were added to the wells and incubated with CHO cells in RPMI 1640 medium. After rinsing the wells to remove unbound cells, bound cells were quantified using a fluorescent plate reader at 485 nm for excitation and at 530 nm for emission. Cell-cell interaction was assayed in the absence (closed bar) or presence (open bar) of the anti-αβ₁ mAb, Y9A2 (10 μg/ml). Note that only approximately 30% of the transfected CHO cells is EGFP positive. Thus, binding of 15–20% of added αβ-K562 cells is substantial. The data suggest that ADAM-15 (wt and truncated) and ADAM-12 on the cell-surface specifically interact with αβ₁ on the surface of apposing cells and mediate cell-cell interaction. D, binding of αβ-K562 cells to CHO cells expressing ADAM-12 or -15. A, a monolayer of CHO cells expressing ADAM-15 before adding αβ-K562 cells; B, αβ-K562 cells on CHO cells expressing EGFP only; C, αβ-K562 cells on CHO cells expressing ADAM-15; D, αβ-K562 cells bound to CHO cells expressing ADAM-15 in the presence of Y9A2. Note that only K562 cells bound to CHO cells after rinsing the wells to remove unbound cells are shown in D–F.
FIG. 5. Inhibition of interaction between membrane-bound ADAM and \( \alpha_9\beta_1 \) by soluble disintegrin domains. CHO cells were transiently transfected with ADAM-12 (top) or ADAM-15 (bottom) cDNA in the IRES/EGFP bicistronic expression vector, and plated in wells of 96-well plates as described under "Experimental Procedures." Labeled \( \alpha_9\)-K562 cells were first precultured with soluble GST-ADAM disintegrin domain fusion proteins, and then incubated with CHO cell monolayer expressing ADAMs. Data are shown as means \( \pm \) S.D. of triplicate experiments. Inhibition (%) of closed square fusion protein; closed circle used as total binding. Background binding, and binding in the absence of added protein was determined in the presence of soluble ADAM-12 or -15 (Fig. 5). The disintegrin domain blocked (with IC\( \text{50} \) of about 0.5 \( \mu M \)), but the ADAM-12 disintegrin domain did not significantly block, ADAM-15/\( \alpha_9\beta_1 \)-mediated cell-cell interaction. The level of maximal inhibition is much higher with the soluble ADAM-15 disintegrin domain than with the ADAM-12 disintegrin domain in both cases.

These results suggest that ADAM disintegrin domains synthesized in bacteria effectively compete with those synthesized in mammalian cells for binding to \( \alpha_9\beta_1 \), and that, consistent with the adhesion and cell-cell interaction results, ADAM-15 disintegrin domain has a higher binding affinity to \( \alpha_9\beta_1 \) than the ADAM-12 disintegrin domain.

**Cation Dependence of \( \alpha_9\beta_1 \) Interaction with ADAM-15 or ADAM-12—**Chen et al. (4) recently reported that the cation and integrin activation states that are required for \( \alpha_9\beta_1 \)/ADAM-2 (fertilin \( \beta \)) interaction are different from those required for \( \alpha_9\beta_1 \)-extracellular matrix protein interaction. They have shown that Ca\(^{2+} \) promotes and Mn\(^{2+} \) inhibits \( \alpha_9\beta_1 \) binding to ADAM-2. In contrast, Ca\(^{2+} \) suppresses and Mn\(^{2+} \) stimulates \( \alpha_9\beta_1 \) binding to laminin (4).

We determined the cation requirement for adhesion of \( \alpha_9 \)-CHO cells to the recombinant disintegrin domain of human ADAM-15 (Fig. 6). We removed the GST portion of the fusion protein, since it generates high background binding to integrins in the presence of Mg\(^{2+} \). We found that Mg\(^{2+} \) and Mn\(^{2+} \) stimulated, but Ca\(^{2+} \) suppressed, \( \alpha_9\beta_1 \) adhesion to the ADAM-15 disintegrin domain. Ca\(^{2+} \) supported \( \alpha_9\beta_1 \) binding to ADAM-15. We also examined the cation requirement for \( \alpha_9\beta_1 \)/ADAM-15-mediated cell-cell interaction (Fig. 7). This interaction is higher in the presence of Mn\(^{2+} \) and Mg\(^{2+} \) than in the presence of Ca\(^{2+} \). We obtained essentially the same results with \( \alpha_9\beta_1 \)/ADAM-12-mediated cell-cell interaction (Fig. 7). These results suggest that the cation requirements for \( \alpha_9\beta_1 \) adhesion to the ADAM-15 disintegrin domain, and for \( \alpha_9\beta_1 \)/ADAM-15- or ADAM-12-mediated cell-cell interaction, are not different from known integrin-extracellular matrix interactions, but are different from the reported ADAM-2/\( \alpha_9\beta_1 \)-mediated cell-cell interaction (4).

**Effect of Activating and Blocking Antibodies on \( \alpha_9\beta_1 \)/ADAM-15, or ADAM-12-mediated Cell-Cell Interaction—**Although the cation requirement for \( \alpha_9\beta_1 \)/ADAM protein-mediated cell-cell interaction is not different from that for known integrin extracellular matrix interactions, it is still possible that a different activation status of \( \alpha_9\beta_1 \) is required for this interaction. To address this question, we studied the effect of the activating anti-\( \beta_1 \) antibody TS2/16, and the inhibitory anti-\( \beta_1 \) antibody AIIB2, on this interaction. These mAbs stimulate or block \( \beta_1 \)
ADAM-15, but AIIB2 completely blocked ADAM-15-mediated extracellular matrix interactions. It is likely that the activation status of cells expressing EGFP and ADAM-15. These results again suggest substantial considering that only approximately 30% of added CHO cells expressing ADAM-15 (10–20% of added cells) is ADAM-mediated cell-cell interaction is very similar to that observed between human and mouse ADAM-12. The present study for the first time demonstrated that ADAM-15 and ADAM-12 binding to α9β1 mediates cell-cell interaction. α9β1 is distributed in tissues including airway epithelia, the basal layer of squamous epithelia, smooth muscle, skeletal muscle, hepatocytes, neutrophils, and monocytes (34–36). α9β1 has been reported to recognize tenasin C type III repeat (22, 37), vascular cell adhesion molecule-1 (36) during inflammation. Thus, integrin α9β1 may have broad ligand specificity similar to integrins of the αv family. Considering a wide distribution of ADAMs and α9β1, ADAM/α9β1-mediated cell-cell interaction may be involved in many developmental and pathological situations, including myoblast fusion, fertilization, and vascular and cartilage remodeling.

Human ADAM-15 potentially participates in vascular remodeling such as in atherosclerosis, since its protein level is increased in the core of atherosclerotic lesions and in intimal

**FIG. 7.** Cation dependence of cell-cell interaction between α9β1 and ADAM-15 or ADAM-12. CHO cells transiently expressing ADAM-15 or ADAM-12 were plated in wells of 96-well plastic culture plates, and grown overnight to near confluence. Fluorescence-labeled α9-K562 (shaded column) or parent K562 cells were added to the wells and incubated with CHO cells in Hepes-Tyrode buffer supplemented with 1 mM EDTA, 2 mM Mg2+, 2 mM Ca2+, or 1 mM Mn2+ at 37°C for 90 min. After rinsing the wells to remove unbound cells, K562 cells bound to CHO cells were quantified utilizing a fluorescent plate reader. Data are shown as means ± S.D. of triplicate experiments. The data suggest that the cation dependence of cell-cell interaction between α9β1 and ADAM-15 or -12 is similar to that for known integrin-extracellular matrix interactions.

**DISCUSSION**

A **Novel Link between ADAMs and Integrin α9β1**—It has not been established whether most of the ADAM disintegrin domains interact with integrins, since they generally lack the RGD motif in their putative integrin binding site. The present study establishes that ADAM disintegrin domains that lack an RGD motif (mouse ADAM-15, human ADAM-15 mutants, and human and mouse ADAM-12) support cell adhesion to α9β1 in an RGD-independent manner. Thus α9β1 may be a major receptor for ADAMs that lack RGD motifs. The human ADAM-15 disintegrin domain, which has the RGD motif, binds to α9β1 in an RGD-dependent manner (15), and to α9β1 in an RGD-independent manner. However, its interaction with α9β1 appears to be physiologically more important, since this interaction is evolutionarily conserved, while the RGD motif of ADAM-15 is not conserved.

The present study for the first time demonstrated that ADAM-15 and ADAM-12 binding to α9β1 mediates cell-cell interaction. α9β1 is distributed in tissues including airway epithelia, the basal layer of squamous epithelia, smooth muscle, skeletal muscle, hepatocytes, neutrophils, and monocytes (34–36). α9β1 has been reported to recognize tenasin C type III repeat (22, 37), vascular cell adhesion molecule-1 (36), and osteopontin (38) in an RGD-independent manner. It has been proposed that α9β1-mediated binding of neutrophils to endothelial cells may be involved in chemotaxis across activated endothelial monolayers by interacting with the endothelial ligand vascular cell adhesion molecule-1 (36) during inflammation. Thus, integrin α9β1 may have broad ligand specificity similar to integrins of the αv family. Considering a wide distribution of ADAMs and α9β1, ADAM/α9β1-mediated cell-cell interaction may be involved in many developmental and pathological situations, including myoblast fusion, fertilization, and vascular and cartilage remodeling.
cells close to the lumen, but not in normal vessel (18). In addition, the mRNA level of ADAM-15 is up-regulated in human osteoarthritic cartilage and neoplastic cartilage (chondrosarcoma), and therefore ADAM-15 has a potential role in cartilage remodeling (39). ADAM-15 has a catalytically active metalloprotease domain with a metalloprotease catalytic site consensus sequence (HEXXH) (17). Since αβ1 is highly and uniformly expressed in neutrophils, and weakly expressed in monocytes (34), αβ6/ADAM-15-mediated cell-cell interaction may be critically involved in recruitment of these cells to inflammatory sites, and subsequent vessel or tissue damage (40). ADAM-15/αβ1 and ADAM-15/αβ2 interaction (15) may play a crucial role during atherosclerosis and cartilage remodeling.

ADAM-12 has been implicated in myoblast fusion during myogenesis (5, 6). The caveolae-rich domain of ADAM-12 has a putative fusion peptide and a short hydrophobic stretch (19, 20). The metalloprotease domain-less mouse ADAM-12, not the full-length ADAM-12, induces fusion of C2C12 myoblastic cells in vitro (5). The myogenic activity of the processed ADAM-12 was also demonstrated in tumor cells expressing a secreted form of human ADAM-12 that has only disintegrin and caveolae-rich domains (6). The function of the metalloprotease domain during myoblast fusion is not established, although ADAM-12 has a catalytically active metalloprotease domain. Integrin αβ1 is highly expressed in skeletal and smooth muscle cells (34), and therefore ADAM-12/αβ1 interaction in vivo through the disintegrin domain may be involved in myogenesis.

Recently Bigler et al. reports that GST-ADAM-2 disintegrin domain fusion protein expressed in bacteria effectively blocks sperm-egg binding and fusion (41). We have shown in the present paper that αβ6 interacts both with GST-ADAM-12, or ADAM-15 disintegrin domain fusion protein that are expressed in bacteria and with ADAM-12 and -15 that are expressed on mammalian cells. Furthermore, we have shown that GST-ADAM disintegrin domains effectively competed with ADAMs on mammalian cells for binding to αβ1. These results suggest that the ADAM disintegrin domains expressed in bacteria are similar in integrin binding function to those expressed in mammalian cells, although the bacterial disintegrin domains lack glycosylation. Additionally, these results suggest that the disintegrin domains primarily mediate interaction with integrins, although we do not rule out the possibility that other domains (e.g. Cys-rich domain) may be involved in this interaction (42).

We have shown that Ntera-2 embryonic carcinoma cells and G361 melanoma cells expressing native αβ1 adhere to ADAM-12 and -15 in an αβ6-dependent manner. Considering the wide distribution of ADAMs, it is possible that αβ6/ADAM interaction may be involved in cell-cell interaction during cancer metastasis. It is also possible that this interaction mediates transduction of proliferative signals through cell-cell interaction in tumor mass. Since αβ1 on these cells requires activation for binding to ADAM disintegrin domains, it is likely that αβ6/ADAM interaction may be regulated by αβ1 activation in these cells, unlike αβ1 on CHO cells, which appears to be constitutively active.

**Activation Status of Integrins during ADAM / Integrin-mediated Cell-Cell Interaction**—Integrin αβ1 on mouse eggs and on α5-transfected cells, has been reported to interact with the disintegrin domain of the sperm surface protein ADAM-2 (fer-tilin β) (2). Additionally, the activation status of αβ1 for ADAM-2 binding has been reported to be different from that for laminin (4). Thus, a major question is whether ADAM-15 or -12-mediated cell-cell interaction is different from known integrin/extracellular matrix interactions. We have shown that ADAM-15- or ADAM-12/αβ1-mediated cell-cell interaction is similar in cation requirement to αβ1 adhesion to ADAM-12, and to their interaction with known integrin-extra-cellular matrix proteins (e.g. fibronectin). Consistently, both ADAM-15/αβ1 and ADAM-12/αβ1 interactions are stimulated by the activating anti-β1 mAb TS2/16, and blocked by the function-blocking anti-β1 mAb A1B2. These results establish that the β1 integrin activation status that is required for ADAM-15 or ADAM-12 interaction with αβ1 is similar to that required for the interaction between β1 integrins and extracellular matrix ligands. Thus, our results do not fit in well with the reported ADAM-2/egg interaction (4). Further studies will be required to resolve this apparent discrepancy.

Very recently, Miller et al. (43) reported that the αβ1 is not essential for sperm-egg fusion using eggs from α null mice and proposed that β1 integrins other than αβ1 might be involved in this process. In our preliminary experiments, we found that αβ1 specifically binds to the disintegrin domain of ADAM-2 synthesized in bacteria, suggesting that αβ1 is a likely candidate integrin that interacts with ADAM-2 during sperm-egg binding and fusion. This is consistent with the idea that αβ1 is a predominant receptor for non-RGD disintegrin domains of ADAMs. Further studies will be required to establish whether αβ1 is really involved in sperm-egg binding and fusion.

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