The Reprolysin Jararhagin, a Snake Venom Metalloproteinase, Functions as a Fibrillar Collagen Agonist Involved in Fibroblast Cell Adhesion and Signaling*

Paola Zigrino‡, Aura S. Kamiguti§, Johannes Eble‡, Claudia Drescher‡, Roswitha Nischt‡, Jay W. Fox§, and Cornelia Mauch‡**

From the ‡Department of Dermatology, University of Cologne, Cologne 50924, Germany, the §Biomolecular Research Facility, University of Virginia, Charlottesville, Virginia 22903, the ¶Department of Hematology, University of Liverpool, Liverpool L69 3BX, United Kingdom, and the ‡Department of Physiology, University of Münster, Münster 48149, Germany

When human dermal fibroblasts are grown in contact with fibrillar collagen type I, a series of events are triggered. Fibroblasts acquire phenotypic tissue-like characteristics that are not observed in fibroblasts grown as monolayer cultures on plastic or on monomeric collagen type I (7, 8). When seeded into these loose networks of collagen fibrils, fibroblasts down-regulate type I collagen expression (9), induce MMP-1 synthesis (10), and activate pro-MMP-2 (11). Furthermore, it has been shown in fibroblasts that collagen binding to the α(1)β(1) integrin contributes to the reorganization and contraction of the collagenous matrix (12, 13) and is responsible for the induction of MMP-1 synthesis (14, 15). The down-regulation of type I collagen synthesis in this system was due to collagen binding to the α(1)β(1) integrin (14). Recently, we observed that, in addition to MPP-1, MT1-MMP is also induced on both the mRNA and protein levels by the ligation of the α(1)β(1) integrin receptor with fibrillar collagen (16). The synthesis of the α(1)β(1) integrin was found to be up-regulated in collagen lattices, whereas the expression of other collagen integrin receptors, such as α(2)β(1) and α(3)β(1), was not affected (13).

The snake venom metalloproteinases (SVMPs) are members of the Reprolysin family (M13) of metalloproteinases. The ADAMs (a disintegrin-like and metalloproteinase) family of proteins, this work demonstrates the potential of the disintegrin-like/cysteine-rich domains in the ADAMS as cellular signaling agents to elicit responses relevant to the biological function of these proteins.

Adhesion of fibroblasts to native type I collagen is mediated by α(1)β(1) and α(2)β(1) integrin receptors (1, 2). Recently, Knight et al. (3) have shown that the sequence GFOGER (O, hydroxyproline) in triple-helical collagen type I and IV is recognized by both α(2)β(1) and α(1)β(1) integrins. Several studies have localized the binding site for collagen within the I-domain of the α-chain integrin subunit. The I-domain is composed of about 200 amino acids and shares homology with the von Willebrand factor A domain (4–6).

* Received for publication, March 1, 2002, and in revised form, July 20, 2002
Published, JBC Papers in Press, August 16, 2002, DOI 10.1074/jbc.M202049200

** To whom correspondence should be addressed: Tel.: 49-221-478-5407; Fax: 49-221-478-5949; E-mail: Cornelia.Mauch@medizin.uni-koeln.de.
ment representing the disintegrin and cysteine-rich domains of jararhagin. The α6β4 integrin can also interact with jararhagin-C, but the interaction seems to be weaker than with jararhagin (23), suggesting that additional N-terminal structures might be involved in jararhagin-α6β4 binding. Interestingly, synthetic peptides based on a sequence in the metalloproteinase domain of jararhagin have been shown to bind to the I-domain of the recombinant α6 integrin chain thereby preventing the binding of the α2-I domain to collagens I and IV, and to laminin-1 (24).

In this study, we have investigated the ability of jararhagin to mimic fibrillar collagen interaction with fibroblasts to modulate the expression of the integrin α6β4 and the matrix metalloproteinases MMP-1 and MT1-MMP. In contrast to previous studies performed in platelet, jararhagin binding to fibroblasts led to cellular activities similar to those induced by fibrillar type I collagen binding via the α6β4 integrin. These results suggest that other disintegrin-like/cysteine-rich domain-containing proteins, such as the ADAMs, may be capable of not only binding to integrins, as has been shown, but also signaling via integrins to alter cellular events such as gene and protein expression.

MATERIALS AND METHODS

Antibodies and Reagents—The following antibodies were used: function blocking mouse monoclonal antibodies directed against the β1 (4B4; Coulter Corporation), α6 (P1E6, BIOMOL), and α2 (BIOMOL) integrin chains; monospecific mouse antibodies to MT1-MMP were raised against a peptide corresponding to the residues 160–175 of human MT1-MMP (114–121; Fuji Chemicals). Rabbit polyclonal antibodies to human MMP-1 were kindly provided by Dr. P. Angel (Deutsches Krebsforschungszentrum, Heidelberg, Germany). Function-blocking mouse antibodies raised against the human α6 integrin chain, mouse antibodies against human ILA-ARO, and antibodies directed against the β1 integrin subunit antibodies used for immunoblotting were from Chemicon. The rabbit polyclonal antibodies directed against jararhagin were a kind gift from Dr. R. D. G. Theakston (Liverpool School of Tropical Medicine, Liverpool, UK). Jararhagin was purified from the venom of B. jararaca as previously described (19). Inactivation of protofibrillar activity was performed by a 5-min treatment with 5 mM 1,10-phenanthroline at 37 °C (25).

Cell Culture Conditions and Immunostaining—Human dermal fibroblasts obtained by outgrowth from explants were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 2 mM glucose, and 150 units/ml each of penicillin and streptomycin. Fibroblasts were used at passages 1–9. Three-dimensional collagen gels were prepared as described previously (9). Briefly, type I collagen (3 mg/ml, Vitrogen) and 10× DMEM were combined in a 1:10 ratio and neutralized by the addition of 0.1 M NaOH. Fibroblasts were seeded into collagen gels at a density of 1 × 10^5 cells/ml and incubated at 37 °C. Alternatively, suspensions of fibroblasts were preincubated with jararhagin, 100 nM, for 20 min at 37 °C followed by seeding into collagen gels. Rates of gel contraction were monitored by determination of the density before plating. Adhesion assays were performed as previously described (26). Briefly, 96-well microtiter plates were coated with 100 µl of active or 1,10-phenanthroline-inactivated jararhagin (4 µg/ml), monoclonal collagen type I (40 µg/ml), or 50% FCS at 4 °C overnight. BSA coating and blockage of nonpecific binding sites were performed by 1-h incubation with heat-denatured BSA (1% BSA in Ca^2+/-Mg^2+-free PBS) at room temperature. After washing the wells twice, cells (2 × 10^4 cells/well) were seeded and incubated for 2 h at 37 °C. Non-adherent cells were removed by washing twice with PBS, and adherent cells were fixed with 3% formaldehyde in PBS, pH 7.6, and stained with 0.5% crystal violet in 20% (v/v) methanol. The dye was released from the cells by addition of 0.1 M sodium citrate in 50% (v/v) ethanol. The optical density of the released dye solution was determined at 595 nm. Values were calculated relative to the values obtained for the control assays (PBS or jararhagin pre-coated plates), which were set at 100%. Statistical analysis was performed with the ANOVA Dunnett multiple comparison test.

Binding of Soluble α6β4 to Immobilized Jararhagin—Recombinant soluble human integrin α6β4 ectodomain heterodimers were prepared in insect cells using an expression plasmid in which the cytoplasmic and transmembrane domains were replaced by Fos and Jun dimerization motifs as described previously (27).

Microtiter plates were coated with jararhagin and bovine type I collagen at concentrations of 4 and 40 µg/ml in Tris-buffered saline (TBS, 50 mM Tris-HCl, pH 7.4, 150 mM NaCl) containing 3 mM MgCl2 and 0.1 mM acetic acid. After overnight incubation, the wells were blocked with heat-denatured BSA and incubated with 6 µg/ml soluble α6β4 integrin in the absence or presence of 10 µg EDTA for 2 h at room temperature. Then the wells were washed twice, and substrate-bound integrin was detected by enzyme-linked immunosorbent assay using a rabbit anti-human β1 integrin antisera and alkaline phosphatase-coupled anti-rabbit IgG antibodies as primary and secondary antibodies, respectively. para-Nitrophenylphosphate was used as the enzyme-linked immunosorbent assay substrate with the product measured at 405 nm. Each value was measured in duplicate, and standard deviations were calculated.

Preparation of Cell Membranes and Western Blot Analysis—For preparation of crude plasma membranes, cells were washed twice with PBS and scraped off the plates with PBS containing the protease inhibitors aprotinin (10 µg/ml), Pefabloc (0.25 mg/ml), and leupeptin (1 µg/ml). Cell suspensions were subjected to three cycles of freeze-thaw in a dry ice-ethanol/37 °C bath, and cell lysis was confirmed microscopically. Lysates were separated from cell nuclei by centrifugation at 500 × g. Then the supernatant was centrifuged at 7,000 × g for 15 min at 4 °C. Bound primary antibodies were detected using a horseradish peroxidase-conjugated secondary antibody (1:2000, Dako) and visualized with the ECL system (ECL™, Amersham Biosciences). For preparation of total lysates, cells were washed twice with PBS and lysed in PBS containing 0.5% Nonidet P-40. Lysates were centrifuged at 15,000 × g for 20 min at 4 °C. Protein concentration was determined using a commercial assay (Bio-Rad).

Analysis of Jararhagin Binding to fibroblasts—Western blotting to detect the amount of protein from the membrane preparations, lysates, or conditioned media were separated on 10% SDS-polyacrylamide gels under reducing conditions and transferred onto Hybond-C Super™ (Amersham Biosciences). After blockage of nonspecific binding sites with 5% skimmed milk in PBS containing 0.5% Tween (v/v), the blots were incubated with the primary antibodies overnight at 4 °C. Bound primary antibodies were detected using a horseradish peroxidase-conjugated secondary antibody (1:2000, Dako) and visualized with the ECL system (ECL™, Amersham Biosciences).

Zymographic Analysis—Cells were cultured as monolayers with or without jararhagin stimulation. At different time points, media were collected and separated (20 µl/well) on 10% SDS-polyacrylamide gels containing 1 mg/ml bovine gelatin (Sigma). Then gels were washed in...
FIG. 1. Jararhagin is a cell-adhesive substrate. A, fibroblasts were seeded on microtiter plates (2 × 10^4 cells/well) coated with either 4 μg/ml jararhagin or 1,10-phenanthroline-inactivated jararhagin, 40 μg/ml collagen type I, or 50% FCS as described under “Materials and Methods.” BSA-coated wells were included as a negative control. After 2 h, non-adherent cells were removed and the adherent cells were stained with crystal violet after fixation. The bars represent the mean ± S.E. of the optical densities determined after release of the dye from three independent experiments performed in duplicates. The mean obtained for FCS-coated dishes (OD_{595 nm} of 0.68 ± 0.08) was arbitrarily set as 100%. B, fibroblasts were incubated with monoclonal antibodies raised against the α1, α2, or β1 integrin chains (2.5 μg/ml) or with a combination of antibodies raised against the α2 and β1 integrin chains (2.5 or 5 μg/ml each). Then the cells were seeded on plates precoated with jararhagin (4 μg/ml) for 2 h. HLA antibodies (5 μg/ml) were used as a control. The bars represent the mean ± S.E. of the optical densities of the released dye from three independent experiments performed in duplicate. The mean of the optical densities obtained for adhesion to jararhagin-coated wells without treatment (OD_{595 nm} of 0.5 ± 0.09) was set arbitrarily as 100%. (*, p < 0.05; **, p < 0.01.)

Northern Blot Analysis—Total RNA was isolated by direct lysis of the cells in guanidine thiocyanate followed by phenol-chloroform extraction (29). Total RNA (5 μg) was resolved in formaldehyde/agarose gels, blotted onto Hybond-N membranes (Amersham Biosciences), and hybridized with random-primed 32P-labeled cDNA probes for MT1-MMP (30), MMP-1 (31), and the α2 integrin chain (32).

RESULTS

Jararhagin Supports Adhesion of Fibroblasts and Inhibits Collagen Lattice Contraction

Fibroblasts showed similar adhesion levels to jararhagin- and type I collagen-coated dishes (Fig. 1A). No significant difference was observed between fibroblast adhesion to jararhagin or 1,10-phenanthroline-inactivated jararhagin. As shown in Fig. 1B, cell adhesion to jararhagin was reduced by ~30% in the presence of blocking antibodies directed against the α2 or β1 integrin subunits; whereas no inhibition was noticed with other function-blocking antibodies directed against α1 or α3 integrins, which both can serve as collagen receptors (2). Using a combination of both the α2 and β1 antibodies, cell adhesion to jararhagin was inhibited by up to ~60% in a dose-dependent manner.

FIG. 2. Jararhagin interferes with collagen gel contraction. Human fibroblasts were grown in collagen gels in the absence (white bars) or in the presence of 100 nM (black bars). At the indicated time points the gel surface area was measured. Contraction is indicated as percentage of the initial gel surface area, which was set arbitrarily as 100%. The results represent the mean ± S.E. of two independent experiments performed in triplicates.

2.5% Triton X-100 for 30 min followed by an overnight incubation in metallocproteinase substrate buffer (50 mM Tris-HCl, pH 8.0, 5 mM CaCl2) (28). Gels were stained with Coomassie Blue R-250 and then destained in water.

Northern Blot Analysis—Total RNA was isolated by direct lysis of the cells in guanidine thiocyanate followed by phenol-chloroform extraction (29). Total RNA (5 μg) was resolved in formaldehyde/agarose gels, blotted onto Hybond-N membranes (Amersham Biosciences), and hybridized with random-primed 32P-labeled cDNA probes for MT1-MMP (30), MMP-1 (31), and the α2 integrin chain (32).

When fibroblasts were pre-treated with jararhagin followed by incubation of the cells within collagen lattices there was a notable delay of lattice contraction (Fig. 2). After 24 h, media were replaced and fresh jararhagin was added. After the indicated time points media were collected and stored at −20 °C until use. In parallel, the cells were used for membrane preparations as described under “Materials and Methods.” 20 μl of the conditioned media (A) and 40 μg of the crude membrane fractions (B) was resolved on 10% SDS-polyacrylamide gels under reducing conditions. After blotting, specific protein bands were visualized by immunodetection using polyclonal antibodies raised against jararhagin (5 μg/ml). The jararhagin-specific bands of 55 and 33 kDa, the latter one presumably representing a degradation product, are marked by arrows. The molecular mass standards are indicated (kDa).

The results suggest that jararhagin delays the contraction by interfering with the α2β1-collagen interaction.

Jararhagin Interaction with the Integrin Receptor α2β1

Previous studies have shown that binding of the α2β1 integrin on platelets by PIII snake venom metallocproteinases results in an inhibition of the signaling events normally induced in collagen-stimulated platelets coupled with a potent inhibition of platelet aggregation (22, 23). To determine whether jararhagin binds to cell surface proteins, both supernatants and crude fibroblast membranes were analyzed by SDS-PAGE after 24 and 48 h of incubation with jararhagin (Fig. 3). Immuno...
Jararhagin, and the transcript levels for MMP-1 and MT1-MMP were assessed by Northern blot analysis (Fig. 5). Control fibroblasts grown in collagen gels showed increased transcript levels for MT1-MMP and MMP-1 at 24 h with a further increase at 48 h culture. At both time points, pre-treatment with jararhagin did not result in significant differences of these transcript levels from those observed in the untreated cells. In addition, there was a similar increase in integrin α2 mRNA level from both untreated and jararhagin-treated fibroblasts. Therefore, pre-treatment of fibroblasts with jararhagin had no apparent effect on fibroblasts grown within collagen lattices.

**Jararhagin Treatment of Fibroblast Monolayer Cultures Results in Similar Changes as Observed for Fibroblasts Grown in Collagen Lattices**

**Morphology**—Analysis of fibroblast cell morphology following treatment with increasing concentrations of jararhagin showed a characteristic elongated shape with protrusions of cell extensions identical to that reported for fibroblasts grown in collagen gels (9). Untreated fibroblasts maintained their characteristic spindle-like morphology with a flattened cell shape (Fig. 6).

**MMP mRNA Expression**—In contrast to fibroblast growth in collagen lattices, in which no significant alterations could be detected, in monolayer cultures treatment with jararhagin produced significant differences as shown in Fig. 5. In monolayer cultures, only very low levels of MT1-MMP and MMP-1 transcripts were observed. However, fibroblasts pre-treated with jararhagin displayed a strong induction of MT1-MMP and MMP-1 mRNA expression together with increased α2-integrin transcript levels. These increases were apparent at 24 h, and by 48 h the increases were comparable to those obtained with fibroblasts cultured within collagen lattices. In addition, the induction of MMP mRNA levels was found to be concentration-dependent, showing maximal stimulation when the cells were pre-treated with 200 nM jararhagin (Fig. 7).

To test whether the metalloproteinase activity of jararhagin is required for the induction of MMP-1 and MT1-MMP expression, monolayer cultures of fibroblasts were treated with active or 1,10-phenanthroline-inactivated jararhagin (22). As shown in Fig. 8, treatment of fibroblasts with proteolytically inactive jararhagin resulted in no significant differences in MMP-1 and MT1-MMP mRNA levels when compared with fibroblasts treated with active jararhagin. The apparent slight reduction
of increasing amounts of jararhagin (50, 100, and 200 nM) were ana-
grown in monolayer cultures for 48 h in the absence (−) or the presence
of increasing amounts of jararhagin (50, 100, and 200 nM) were ana-
yzed by light microscopy. Bar, 25 μm.

Fig. 6. Jararhagin alters fibroblasts morphology. Fibroblasts
grown in monolayer cultures for 48 h in the absence (−) or the presence
of increasing amounts of jararhagin (50, 100, and 200 nm) were ana-
yzed by light microscopy. Bar, 25 μm.

| Jararhagin (nM) | Co | 50 | 100 | 200 |
|----------------|----|----|-----|-----|
| - MT1-MMP      |    |    |      |     |
| - MMP-1        |    |    |      |     |
| - 18S          |    |    |      |     |

Fig. 7. Alterations induced by jararhagin are dose-dependent.
Fibroblasts grown in monolayer cultures for 48 h in the absence (Co) or
the presence of increasing amounts of jararhagin (50, 100, and 200 nm) were analyzed by Northern blot analysis and by gelatin zymography. 5 μg of total RNA was resolved on formaldehyde-agarose gels, transferred to nylon membranes, and hybridized with 32P-labeled cDNA probes for MT1-MMP and MMP-1. Equal loading of RNA was assessed by hybridization of 18 S rRNA.

MT1-MMP - m
MMP-1 - m+J
-18S - m+Ph

Fig. 8. Inactivation of the metalloproteinase domain of jarar-
hagin does not affect mRNA levels. Fibroblasts were cultured in monolayer (m) for 48 h in the absence or in the presence of 100 mM jararhagin (+J), 100 mM 1,10-phenanthroline-inactivated jararhagin (+Ph), or 100 mM 1,10-phenanthroline (+Ph). After 48 h, total RNA was isolated and 5 μg/lane separated on a formaldehyde-agarose gel. After transfer of the RNA, the nylon membrane was hybridized with 32P-labeled cDNA probes for MT1-MMP and MMP-1. Equal loading of RNA is shown by hybridization of 18 S rRNA.

DISCUSSION

In vivo, skin fibroblasts are surrounded by extracellular matrix components, including fibrillar collagens. As an approach to the in vivo situation, an in vitro model was established several years ago, which is believed to resemble some aspects of the in vivo environment (9, 35). In the in vitro model, fibroblasts are embedded in a three-dimensional matrix consisting mainly of fibrillar type I collagen. The collagenous environment causes α2β1-mediated intracellular signaling events that result in changes in the expression of a variety of proteins, including MMP-1, MT1-MMP, and α2β1 integrin (10, 16, 13). Recently, it has been demonstrated that fibroblasts, when adhered within three-dimensional matrices compared with adherence as monolayers, have very different focal adhesion complexes, morphologies, and biological activities (36). These results underscore
the concept that cells respond differently to different architectures of the extracellular matrix to which they are adhering.

The snake venom metalloproteinase jararhagin inhibits collagen-induced platelet aggregation by binding to α2β1 integrin, thereby blocking collagen binding and its subsequent cell surface receptor-mediated signaling (18, 19). Our interests were in examining whether the PIII SVMP, jararhagin, could interfere with the α2β1-mediated changes in fibroblasts that are observed upon contact with fibrillar collagen.

We demonstrated that jararhagin does bind to the fibroblast cell surface, and, interestingly, the level of binding increases over time. This can be explained by the increase in cell surface expression α2β1 receptor observed following initial incubation of fibroblasts with jararhagin. We consider the interaction of jararhagin with fibroblasts to occur via one or more specific interactions of jararhagin with the cell surface, because bound labeled jararhagin could be specifically displaced by fibrillar collagen (Fig. 4A).

Fibroblasts binding to immobilized jararhagin was not dependent on the proteolytic activity of the SVMP (Fig. 8). Preincubation of the fibroblasts with antibodies against the subunits of the collagen binding integrins (α2, α1, α3, and β1) or combinations of antibodies against the α2 and β1 integrin chains blocked binding to a level of ≈40% of the control (Fig. 1B). One possible explanation for the incomplete inhibition could be that, in addition to α2β1 integrin, other integrins or matrix binding receptors may be involved in fibroblast adhesion to jararhagin. This is not necessarily surprising, given the multiple domains of the protein (metalloproteinase, disintegrins-like, and cysteine-rich), each of which could be involved in cell surface interactions (37). We demonstrated that direct binding of the ectodomain of recombinant α2β1 to jararhagin occurred in a non-cation-dependent manner (Fig. 4B). Most matrix components, including collagen, bind to the α2β1 integrin in a cation-dependent manner (38). However, recent reports describe the presence of a non-cation-dependent site on the I-domain of the α2 subunit that supports binding to pro-MMP-1 (39). Therefore, jararhagin binds to fibroblasts via interaction with the α2β1 integrin, albeit at a site different from the cation-dependent site, and hence the anti-α2 antibodies we used may not function as effectively to block binding at the non-cation-dependent site on the I-domain. This offers a possible explanation for the incomplete blocking of jararhagin to soluble α2β1. Several reports have indicated α2β1 integrin as binding partner for jararhagin (18, 19, 23), and our results corroborate this. However, we cannot exclude that other receptors may also be involved in the binding of jararhagin to the cell surface.

In contrast to fibroblasts grown as monolayers, distinctive changes in gene expression were observed for fibroblasts grown in collagen lattices comprised of fibrillar type I collagen (16). Therefore, we anticipated that treatment of fibroblasts with jararhagin prior to growth in collagen lattices would compete with the collagen fibrils for α2β1 binding and block the typical α2β1-mediated signaling responses observed for fibroblasts...
grown in collagen lattices. However, treatment of fibroblasts
grown in collagen lattices with jararhagin failed to show any
inhibition of the collagen-induced up-regulation of MT1-MMP
and MMP-1 transcript levels. Jararhagin treatment did signif-
icantly delay collagen lattice contraction, a phenomenon that
has been shown to be mediated by the integrin αvβ3 (11, 13, 14).
From our data the binding of jararhagin to the αvβ3 integrin
receptor at the concentrations tested could only partially com-
pete with the native substrate collagen. Other investigators
have shown a concentration-dependent inhibition of platelet
adhesion to monomeric collagen in the presence of increasing
amounts of jararhagin or jararhagin-C (23).

Although collagen binding was partially competed by jarar-
hagin treatment of the fibroblasts (as indicated by the delayed
collagen gel contraction), the collagen-induced MT1-MMP and
MMP-1 mRNA expression was unchanged. This suggests that
in the jararhagin-bound αvβ3 integrin population a similar
signaling phenomenon was occurring that recapitulates the
binding of the integrin to fibrillar collagen. This was further
substantiated by the results in monolayer cultures, whereby
jararhagin induced responses resembling those observed with
fibroblasts grown in collagen lattices (16). These included the
induction of MMP-1 and MT1-MMP expression and pro-MMP-2
activation. The apparent increase in MT1-MMP is modest;
however, it was sufficient to produce the functional stoichiom-
etry between MT1-MMP, TIMP-2, and pro-MMP-2 such that
there is an overall increase in pro-MMP-2 activation as ob-
served by zymography.

Because the effects observed in jararhagin-treated mono-
layer cultures are similar to those occurring within collagen
gels, which are mediated by the engagement of αvβ3 integrin,
we infer that jararhagin essentially mimics the effects of the
physiological ligand. This is corroborated by the finding that
jararhagin binds to the soluble ectodomain of the αvβ3 integrin.

Although jararhagin could support the binding of the αvβ3
ectodomain, it was somewhat surprising that this was not
dependent on divalent cations, because EDTA did not inhibit
the process. Although most of the ligand-integrin interactions
are dependent upon divalent ions (38), there are recent reports
suggesting that collagen binding to the αv I-domain can occur
in the absence of metal ions (40). These authors suggest that
collagen binding could occur to the “open,” metal-dependent,
and “closed,” metal-independent, conformations of the I-
domain. Additional studies are planned to better characterize
the binding site for jararhagin on the αvβ3 integrin.

The question as which region or regions of jararhagin is/are
responsible for the activities observed on treatment of fibro-
blasts is unclear. Different regions of PIII SVMPs have been
shown to bind to the αvβ3 integrin. One of these regions is
represented by the ECD motif located in the disintegrin-like
domain (41). The second region, the RKKH motif of jararhagin,
is located in the metalloproteinase domain (24). A third possi-
ble site is the cysteine-rich domain. The recombinant cysteine-
rich domain of atrolysin A, a PIII SVMP isolated from Crocata
lus atrox venom, has been shown to inhibit collagen-stimulated
platelet aggregation thereby indicating an ability to bind plate-
let αvβ3 integrin (37). Preliminary studies in our laboratory
(data not shown) using venom proteins containing only the
disintegrin-like/cysteine-rich domains have indicated that
these domains can induce similar activities as observed with
jararhagin when used to treat fibroblast. Therefore, it is likely
that the proteinase domain does not play a significant role in
these activities.

Data from Kamiguti and colleagues (25) indicated that inhi-
bition of collagen-induced platelet aggregation occurs by bind-
ing of jararhagin to the I-domain of the αv integrin subunit on
platelets. In contrast, when fibroblasts are treated with jarar-
hagin, activation of the αvβ3 integrin receptor was observed as
evidenced by the up-regulation of MMP synthesis. Kamiguti
and colleagues (22) also showed that inhibition of collagen-
induced platelet aggregation results in a reduced collagen-
stimulated phosphorylation of the tyrosine kinase pp7226.

In platelets, activation and aggregation induced by collagen
depends on the cooperative action of αvβ3, glycoprotein VI, and
αIβ2 (42), and therefore the binding of jararhagin to αvβ3 may
not be sufficient for effective signal transduction. Furthermore,
the binding of jararhagin to platelet αvβ3 may preclude the suc-
cessful binding of collagen to αvβ3 and/or glycoprotein VI given
the close proximity of these two receptors. These events could
lead to the overall effect of inhibiting platelet aggregation.

However, the situation with fibroblasts is rather different. The
binding of jararhagin to αvβ3 integrin is sufficient to induce
cellular signaling events essentially identical to that observed
for fibrillar collagen. Therefore, it seems that, although differ-
cent cells may use identical integrins to transduce signals, the
presence of other receptors or cell signal pathways give rise to
different activities. As shown by our experiments, in platelets
jararhagin can engage the αvβ3 receptor to block platelet ag-
rgregation, yet in fibroblasts jararhagin promotes αvβ3 receptor-
mediated signaling to promote the expression of MMP-1, MT1-
MMP, and the αvβ3 integrin. Therefore, not unexpectedly,
when considering the activities resulting from the binding of
ligands to signal-transducing receptors, care must be given to
fully understand the relationship of the receptor to other cell
surface receptors as well as the pathways available for trans-
mitting the signals.

Members of the ADAMs family of Reprolysin have been
demonstrated to bind to various integrins (43). For instance,
ADAM 12 and ADAM 15 have been shown to bind to the αvβ3
integrin through their disintegrin-like/cysteine-rich domains
mediating thereby cell-cell contacts (44). In another case,
ADAM 23 was shown to bind to the αvβ3 integrin and mediate
cell interactions in cells of neural origin (45). Although this
event promotes sperm-egg fusion, very little data is available
on signal transduction following the ADAM-integrin interac-
tion. Based on the data presented here, we would suggest that
future investigations examining the potential of cell signaling
following integrin engagement by an ADAM may be a produc-
tive path to fully understand the biological activities of the
ADAMs.

In summary, our studies have demonstrated that the snake
venom metalloproteinase jararhagin is a useful tool for study-
ing the molecular basis of collagen-induced cellular activities
in human skin fibroblasts for comparison and contrast with other
cell types.

Acknowledgments—We are grateful to Drs. B. Eckes, T. Krieg, and
M. Aumailley (University of Cologne, Cologne, Germany) for critical
discussions. We thank Dr. G. D. Laing (Liverpool School of Tropical
Medicine, Liverpool, United Kingdom) for helping in the purification
of jararhagin.

REFERENCES
1. Hemler, M. E. (1990) Ann. Rev. Immunol. 8, 365–400
2. Hynes, R. O. (1992) Cell 99, 11–25
3. Knight, C. G., Morton, L. F., Peachey, A. R., Tuckwell, D. S., Farndale, R. W.,
and Barnes, M. J. (2000) J. Biol. Chem. 275, 35–40
4. Hughes, A. L. (1992) Mol. Biol. Evol. 9, 216–234
5. Eble, J. A., Golbik, R., Mann, K., and Kühl, K. (1993) EMBO J. 12, 4795–4802
6. Tuckwell, D. S., Calderwood, D. A., Green, L. J., and Humphries, M. J. (1995)
J. Cell Sci. 108, 1629–1637
7. Grinnell, F. (1994) J. Cell Biol. 124, 401–404
8. Grinnell, F., Ho, C.-H., Lin, Y.-C., and Skuta, G. (1999) J. Biol. Chem. 274,
918–923
9. Mauch, C., Hatamochi, A., Scharffetter, K., and Krieg, T. (1988) Exp. Cell Res.
178, 493–503
10. Mauch, C., Adelmann-Grill, C. B., Hatamochi, A., and Krieg, T. (1989) FEBS
Lett. 250, 301–305
11. Seltzer, J. L., Lee, A.-Y., Akers, K. T., Sudbeck, B., Soutouh, E. A., Wayner,
