Integrin Activation by Dithiothreitol or Mn\(^{2+}\) Induces a Ligand-occupied Conformation and Exposure of a Novel NH\(_{2}\)-terminal Regulatory Site on the \(\beta_1\) Integrin Chain*  

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Integrins can be expressed in at least three functional states (i.e. latent, active, and ligand-occupied). However, the molecular bases for the transitions between these states are unknown. In the present study, changes in the accessibility of several \(\beta_1\) epitopes (e.g. N29, B44, and B3B11) were used to probe activation-related conformational changes. Dithiothreitol or Mn\(^{2+}\) activation of integrin-mediated adhesion in the human B cell line, IM9, resulted in a marked increase in the exposure of the B44 epitope, while N29 expression levels were most sensitive to dithiothreitol treatment. These results contrasted with the epitope expression patterns of spontaneously adherent K562 cells, where N29 was almost fully accessible and B44 was low. Addition of a soluble ligand resulted in a marked increase in B44 levels, suggesting that this antibody detected a ligand-induced binding site. The N29 epitope was mapped to a cysteine-rich region near the NH\(_{2}\)-terminus of the \(\beta_1\) integrin chain, thus defining a novel regulatory site.  

These studies indicate that the activation of integrin function by different stimuli may involve related but nonidentical conformations. Both Mn\(^{2+}\) and dithiothreitol appear to induce localized conformational changes that mimic a ligand-occupied receptor. This differs from the “physiologically” activated integrins on K562 cells that display a marked increase in overall epitope accessibility without exposure of the ligand-induced binding site epitopes. The increased exposure of the N29 site on K562 cells may indicate a role for this region in the regulation of integrin function.  

Members of the integrin family mediate cellular interactions with elements of their microenvironment (1–3). These contacts can lead to cellular adhesion, migration, and activation (4–6). In a number of cell types, such as platelets and leukocytes, the activities of integrins are tightly regulated such that host cell activation is required before cell binding can proceed (7–9). This prerequisite ensures that integrin function is operative only at the appropriate anatomical or pathological sites.  

Although the structural basis for the underlying changes associated with the acquisition of integrin functionality is unknown, data from a number of different biochemical and immunological approaches clearly demonstrate activation-associated alterations in integrin conformation (10–12). Antibody-binding studies and protease-susceptibility studies have shown that there are activation-associated changes in the accessibility of regions of the complex (10, 11). Fluorescent energy transfer studies on \(\alpha_{1b}\beta_3\) have also demonstrated that there are alterations in the spacing and interaction of \(\alpha_{1b}\) and \(\beta_3\) in the activated integrin structure (12). Changes in epitope expression are also observed following receptor occupancy (13–16). Collectively the data suggest that the activated integrin complex acquires a more open conformation than is observed in the latent structure.  

Recently models of integrin activation have been proposed that involve allosteric mechanisms for the acquisition of an adhesion-competent conformation (17, 18). Support for such a model derives from the observations that the binding of ligand to purified integrin inhibits the binding of an inhibitory antibody to the \(\beta_1\) chain (19). The pattern of inhibition displays characteristics that are most compatible with an allosteric mechanism. However, as pointed out by Mould (17), the situation with the integrins is more complex than a classical allosteric mechanism, as the “active” integrin does not necessarily acquire a conformation that approximates the ligand-bound receptor. Thus the existence of multiple intermediate conformational changes have been suggested.  

Activation of integrin function can be achieved by a variety of stimuli (20–26). Mn\(^{2+}\) and the bifunctional reducing agent, DTT,\(^{1}\) have been shown to activate integrin binding in a number of systems (22–26). Since both of these agents activate purified integrins, it would appear that their effects on adhesion might be directly on the receptor complex (19, 24). These agents may provide useful probes for the analysis of the changes associated with integrin activation and ligand binding.  

We have previously described a panel of regulatory antibodies to the human \(\beta_1\) integrin chain and localized their continuous epitopes (27–29). Three noncompeting groups of antibodies were identified, and one set of antibodies was shown to react with the membrane proximal \(\beta_1\) region (28). The present study localizes a novel stimulatory region to the cysteine-rich amino-terminal portion of the \(\beta_1\) chain. Furthermore, it is demonstrated that it is possible to generate functionally “activated” integrins with overlapping but nonidentical conformations.  

EXPERIMENTAL PROCEDURES  

Materials—Unless otherwise indicated, all chemicals were purchased from Sigma. Media, fetal bovine serum, and GRDS/GRES peptides were obtained from Life Technologies, Inc. Purified human plasma fibronectin was obtained from Chemicon Intl., Temecula, CA. Custom-synthesized peptides were purchased from Research Genetics, Huntsville, AL.  

1 The abbreviation used is: DTT, dithiothreitol.
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Table I

| Antibody       | Activity | Epitope location | Reference |
|----------------|----------|------------------|-----------|
| J1B1/C30B/D11B | Inhibitory| 82–87            | 30        |
| 3S3            | Inhibitory| Discontinuous?   | 31        |
| B3B11          | Stimulatory| 660–668         | 28        |
| N29            | Stimulatory| 15–54            | 28, this paper |
| B44            | Stimulatory| Linear?          | 28        |

![Graph](image)

**Fig. 1.** The relative levels of β1 epitope expression on IM9 cells. IM9 cells were stained with the indicated antibodies and their relative levels of binding expressed as a percentage of J1B1A MF1 levels. The average and ranges of two representative experiments are provided.

**Antibodies**—The production, properties, and purification of the antibodies to β1 (Table I), J1B1A (30), B3B11, B44, N29 (28), and 3S3 (31) have been previously described in detail. Dr. C. Damsky provided the anti-β1 AIIb2 (32).

**Cells and Culture**—The human cell lines IM9 (B cell), Jurkat (T leukemia), and K562 (erythroleukemia) were obtained from the ATCC. They were maintained in RPMI 1640 supplemented with 10% fetal bovine serum.

**Cell Binding Assay**—The assays were performed as described previously (28). Nontissue culture treated microtiter wells were coated with purified plasma fibronectin (5 μg/ml) in bicarbonate buffer at 4 °C overnight. The wells were washed and blocked with 1% bovine serum albumin in RPMI. In studies involving Mn2+, the cells were washed and resuspended in Puck’s saline A alone or in the presence of the indicated concentration of cation. When cells were pretreated with DTT, they were washed to remove the DTT prior to their addition to the binding assays.

Cells were preincubated with the indicated stimuli for 30 min at room temperature and then added (2 × 105 cell/well) to the coated wells and incubated for 60 min at 37 °C. The nonadherent cells were removed by centrifugation of the inverted plates for 5 min at 700 g, and the supernatants were removed. The adherent cells were stained for 60 min with 0.5% crystal violet in a 30% solution of methanol in water. The plates were washed with tap water to remove unbound dye. The residual dye was solubilized in methanol, and the absorbance at 550 nm was determined. In all assays the adherence to bovine serum albumin (OD < 0.1) was subtracted from the values obtained for the fibronectin or antibody coated wells. Unless indicated otherwise, all assays were performed at least three times in sextuplicate.

**Flow Cytometry Analysis**—Cells were preincubated with the indicated stimuli at room temperature and then incubated with the indicated antibody (5 μg/ml) for 30 min at 37 °C. The cells were washed twice with phosphate-buffered saline and incubated for 60 min at 4 °C with a fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin (Chemicon). All assays included cells incubated with the second antibody alone as a control for nonspecific binding. Fluorescence analysis was performed with a BD FACScaliber.

For the studies involving ligand binding to K562, the cells were preincubated with the indicated peptides (1 mM) or fibronectin (100 μg/ml) for 1 h at room temperature. Antibodies were then added to this mixture for 30 min at 37 °C, and the cells were processed for fluorescence-activated cell sorter analysis as described above.

**Fig. 2.** The induction of IM9 adherence to fibronectin following treatment with either Mn2+ (A) or DTT (B). IM9 cells were incubated with the indicated concentration of stimulus and tested for adherence to immobilized fibronectin. Representative results of one of four independent experiments are shown. The standard errors for all samples were less than 15%.

**Epitope Library Production and Screening**—Libraries were constructed using the Novatope System (Novagen Inc.) according to the manufacturer’s instructions. The method based on the use of modified pET vectors for the expression of β1-T7 gene 10 fusion proteins consisted of digesting pFnRj1 (33) with DNsae I in the presence of Mn2+ and size fractionating the random fragments. The 250–350-base pair fragments were flush ended with T4 DNA polymerase, single dA tailed and ligated into the EcoRV site of the pTOPE-1b(+)-plasmid. Novabase (DE3) cells were transformed with the plasmid, and colonies were immunoscreened with anti-β1 monoclonal antibodies and an alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin. Positive colonies were subcloned and examined for reactivity with the antibodies. The inserts from individual colonies were sequenced using T7 gene primes. The inserts were then aligned with the β1 chain 5′ primer pair GTGAAATTCATATGCAAACAGATGAAAATAG. The 3′ primer pair GTGAAATTCATATGCAAACAGATGAAAATAG was also designed to amplify the β1 gene sequence. The resulting insert was codified to code for residues Gin5 through Pro22 of the mature β1 chain. The corresponding fusion protein was expressed in competent Escherichia coli BLR(DE3)plyss strain, purified with Ni2+ columns, and...
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**RESULTS**

Differential Expression of \( \beta_1 \) Epitopes on IM9 Cells—A comparison of the binding levels of a panel of anti-\( \beta_1 \) monoclonals to IM9 cells indicated that there were marked differences in their levels of expressions (Fig. 1). A calculation of their expression levels relative to the total \( \beta_1 \) expression detected by JB1A or C30B indicated that B3B11, B44, and N29, respectively, were present on 18, 2, and 10% of the integrins. Previous studies had determined that these antibodies recognized continuous epitopes in the nonpolymorphic extracellular domain of the \( \beta_1 \) chain (28). Thus it appeared that their low expression levels were indicative of a sequestration of the regions containing these epitopes. As IM9 cells express \( \alpha_4 \beta_1 \) but do not spontaneously adhere to fibronectin, it was speculated that the negative correlation of expression of B44, B3B11, and N29 epitopes with adhesive function might indicate that they were reporters of integrin activity.

Treatment of the cells with \( \text{Mn}^{2+} \) or DTT resulted in a marked increase in adherence. The half-maximal stimulatory concentration for \( \text{Mn}^{2+} \) was 70 \( \mu \text{M} \) (Fig. 2A). The situation with DTT was somewhat more complex with half-maximal activity at 2–5 mM and a loss in adhesion at concentrations in excess of 50 mM (Fig. 2B). The adhesion induced by both stimuli was inhibited by more than 60% by anti-\( \alpha_4 \) and anti-\( \beta_1 \), suggesting that \( \alpha_4 \beta_1 \) was mediating a significant proportion of the induced binding (data not shown). Neither of the stimuli caused any change in total \( \beta_1 \) levels, indicating that the adhesive changes related to altered integrin activity rather than increases in expression levels (Figs. 3 and 4).

Cells treated with \( \text{Mn}^{2+} \) displayed a 40–50-fold increase in the levels of B44 expression such that 30–40% of the \( \beta_1 \) displayed this epitope (Fig. 3). Although there was a doubling of the N29 levels, the majority of integrins did not express this epitope. The binding of B3B11 and 3S3 were relatively unaffected under these conditions. DTT treatment caused a comparable increase in the level of B44 binding (Fig. 4). However, unlike the case for the \( \text{Mn}^{2+} \)-treated cells, there was almost 100% exposure of the N29 epitope and a small increase in B3B11 binding.

\( \beta_1 \) Epitope Expression Patterns on K562 Cells—The results of the above studies supported the concept that the expression of the B44 and possibly of the N29 epitope might relate to the activational status of the integrin. As an approach to testing this possibility K562 cells were examined for their antibody binding patterns. These cells spontaneously adhere to fibronectin, and their receptors have been shown to be in an intermediate affinity state (15). There were increases in the proportions of N29 and B3B11 expressed on these cells (Fig. 5). However, there was almost a complete absence of B44 binding, suggesting that this antibody was not a marker of integrin functionality.

Preliminary studies had indicated that \( \text{Mn}^{2+} \) induced the expression of the B44 epitope on K562 cells. Since it had been

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**Fig. 3. The effects of Mn\(^{2+}\) treatment of IM9 cells on \( \beta_1 \) epitopes expression.** Cells were treated with Mn\(^{2+}\) (1 mM), stained with the indicated antibodies and analyzed by flow cytometry. The control, untreated, and Mn\(^{2+}\)-treated cell profiles are represented by the dashed, solid, and dotted lines, respectively. The arrows indicate the profiles of the Mn\(^{2+}\)-treated cells.
observed that this cation could induce conformational states which resembled those of a ligand-occupied integrin, the effects of ligand binding on B44 expression were examined. Treatment of the cells with fibronectin or RGDS-containing peptides resulted in a 2–3-fold increase in the B44 levels (Fig. 5). The expression levels of the other epitopes were not significantly changed by this treatment. Furthermore, the control peptide RGES did not induce these changes, indicating that the effects were specific to integrin ligands.

Location of the N29 Epitope—A \( \beta_1 \) epitope library was screened with N29 and B44 and a single N29 reactive clone, B105, was identified. DNA sequencing of B105 indicated that this clone contained the first 105 residues of the \( \beta_1 \) chain (data not shown). Previous studies had determined that the JB1A epitope consisted of residues 82–87 and that a panel of monoclonal antibodies to the \( \beta_1 \) chain including N29 did not react with fusion proteins containing a fragment spanning residues 55–105 (29).

Expression of \( \beta_1 \) residues 1–57 as a fusion protein resulted in a product that was reactive with N29 under reducing conditions (Fig. 6). In contrast, N29 did not react with a gonococcal porin (1b) fusion protein expressed in the same vector system. The specificity of the reaction was further demonstrated by the fact that N29 but neither B3B11 nor JB1A reacted with the fusion protein (Fig. 7A). Furthermore, preincubation of N29 with the purified 1–57 fragment specifically inhibited the binding of N29 to purified \( \beta_1 \) (Fig. 7B).

Honda et al. (40) have described a stimulatory antibody to \( \beta_3 \), AP-5, which recognized a cation-sensitive epitope containing residues 1–6 of the \( \beta_3 \) integrin (34). It was therefore questioned if the N29 epitope might be located in a homologous region of the \( \beta_1 \) chain. Pretreatment of N29 with a synthetic peptide containing residues 1–14 did not influence the ability of the antibody to bind to purified \( \beta_1 \) (Fig. 7B). These results indicate that the N29 epitope was located between residues 15 and 54 of the \( \beta_1 \) chain.

**DISCUSSION**

The present studies provide several new pieces of data relevant to integrin activation. 1) The stimulatory antibody, N29, recognizes a new regulatory region located near the NH2 terminus of the \( \beta_1 \) molecule. 2) The stimulatory antibody, B44, identifies an epitope, which is exposed on ligand binding. 3) Mn\(^{2+}\) and DTT induce changes in \( \beta_1 \) epitope accessibility, which resemble those observed in the ligand occupied receptor. 4) The overall accessibility of epitopes in physiologically active integrins is increased relative to those on nonadherent cells or on Mn\(^{2+}\) and DTT-activated cells.

The initial assumption that N29 might identify an activation epitope does not appear to be fully supported by the results of this study. In the case of DTT-treated cells, there was an almost total exposure of the N29 epitope associated with activation of adhesion. However, the N29 levels on spontaneously adhesive cells such as K562 and Jurkat \(^2\) or following treatment with Mn\(^{2+}\) were elevated 2–4-fold, such that 20–30% of the \( \beta_1 \) integrins displayed this epitope. There were also low but detectable levels of N29 exposure on nonadherent cells. Thus the correlation between integrin functional status and N29 accessibility appeared to be semiquantitative rather than a qualitative one.

\(^2\) H. Ni and J. A. Wilkins, unpublished results.
The antibody B44 identifies an epitope, which under normal conditions appears to be of very limited accessibility. Thus the expression levels of this epitope on adhesion competent cells such as Jurkat and K562 are significantly lower than the total integrin levels. However, occupancy of integrin by ligand or by an RGD-containing antagonist results in a marked increase in B44 expression. The B44 epitope is reduction resistant under SDS-polyacrylamide gel electrophoresis conditions, implying that the antibody detects a continuous peptide sequence. It appears that ligand binding exposes the cryptic epitope to the solvent and renders it antibody accessible. However, it is unlikely that this epitope represents a ligand contact site as B44 binding has been shown to induce adherence in Jurkat cells (28). The properties of B44 most resemble those of two other antibodies, 15/7 (35) and HUTS-21 (36), which detect integrins in a ligand-occupied or high affinity state. These antibodies have been shown to react with epitopes that are located in the cysteine rich region of the β1 (residues 355–425). However, to date it has not been possible to determine the location of the B44 epitope.

Fig. 5. The effects of ligand binding on the β1 epitope expression of K562 cells. Cells were treated with fibronectin or RGDS/RGES peptides, and stained with the indicated antibodies. The panel labeled B44* indicates the line patterns used for each treatment group. Note that the negative control cells are omitted from this panel for clarity. Control binding is indicated in all other panels by the line with large dashes.

The treatment of IM9 cells with Mn\textsuperscript{2+} induces B44 epitope expression. The implication is that the Mn\textsuperscript{2+} induces alterations that resemble those caused by ligand binding to a competent integrin. It has been suggested that Mn\textsuperscript{2+} may stimulate adhesion by forming a co-ordination complex with residues in the cation binding domains of the integrin and the aspartate residue of the ligand (37, 38), or by facilitating the ligand entry to the binding site via an exchange mechanism (18). Recently it has been proposed that Mn\textsuperscript{2+} may induce a conformation resembling the ligand occupied receptor thus permitting ligand access to the binding region of the integrin (17). The binding pattern of B44 is compatible with the latter explanation of Mn\textsuperscript{2+} action. However, it does not address the issue of the relative contributions of Mn\textsuperscript{2+} to cation-facilitated exchange and ligand co-ordination.

Activation of adhesion by reducing agents has also been
described in several systems. Edwards et al. (25) noted that there was an obligate requirement for a bifunctional thiol with a minimal spacing of four carbons between the two –SH groups. Early studies on the activation of platelet adhesion by DTT indicated that there were changes in $\alpha_{IIb}\beta_3$ electrophoretic mobility associated with activation by this agent (26). The DTT-dependent activation of mutant $\alpha_{IIb}\beta_2$ in platelets from a patient with Glanzmann’s thrombasthenia by DTT was shown to be associated with the appearance of activation epitopes (39). However, DTT-induced activation of $\alpha_L\beta_2$ mediated adhesion of natural killer cells to intercellular adhesion molecule 1-expressing target cells failed to reveal conformational changes using two reporter antibodies (25). Furthermore, these authors could not demonstrate the appearance of free thiol groups in the $\alpha_L\beta_2$ complex, implying that the integrin chains were not directly modified by DTT treatment (25). The present data clearly indicate that significant conformational changes are induced by DTT as access to the B44 and the N29 epitopes are markedly increased.

The increased B3B11 and N29 expression on K562 cells implies that physiologically activated integrins undergo changes that allow an increased accessibility to the membrane proximal and NH$_2$-terminal regions of the molecule. Although the integrins on these cells are in an adhesion competent state, ligand binding is required for B44 epitope expression. These results would seem to suggest that there is an intermediate conformation in which the integrin is adhesion-competent but unoccupied. The fact that agents such as Mn$^{2+}$ and DTT induce conformations that resemble the ligand-occupied state suggests that they stimulate adhesion competence by generating integrin intermediates that are distinct from the native active forms observed in K562. Although different functional forms of integrins have been described or postulated (16, 17, 40), it is unclear at this point whether active forms such as those induced by DTT or Mn$^{2+}$ are representative of physiological integrin intermediates. These observations suggest that caution should be exhibited when attempting to correlate competent states induced by these agents with those found in physiologically activated integrins.

The localization of the N29 epitope between residues 15 and 54 places it in a highly conserved cysteine-rich region (41). This area has not previously been identified as a regulatory site, although it is adjacent to region that has been shown to be a cation and ligand sensitive in the $\beta_3$ chain (34). Unlike the $\beta_3$ situation, the binding of N29 is relatively insensitive to the cationic composition of the extracellular milieu. Thus if a homologous region exists in the $\beta_1$ chain it would appear that it is not located in the N29 reactive 15–54 sequence of the molecule.

The antibodies N29, B44, and B3B11/JB1B were originally identified because of their abilities to stimulate Jurkat adher-
ence to collagen and fibronectin (28). It is noteworthy that in those cases where their corresponding epitopes are identified (28, 29), the stimulatory epitopes map to regions that are in close proximity to residues that are predicted to be involved in disulfide bonds between sequentially distant cysteines (i.e. Cys7–Cys415 and Cys444–Cys671). The present results extend those of others employing interspecies sorter analysis.

The differentiation of these possibilities. Studies with purified integrin may permit in the patterns of integrin-associated proteins, or to both of these mechanisms. Activation, the basis for these changes are unknown. They could relate to integrin conformational changes, to alterations in the accessibility of previously buried residues. It is important to bear in mind that, although the results of the antibody studies indicate changes in the accessibility of β1 integrin epitopes following activation, the basis for these changes are unknown. They could relate to integrin conformational changes, to alterations in the patterns of integrin-associated proteins, or to both of these mechanisms. Studies with purified integrin may permit the differentiation of these possibilities.

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REFERENCES
1. Hynes, R. O. (1992) Cell 69, 11–25
2. Clark, E. A., and Brugge, J. S. (1995) Science 268, 233–239
3. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Ann. Rev. Cell Dev. Biol. 11, 549–599
4. Sjaastad, M. D., and Nelson, W. J. (1997) Bioessays 19, 47–55
5. Haazenberger, D., Klominek, J., Holgersson, J., Bergstrom, S.-E., and Sundqvist, K.-G. (1997) J. Immunol. 158, 76–84
6. Pytel, R., Pierschbacher, M. D., and Rusoalhti, E. (1985) Cell 40, 191–198
7. Marburger, G. A., and Plow, E. F. (1981) Biochemistry 20, 1074–1080
8. Wright, S. D., and Meyer, B. C. (1986) J. Immunol. 136, 1759–1764
9. Shimizu, Y., Van Seventer, G. A., Morgan, K. J., and Shaw, S. (1990) Nature 345, 250–253
10. Calvette, J. J. (1994) Thromb. Haemostasis 72, 1–15
11. Calvette, J. J., Mann, K., Schafer, W., Fernandez-Lafuente, R., and Guisan, J. M. (1994) Biochem. J. 298, 1–7
12. Sims, P. J., Ginsberg, M. H., Plow, E. F., and Shattil, S. J. (1991) J. Biol. Chem. 266, 17106–17111
13. Frelinger, A. L. III, Du, X., Plow, E. F., and Ginsberg, M. H. (1991) J. Biol. Chem. 266, 17106–17111
14. Mould, A. P., Gauntt, A. N., Askari, J. A., Akiyama, S. K., and Humphries, M. J. (1995) FEBS Lett. 363, 118–122
15. Fault, R. J., Kovach, N. L., Harlan, J. M., and Ginsberg, M. H. (1993) J. Cell Biol. 121, 155–162
16. Bazzoni, G., Shih, D.-T, Buck, C. A., and Hemler, M. E. (1995) J. Biol. Chem. 270, 25570–25577
17. Mould, A. P. (1996) J. Cell Sci. 109, 2613–2618
18. Lee, O. J., Rieu, P., Arnautou, M. A., and Liddington, R. (1995) Structure 3, 1333–1340
19. Mould, A. P., Akiyama, S. K., and Humphries, M. J. (1996) J. Biol. Chem. 271, 20365–20374
20. Wilkins, J. A., Stupack, D. G., Stewart, S., and Caixia, S. (1991) Eur. J. Immunol. 21, 517–522
21. Chan, B. M., Wong, J. G., Rao, A., and Hemler, M. E. (1991) J. Immunol. 147, 398–404
22. Dransfield, I., Cabanas, C., Craig, A., and Hogg, N. (1992) J. Cell Biol. 116, 219–226
23. Elices, M. J., Urry, L. A., and Hemler, M. E. (1991) J. Cell Biol. 112, 169–181
24. Davis, G. E., and Camarillo, C. W. (1993) J. Immunol. 151, 7138–7156
25. Edwards, B. S., Curry, M. S., Southon, E. A., Chong, A. S.-F., and Graf, L. H. (1995) Blood 86, 2288–2301
26. Ferschmehl, R. E. (1995) Thromb. Haemostasis 73, 862–867
27. Stupack, D. G., Shen, C., and Wilkins, J. A. (1994) Cell. Immunol. 155, 237–245
28. Wilkins, J. A., Li, A. N., Shih, D.-T, and Shen, C. (1996) J. Biol. Chem. 271, 3046–3051
29. Ni, H., and Wilkins, J. A. (1997) Cell Adhes. Commun., in press
30. Stupack, D. G., Stewart, S., Carter, W. G., Wayner, E. A., and Wilkins, J. A. (1991) Scand. J. Immunol. 34, 761–769
31. Gao, J. X., Wilkins, J. A., and Issekutz, A. C. (1995) Cell. Immunol. 163, 178–197
32. Brown, D. L., Phillips, D. R., Danasky, C. H., and Char, J. S. (1989) J. Clin. Invest. 84, 366–370
33. Gianetti, P. G., and Rusoalhti, E. (1990) Cell 60, 849–859
34. Honda, S., Taniyama, Y., Pelletier, A. J., Matsu, T., Honda, Y., Orchekowski, R., Ruggeri, Z., and Kunicki, T. J. (1995) J. Biol. Chem. 270, 11947–11954
35. Puzon-McLaughlin, W., Yednock, T. A., and Takada, Y. (1996) J. Biol. Chem. 271, 16580–16585
36. Luque, A., Gomez, M., Puzon, W., Takada, Y., Sanchez-Madrid, F., and Cabanas, C. (1996) J. Biol. Chem. 271, 11067–11075
37. Bergelson, J. M., and Hemler, M. E. (1995) Curr. Biol. 5, 615–617
38. D'Souza, S. E., Haas, T. A., Pietrowicz, R. S., Byers-Ward, V., McGrath, D. E., Soule, H. R., Cierniewski, C., Plow, E. F., and Smith, J. W. (1994) Cell 78, 659–667
39. Kouns, W. C., Steiner, B., Kunicki, T. J., Mong, S., Jutzi, J., Jennings, L. K., Cazenave, J.-P., and Lanza, F. (1994) FEBS Lett. 363, 118–122
40. Takeda, Y., and Puzon, W. (1993) J. Biol. Chem. 268, 17597–17601
41. Fault, R. J., Wang, J., Leavensley, D. J., Puzon, W., Ruse, G. R., Vestweber, D., and Takada, Y. (1996) J. Biol. Chem. 271, 25099–25106
42. Shih, D.-T, Edelman, J. M., Horowitz, A. F, Grunwald, G. B., and Buck, C. A. (1993) J. Cell Biol. 122, 1361–1371
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