Differential Effects of Integrin α Chain Mutations on Invasin and Natural Ligand Interaction*

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To determine if recognition of the Yersinia pseudotuberculosis invasin protein and natural substrates requires identical integrin residues, a region of the human α3 integrin chain predicted to be involved in substrate adhesion was targeted for mutation. One point mutation located in a region of the third N-terminal repeat of the α3 chain, α3-W220A, failed to promote adhesion to the natural αβ1 substrate epiligrin but maintained near wild type levels of adhesion to invasin. A second nearby mutation, α3-Y218A, which showed no detectable adhesion to epiligrin, was only partially attenuated for invasin binding as well as invasin-mediated bacterial uptake. A third substitution, α3-D154A, predicted to be in the second N-terminal repeat not known to be implicated in cell adhesion, was competent for invasin-promoted adhesion events and appeared to encode a receptor of increased activity, as it had a higher efficiency than wild type receptor for adhesion to epiligrin. Cell lines expressing this derivative were not recognized by function blocking anti-α3 antibody, indicating that the second and third repeats of the α3 chain are either closely linked in space or the second repeat can modulate activity of the third. Differential effects on substrate adhesion do not appear to be associated with all integrin α chain mutations, as α3 chain mutations affecting the divalent cation binding domains depressed adhesion to invasin to a significant extent.

Invasin is a 986-amino acid outer membrane protein encoded by the Gram-negative pathogen Yersinia pseudotuberculosis (1, 2) that binds at least five different integrins containing the β1 chain, including αβ1 and αβ3 (3). Attachment to integrins by invasin leads to internalization of the bacterium (1, 2). Cross-inhibition studies with human fibronectin indicate that invasin recognizes a site on the integrin identical or nearby to that utilized by natural substrates (4).

Substrate recognition of natural substrates appears to involve the extracellular domains of both the α and β integrin chains (5–8). Each α chain has a series of 7 nonidentical N-terminal repeats, approximately 60 amino acids in length (Fig. 1; see Refs. 9 and 10). Analyses of function-blocking monoclonal antibodies and site-directed mutagenesis indicate that the third α chain N-terminal repeat is required for substrate recognition (11–16). One striking feature of the identified critical α chain residues found within this repeat is that aromatic side chains are important for ligand recognition (Fig. 1), reminiscent of previous observations regarding human growth hormone interaction with its receptor (17). Also found in the α chain N-terminal region are three predicted divalent cation-binding motifs within repeats V–VII. Each putative cation-binding N-terminal repeat lacks a potential coordinating residue at the most C-terminal position of the cation-binding loop (26, 27). One possible model is that a residue from a ligand may complete metal coordination within one of these domains to stabilize ligand binding (28), although data regarding substitution mutations in this region are inconsistent with this proposition (29).

Recent molecular modeling of the 7-repeat N-terminal domain of the integrin α chain has led to the proposal that it is arranged in a 7-bladed “propeller” structure (18). This model uses intradomain contacts to bury hydrophobic residues and provides an explanation for why ligand-derived peptides can cross-link to multiple α chain repeats. This is based on the hypothesis that the ligand contact surface of the integrin chain may involve several neighboring repeats binding to a single substrate (19–23).

To investigate the model that identical integrin residues are involved in invasin and natural substrate adhesion, the following work examines three derivatives harboring mutations in a region of α3 homologous to the regions shown to be important for substrate recognition (13, 14). Two mutations (α3-Y218A and α3-W220A) lie in the third N-terminal repeat in a region predicted to adopt a β-turn structure on the surface of the α chain integrin subunit (13, 16), whereas the third mutation (α3-D154A) lies in the second N-terminal repeat. The results indicate that loss of side chains previously implicated in natural substrate recognition of the α subunit is not sufficient to cause loss of recognition of invasin by integrin receptors. Invasin and natural substrates may recognize an overlapping, but nonidentical, set of residues on the integrin receptor during adhesion.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Bacterial and Eukaryotic Cell Growth Conditions—Bacterial strains DH5α (supE44 ΔlacU169 (λ9lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1), JM109 (recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi-1 Δ(lac-proAB)), and BM171–18 (thi supE Δ(lac-proAB) mutS Δ(Tn10) were grown in L broth or 2× YT supplemented with 100 µg/ml ampicillin or 10 µg/ml tetracycline as required. Escherichia coli MC4100 (araD Δ(lac)X74 rpsL galE galK)
haboring plasmids pRI203 (inv+) ; see Ref. 2), pL277 (inv- ); see Ref. 33), or pT-L12 (inv- ); see Ref. 32) were used to study bacterial uptake, as described below. E. coli BHI 71-18, harboring oligonucleotide-modified pSelect plasmids (Promega, Madison, WI), was grown over night at 37°C in L broth containing 100 μg/ml ampicillin. DNA was isolated from a single colony of strain JM109. K562 cells were grown in RPMI 1640 (Irvine Scientific) containing 10% FCS (Sigma), penicillin, and streptomycin. K562 transfectants expressing wild type α3 integrin (KA4), three derivatives harboring α3 mutations (N283E, D346E, and D408E), or vector with no insert (KpF) were as described (29). Stably transected K562 cells were grown in medium supplemented with 400 μg/ml G418. The entire e10, the ligase deficient bacteria internalized/bacteria added.

Conjugation—Geneticin (G418) was purchased from Life Technologies, Inc. Mouse anti-α3 mAb B-5G10, anti-α3 A3-X8, and anti-α3 A3-IVa5 were as described (30, 34). Mouse anti-α3 antibody P1B5 was purchased from Telios Pharmaceuticals (San Diego, CA). Mouse anti-α3 antibody CD1 was described previously (4). Rabbit anti-mouse fluorescein isothiocyanate conjugate was purchased from Sigma. Plasmid pSelect and related reagents were purchased from Promega Biotech (Madison, WI). An invasin derivative containing the C-terminal 497 amino acids of E. coli invasin (11), was a generous gift of Dr. R. E. Burgeson and a colorimetric enzyme-linked immunosorbent assay. To measure adhesion, 25 μl of cells, resuspended at 2 × 10^6 cells/ml in Binding Buffer, were mixed with 25 μl of either undiluted supernatant of the blocking anti-α3 antibody A3-IVa5 or a 1:100 dilution of the blocking anti-α3 antibody P1B5 ascites in Binding Buffer. Cells were incubated with antibody for 1 h at room temperature, and the 50-μl mixture was added to a 1:2 dilution of culture supernatants of hybridomas producing VD1 or 25 μl of an equal mixture of the hybridoma supernatant and mAb A3-IVa5. The concentration of VD1 was sufficient to eliminate detectable binding to α3β1 (see Fig. 5).

Assay of Bacterial Uptake via α3β1 and α3β2. Integrins—K562 transfectants expressing wild type or mutant α3 chains were pelleted, washed twice with PBS to remove G418 and penicillin/streptomycin antibiotics, grown overnight in RPMI 1640 containing 10% FCS, washed once with PBS, and grown overnight again in RPMI 1640, 10% FCS. Cells were pelleted and washed twice by resuspending them in Binding Buffer at 1 × 10^6 cells/ml, and 500-μl aliquots were added to 25 μl of 1:2 dilution of the blocking anti-α3 antibody P1B5 ascites in Binding Buffer at 1 × 10^6 cells/ml, and 500-μl aliquots were added to 25 μl of 1:2 dilution of culture supernatants of hybridomas producing VD1 or 25 μl of an equal mixture of the hybridoma supernatant and mAb A3-IVa5. The concentration of VD1 was sufficient to eliminate detectable binding to α3β1 (Fig. 5).

RESULTS

Site-directed α3 Mutants Fail to Bind Epiligrin—Analyses of blocking antibodies as well as site-specific mutagenesis indicate that in the α4, α5, and α6 chains, there exists a region within the third “FG-GAP” repeat (Fig. 1) that is critical for adhesion (11-16). To begin to analyze the role of the α3 chain in invasin-mediated uptake, and to determine if residues recognized by invasin are identical to those recognized by natural substrates, site-directed mutagenesis was used to generate five substitutions of the human α3 chain. The substitutions changed residues of the third N-terminal repeat shown to be important in substrate recognition in other α chains, as well as a residue located in the second N-terminal repeat. The derivatives α3-Y218A, α3-W220A (third repeat), and α3-D154A (second repeat) were stably expressed in K562 cells as determined by FACS analysis using the anti-α3 monoclonal antibody P1B5 (Fig. 2), whereas α3-N219A and α3-G222A were not expressed (data not shown).

Stable cell lines expressing α3 substitutions were tested for adhesion to invasin and to an extracellular matrix containing epiligrin, a natural α3β1 integrin ligand comprised of a mixture of laminins 5 and 6 (38). A dose-response curve of cell adhesion to increasing amounts of epiligrin performed by FACS analysis indicated that epiligrin was microfibrillar matrix that had been chemically deposited by ECM deposited by A431 cells (see “Materials and Methods”). The concentration of laminin 5 deposited in the wells was dependent on the density of A431 cells (Fig. 3A). K562 cells harboring substitutions α3-Y218A and α3-W220A showed no detectable adhesion to the laminin 5-containing matrix in the absence of blocking anti-α3 antibody, even at the highest concentrations of
laminin 5 (Fig. 3B). In contrast, the derivative α3-D154A containing a substitution in the second repeat adhered considerably better than the wild type α3 (α3-wt) transfectant (Fig. 3B). This high adhesion efficiency was clearly dependent on the presence of the α3 chain, as both wild type receptor and the D154A derivative were unable to promote adhesion to matrix if anti-α3 mAb P1B5 was included in the adhesion mix (Fig. 3C). These results indicate that residues present in repeat III of α3 that are predicted to contribute to adhesion to natural substrate are required for adhesion by α3β1. Furthermore, there appears to be a region within repeat II that modulates the binding efficiency of the integrin to natural substrate (Fig. 3B).

Fig. 1. A, schematic of the structure of the integrin α3 chain. Shown are the seven N-terminal repeats, the putative metal-binding sites (Me2+), and the presumed substrate adhesion repeat III. B, sequence alignment of 5 of the N-terminal repeats from integrin α3, α4, α5, and αIIb chains. Sequences were manually aligned according to recent molecular modeling (18). The FG and GAP sequences are aligned separately, and repeat domains are terminated with the GXXGXXG consensus in which the 4th position is typically aromatic and the final position is hydrophobic (10). The cation binding domains in repeats V–VII are in bold. Integrin αIIb peptides that inhibit ligand binding correspond to the divalent cation-binding region of repeat V (24, 25). Boldface residues in the C terminus of the third repeat of each α chain represent sites in which mutagenesis results in defective adhesion (see Refs. 13, 14, and this study). Asp-154 in the second repeat of α3 (bold) is the site that was changed as a control in this study.

Fig. 2. FACS analysis of K562 transfectants expressing the integrin α3 chain. Cells were incubated in the presence of the anti-α3 antibodies P1B5 (A–E; see Ref. 39) or A3-IVA5 (F–J; see Ref. 34) followed by labeling with a rabbit anti-mouse antibody conjugated to fluorescein isothiocyanate (see “Materials and Methods”). FACS analysis of derivatives in which the primary antibody was omitted (data not shown), or in which no α3 chain was expressed (Mock), showed only background fluorescence. Each markation on the y axis represents 100 cells.
A second result that implicated a role for repeat II in adhesion to substrate was the fact that transfectants expressing $\alpha_3$-D154A were not blocked for adhesion to matrix by the anti-$\alpha_3$ mAb A3-IVA5 (Fig. 3B) because they were not recognized by this reagent. Antibody A3-IVA5 failed to elicit homotypic attachment in the presence of the blocking anti-$\alpha_3$ antibody A3-IVA5, and cell adhesion was determined (see “Materials and Methods”)(37). Wells were coated with invasin (B) in the presence of anti-$\alpha_3$ mAb VD1 (D); invasin, attachment in the presence of anti-$\alpha_3$ mAb VD1 (E); invasin, attachment in the presence of anti-$\alpha_3$ mAb VD1 and anti-$\alpha_3$ mAb A3-IVA5 (F); or bovine serum albumin (G). Error bars represent S.D. of the data over three measurements for all experiments.

**FIG. 3. Adhesion to ECM by K564 transfectants expressing $\alpha_3\beta_3$ derivatives.** A, microtiter wells were coated with dilutions of ECM containing epiligrin by growing serial dilutions of A431 cells for 2 days on the wells. Epiligrin concentration was determined as described (see “Materials and Methods”). B, wells coated with serial dilutions of epiligrin, prepared in parallel to wells in A, were challenged with 5 $\times$ 10^5 cells of K562 transfectants in the presence or absence of the blocking anti-$\alpha_3$ antibodies A3-IVA5, K562; $\bigcirc$, wt-$\alpha_3$; $\square$, D154A-$\alpha_3$; $\triangle$, Y218A-$\alpha_3$; $\blacklozenge$, W220A-$\alpha_3$; $\bullet$, wt-$\alpha_3$ + A3-IVA5; and $\Diamond$, D154-$\alpha_3$ + A3-IVA5. C, microtiter plates were challenged in the presence (B) or absence (A) of the blocking anti-$\alpha_3$ antibody P1B5, and cell adhesion was determined (see “Materials and Methods”, $n = 3$). Error bars represent S.D. of the data over three measurements for all experiments.

**FIG. 4. Adhesion of K562 transfectants expressing $\alpha_3\beta_3$ derivatives to invasin-coated microtiter plates.** Microtiter wells coated with 1 $\mu$g/ml (A) or 5 $\mu$g/ml (B) of MBP-INV497 fusion protein were challenged with 50 $\mu$l of ~1 $\times$ 10^6 cells of K562 transfectants in the presence or absence of blocking anti-$\alpha_3$ antibody VD1 and blocking anti-$\alpha_3$ antibody A3-IVA5, and cell adhesion was determined (see “Materials and Methods”). Wells were coated with invasin (B) in the presence of anti-$\alpha_3$ mAb VD1 (C); invasin, attachment in the presence of anti-$\alpha_3$ mAb VD1 (D); or bovine serum albumin (E). Error bars represent S.D. of the data over three measurements.
expressing invasin was determined. K562 transfectants were challenged with bacteria in the presence or absence of anti-α₅ and anti-α₃ antibodies, and uptake was assayed (see “Materials and Methods”). Transfectants harboring the α₃-wt, α₃-Y218A, α₃-W220A, and α₃-D154A derivatives were all capable of internalizing bacteria expressing invasin, although uptake promoted by α₃-Y218A was consistently 50–75% of that seen with α₃-wt (Fig. 5). Interestingly, in the absence of anti-α5 antibody, both α₃-Y218A and α₃-W220A displayed reduced uptake levels relative to wild type α₃ transfectants (Fig. 5), indicating that binding to α₂β₁ may be less active in promoting bacterial internalization than α₂β₃, and binding of bacteria to α₂β₃ may interfere with access to α₂β₁. The antibody A3-IV5 was able to inhibit uptake by all α3 derivatives, except α₃-D154A, indicating uptake was dependent on α₂β₁ (Fig. 5).

**Ligand-binding Defects of Three α₃ Derivatives Harboring Mutations In Their Divalent Cation Binding Domains**—As the effects of α₃ chain mutations that eliminate integrin adhesion to natural substrate had little or no effect on cellular adhesion to invasin or on invasin-promoted uptake, we decided to compare these results to the analysis of other previously characterized mutants within human α chains. Potentially, the higher affinity of invasin for integrin receptors relative to natural substrates means that single amino acid substitutions that affect natural substrate adhesion are not sufficiently drastic to eliminate adhesion to invasin.

Masumoto and Hemler (29) isolated three integrin α₄ substitutions containing the conservative substitutions N283E, D346E, or D408E at position 3, respectively, of the three putative divalent cation binding domains of integrin α₄ previously shown to be defective for natural ligand binding (29) were severely defective for invasin-mediated bacterial uptake (Fig. 8). These results suggest that although ligand recognition may be mediated by the integrin α chain third N-terminal repeat (11–16), mutational change of any single residue in this region results in a receptor with considerable residual invasin binding activity. This is very different from results with natural ligands, in which substitutions of single critical residues eliminate adhesion. Furthermore, sequences in repeat II (Fig. 1) may play an important role in modulating the adhesion activity of the integrin, as a mutation in this repeat simultaneously stimulates adhesion to epiligrin and results in failure to bind a function blocking mAb.

Although the α₃ substitution mutants were much more defective for adhesion to epiligrin than invasin, one derivative, α₃-Y218A, was more susceptible to antibody A3-IV5 inhibition of invasin adhesion than was wild type receptor, consistent with this derivative being reduced in its affinity for invasin. Incomplete antibody inhibition of α₃-W220A and α₃-wt adhesion to invasin is most likely a reflection of the high affinity interaction of invasin with integrins (Fig. 4; see Ref. 4). Similar results were found with the anti-α₃ antibody P1B5 (data not shown).

The unusually strong affinity of integrin binding to invasin probably plays a role in the observed differences in recognition of altered integrin derivatives by invasin and epiligrin. Presumably, an overlapping set of residues on the α₃ chain is required for adhesion to both invasin and natural substrates. Consistent with this hypothesis, cell lines expressing α₃-Y218A showed defective adhesion to both a natural ligand and invasin, and mAb A3-IV5 blocked interaction with both ligands. The high affinity of invasin for integrin receptors has the presumed consequence that single amino acid substitutions eliminating natural substrate adhesion are not sufficiently drastic to greatly affect adhesion to invasin. There are two likely explanations for why adhesion to invasin is more tolerant of such changes. First, during adhesion, invasin may engage more residues on the integrin receptor than do natural ligands. The overall binding energy contributed by these novel contacts may be sufficient to support adhesion to invasin in the absence of residues necessary for adhesion to natural substrates. Second, the residues engaged by invasin and natural substrates may be determined if the small effects on adhesion had strong effects on uptake (see “Materials and Methods”). In contrast to the subtle defect of these mutants in adhesion to invasin-coated microtiter wells, the three α₃ mutants displayed strong defects in α₂β₃-dependent, invasin-mediated bacterial uptake (Fig. 8). The strong defects observed support the model that moderate differences in adhesion can result in more pronounced defects in bacterial uptake (40). These results also indicate that mutations that cause defects in cellular adhesion to natural substrates can cause drastic defects in invasin-mediated bacterial uptake, in contrast to the results with the mutations in repeat III of the α₅ chain.

**DISCUSSION**

The work presented here analyzed the relative effects of substitutions in the integrin α chain on substrate adhesion. Whereas mutations in the third N-terminal repeat of α₃ abolished adhesion to the natural integrin ligand epiligrin (laminin 5; Fig. 3), integrins harboring these changes showed only small defects in adhesion to invasin (Fig. 4) even when tested in the more stringent assay of bacterial uptake (Fig. 5). A control mutation in the analogous region of the second N-terminal repeat, α₃-D154A, gave the surprising result of enhanced adhesion to epiligrin. In contrast, integrins harboring substitutions in any of the three putative divalent cation binding domains of integrin α₄ previously shown to be defective for natural ligand binding (29) were severely defective for invasin-mediated bacterial uptake (Fig. 8). These results suggest that although ligand recognition may be mediated by the integrin α chain third N-terminal repeat (11–16), mutational change of any single residue in this region results in a receptor with considerable residual invasin binding activity. This is very different from results with natural ligands, in which substitutions of single critical residues eliminate adhesion. Furthermore, sequences in repeat II (Fig. 1) may play an important role in modulating the adhesion activity of the integrin, as a mutation in this repeat simultaneously stimulates adhesion to epiligrin and results in failure to bind a function blocking mAb.
identical, but the binding energy contributed by the individual unaltered residues may be greater for adhesion to invasin than to other substrates. As a result, the relative contributions of the mutated residues to invasin adhesion would be less than that observed for adhesion to a natural substrate.

The results presented here suggest that the \( \alpha_3 \beta_1 \) integrin is more active than \( \alpha_5 \beta_1 \) at promoting bacterial uptake. Substitution derivative \( \alpha_3 \)-W220A showed no defect in the invasin adhesion assay, yet it appeared to be attenuated, compared with wild type \( \alpha_3 \), in its competition with endogenous \( \alpha_5 \beta_1 \) for promoting bacterial uptake (Fig. 5). This is consistent with the result that an anti-\( \alpha_5 \) antibody stimulated bacterial uptake in cell lines expressing \( \alpha_5 \)-W220A or \( \alpha_5 \)-Y218A, whereas the wild type derivative showed no stimulation of bacterial uptake by this treatment (Fig. 5). The reduced bacterial uptake by \( \alpha_3 \)-

Y218A and \( \alpha_3 \)-W220A in absence of anti-\( \alpha_5 \beta_1 \) antibody is apparently a by-product of two phenomena. When \( \alpha_3 \beta_1 \) competes with \( \alpha_5 \beta_1 \) for adhesion to invasin, derivatives \( \alpha_3 \)-Y218A and \( \alpha_3 \)-W220A are presumably less efficient competitors of \( \alpha_5 \beta_1 \) than is \( \alpha_3 \)-wt. In addition, bacterial adhesion to \( \alpha_5 \beta_1 \) would be predicted to result in more efficient phagocytosis than adhesion to \( \alpha_3 \beta_1 \). \( \alpha_3 \beta_1 \) has recently been shown to be specifically associated with tetraspan membrane proteins that are involved in a variety of signaling activities (41), whereas other integrins are apparently not associated with these proteins. Perhaps these integrin-associated proteins may provide important signals for invasin-mediated uptake.

All three \( \alpha_3 \) derivatives tested were dramatically defective for invasin-mediated bacterial uptake relative to wild type receptor, indicating that substitution in three different N-terminal repeats affected invasin binding. As the bacterial uptake defect of each of the \( \alpha_3 \) mutants is approximately equal (Fig. 8), this suggests that substitution within any one cation-binding loop is no more detrimental to ligand interaction than another. These three cation binding domains may regulate the structure of the integrin \( \alpha \) subunit, affecting the ligand-binding site at a distance.

It has recently been suggested that the integrin \( \alpha \) chain N-terminal repeats adopt a 7-bladed propeller structure resembling G-protein \( \beta \) subunits (18, 42, 43). According to this model, \( \alpha_3 \) integrin chain residues Tyr-218 and Trp-220 reside on a solvent-exposed surface of the third blade (repeat) of the propeller. The finding that the \( \alpha_5 \) integrin substitution D154A in the second repeat is not recognized by the function-blocking antibody A3-IVA5 (Fig. 2) and stimulates ligand binding is surprising (Fig. 3) but consistent with this model. There are several explanations for the results regarding repeat II. The
second and third repeats may be in sufficiently close proximity to allow a single antibody to bind to the repeats or allow steric interference of ligand binding to repeat III by an antibody bound to the neighboring repeat II. Antibody binding to one domain may also disrupt the structure of its neighbor by causing a conformational change. Additionally, sequences that are proximal in the linear sequence to Asp-154 may be required for ligand interaction. Finally, sequences in repeat II could modulate binding of substrate to repeat III. The D154A mutation may lock the integrin into a high affinity state that allows high efficiency substrate adhesion. In the propeller model, sequences in repeat II could modulate a conformational change. Additionally, sequences that are close to the solvent-exposed surface of the molecule, consistent with each of these scenarios (18). Physical studies on the interaction of this region with substrates will directly test the validity of these models and provide an explanation for the apparent interaction between neighboring repeats.

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REFERENCES
1. Isberg, R. R., and Falkow, S. (1985) Nature 317, 262–264
2. Isberg, R. R., Voorhis, D. L., and Falkow, S. (1987) Cell 50, 769–778
3. Isberg, R. R., and Leung, J. M. (1990) Cell 60, 861–871
4. Van Nhieu, G., and Isberg, R. R. (1991) J. Biol. Chem. 266, 24367–24375
5. Hynes, R. O. (1992) Cell 69, 11–25
6. Loftus, J. C., O'Toole, T. E., Plow, E. F., Glass, A., Frelinger, A. L., III, and Ginsberg, M. H. (1990) Science 249, 915–918
7. Bajt, M. L., and Loftus, J. C. (1994) Arch. Biochem. Biophys. 325–331
8. Bajt, M. L., Goodman, T., and McGuire, S. L. (1995) J. Biol. Chem. 270, 94–98
9. Corbi, A. L., Miller, L. J., O'Connor, K., Larsen, R. S., and Springer, T. A. (1997) EMBO J. 16, 4023–4028
10. Tuckwell, D. S., Hemler, M. E., and Burgeson, R. E. (1991) J. Biol. Chem. 266, 23438–23444
11. Weitzman, D. B., Pausquinini, R., Takada, Y., and Hemler, M. E. (1993) J. Cell Biol. 123, 245–253
12. Arroyo, A. G., Sanchez Mateos, P., Campanero, P. M., Martin Padura, I., Dejana, E., and Sanchez Madrid, F. (1992) J. Cell Biol. 117, E59–E67
13. Saltman, L. H., Lu, Y., Zaharias, E. M., and Isberg, R. R. (1996) J. Biol. Chem. 271, 1361–1368
14. Kamata, T., Irie, A., Tokuhira, M., and Takada, Y. (1996) J. Biol. Chem. 271, 18610–18615
15. Loftus, J. C., Halloran, C. E., Ginsberg, M. H., Feigens, L. P., Zablocki, J. A., and Smith, J. W. (1996) J. Biol. Chem. 271, 2033–2039
16. McKay, B. S., Annis, D. S., Honda, S., Christie, D., and Kunicki, T. J. (1996) J. Biol. Chem. 271, 30544–30547
17. Clackson, T., and Wells, J. A. (1995) Science 267, 383–386
18. Springer, T. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 65–72
19. D’Souza, S. E., Ginsberg, M. H., Burke, T. A., and Plow, E. F. (1990) J. Biol. Chem. 265, 3440–3446
20. Smith, J. W., and Cheresh, D. A. (1990) J. Biol. Chem. 265, 2168–2172
21. Calvete, J., Schauer, W., Mann, K., Henschel, A., and Gonzalez-Rodriguez, J. (1992) Eur. J. Biochem. 206, 759–765
22. Janatova, J., Reid, K. B., and Willis, A. C. (1989) Biochemistry 28, 4754–4761
23. Sottile, J., and Mosher, D. F. (1993) Biochemistry 32, 1641–1647
24. D’Souza, S. E., Ginsberg, M. H., Matsuura, G. R., and Plow, E. F. (1991) Nature 350, 66–68
25. Taylor, D. B., and Gartner, T. K. (1992) J. Biol. Chem. 267, 11729–11733
26. Takada, Y., Elies, M. J., Crouse, C., and Hemler, M. E. (1989) EMBO J. 8, 1361–1368
27. Tuckwell, D. S., Brass, A., and Humphries, M. J. (1992) Biochem. J. 283, 325–331
28. Dransfield, I., Buckle, A.-M., and Hogg, N. (1990) Immunol. Rev. 114, 29–44
29. Masumoto, A., and Hemler, M. E. (1993) J. Cell Biol. 123, 245–253
30. Arroyo, A. G., Sanchez Mateos, P., Campanero, P. M., Martin Padura, I., Dejana, E., and Sanchez Madrid, F. (1992) J. Cell Biol. 117, E59–E67
31. Saltman, L. H., Lu, Y., Zaharias, E. M., and Isberg, R. R. (1996) J. Biol. Chem. 271, 1361–1368
32. Leong, J. M., Fournier, R. S., and Isberg, R. R. (1990) EMBO J. 9, 1979–1989
33. Leong, J. M., Morrissey, P. E., Marra, A., and Isberg, R. R. (1996) EMBO J. 14, 422–431
34. Weitzman, D. B., Pausquinini, R., Takada, Y., and Hemler, M. E. (1993) J. Cell Biol. 120, 8651–8657
35. Pierschbacher, M., Hayman, E., and Ruoslabi, R. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1224–1227
36. Carter, W. G., Kaur, P., Gil, S. G., Gahr, P. J., and Wayner, E. A. (1990) J. Cell Biol. 111, 3141–3154
37. Marinovitch, M. P., Lunstrum, G. P., and Burgeson, R. E. (1992) J. Biol. Chem. 267, 17900–17906
38. Carter, W. G., Ryan, M. C., and Gahr, P. J. (1991) Cell 65, 599–610
39. Wayner, E. A., and Carter, W. G. (1987) J. Cell Biol. 105, 1873–1884
40. Tran Van Nhieu, G., and Isberg, R. R. (1993) EMBO J. 12, 1887–1895
41. Berditchevski, F., Zutter, M. M., and Hemler, M. E. (1996) Mol. Biol. Cell 7, 193–207
42. Lambright, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E., and Sigler, P. B. (1996) Nature 379, 311–319
43. Wall, M. A., Coleman, D. E., Lee, E., Iniguez Lhuillier, J. A., Posner, B. A., Gilman, A. G., and Sprang, S. R. (1995) Cell 83, 1047–1058.