The functional status of an integrin depends on the conformation of its extracellular domain, which is controlled by the cell expressing that receptor. The transmission of regulatory signals from within the cell is considered to be via propagated conformational changes from the receptor’s cytoplasmic tails to the extracellular ligand binding “pocket.” The end result is increased accessibility of the ligand binding pocket in the high affinity (“active”) form of integrins. We report an increased accessibility of the ligand binding pocket in the cysteine-rich repeats in the integrin αβ1 chain. The QE.2E5 epitope is located approximately 200 residues both from the predicted binding site for fibronectin and from the epitopes recognized by other activating anti-β1 monoclonal antibodies. It is also expressed on β1 integrins from a number of nonhuman species. Although they have the same functional effects, the binding of QE.2E5 and another activating antibody (8A2) to the receptor have contrasting effects on the expression of an activation-dependent epitope in the β1 chain. We propose that the cysteine-rich repeats contain a regulatory region that is distinct from those previously described in the integrin β1 chain.

The integrins are a supergene family of membrane-spanning αβ heterodimers that recognize multiple ligands (1, 2). The subgroup of β1 integrins are predominantly receptors for extracellular matrix molecules (fibronectin, collagen, and laminin), although one (α4β1) is also a receptor for the membrane-bound vascular cell adhesion molecule-1 (3). The site of ligand binding in integrins maps close to the amino-terminal end of the integrin molecule, and the α and β chains each contribute to ligand binding (4–8). Electron microscopy images (9–11) show that ligand physically associates with the complex globular head of the receptor, which is made up of the amino termini of the α and β chains. This region is attached to the cell via the trailing carboxyl ends of each chain and projects a substantial distance (greater than 10 nm) away from the cell surface.

A distinctive characteristic of integrins is that their affinity for ligands is precisely regulated. This has been directly demonstrated for some (α5β1, αLβ2, αMβ2, and αIIbβ3) using soluble ligand binding assays (12–21), and it is probably a general property across the family. It is considered that cells are able to alter the conformation of an integrin’s external domain when appropriately stimulated, which in turn renders the ligand binding “pocket” more accessible to macromolecular ligands (22–25). The process whereby cells convert intracellular signaling events into changes in the physical properties of receptors has been termed “inside-out signaling” (2). Conversely, engagement of ligand with integrin generates signals that are transmitted to the cell’s interior, a process known as “outside-in signaling” (2). These signals appear to be transmitted along the integrin molecule via propagated conformational changes, which convert physical alterations in the cytoplasmic tails into functional changes in the ligand binding region and vice versa. The β chains of the integrins have a key role in this propagation of signals and in the status of receptor function. Mutations within the predicted ligand binding domain of the β chain (residues 119 and 214 in β3 and 130 in β1) render the receptor inactive and unable to be activated (26–29). A mutation in the cytoplasmic tail of β3 also interferes with receptor activation (30), as does complete removal of the tail (31). Further insights into receptor activation and signal propagation have been provided by characterization of anti-β1 chain monoclonal antibodies (mAbs),1 which directly activate receptor function (32–38). Some of these against the integrin β3 chain recognize epitopes that are induced by receptor interaction with ligand (or ligand-mimetic peptides) (32–34). In other words, they recognize conformation-sensitive epitopes, and in turn, their binding induces an activating conformational change in the receptor (or stabilizes the active conformation of the receptor). The binding site of one of these antibodies has been mapped to a region adjacent to the transmembrane portion of the β chain, which is a significant distance from the ligand binding site (39). Hence the binding of this antibody induces a long-distance conformational shift that may mimic the changes occurring during physiological receptor activation.

Several activating anti-β1 mAbs recognize epitopes in the predicted ligand binding region within several residues of the epitopes of a number of mAbs that inhibit receptor function (40). This suggests that subtle conformational changes within the ligand binding pocket induce profound alterations in receptor function. We now report an activating anti-human β1 mAb (QE.2E5) that defines a novel regulatory domain in the β1 chain. Its binding site is located within or adjacent to a series

---

1 The abbreviations used are: mAb, monoclonal antibody; CHO, Chinese hamster ovary.
Novel Activating Anti-β1 Integrin Antibody

of well conserved cysteine-rich repeats and is at least 194 amino acid residues from the predicted ligand-binding domain. The antibody also binds to and activates β1 integrins from other species, suggesting that this region may have been conserved because it is crucial to normal receptor function.

MATERIALS AND METHODS

Antibodies—The murine anti-human β1 mAb SA2 (36) and A1A5 (41) were the gifts of Dr. Nicholas Kovač (University of Washington, Seattle, WA) and Dr. Martin Hemler (Dana-Farber Cancer Institute, Boston, MA), respectively. The rat anti-human β1 mAb A11B2 (42) was a gift from Dr. Caroline Damsky (University of California, San Francisco, CA). The murine anti-human αβ1 mAb PHM2 (43) was a gift from Professor Robert Atkins (Monash Medical Centre, Melbourne, Australia). PHM2 immunoprecipitates material with the migration characteristics of αβ1 (SDS-polyacrylamide gel electrophoresis), inhibits αβ1-mediated cell adhesion, and does not compete with QE.2E5 for binding to β1 integrins.6 The murine anti-human fibronectin mAb Fn-11 (4) was a gift from Dr. Ron Bowditch (The Scripps Research Institute, La Jolla, CA). Fn-11 blocks αβ1-mediated adhesion to fibronectin. The rat mAb 9EG7 recognizes an activation epitope on the integrin β1 chain (45).

Production and Characterization of QE.2E5—The mAb QE.2E5 was found during a search for novel activation antigens on human endothelial cells. Balb/c mice were immunized with cultured human umbilical vein endothelial cells that had been stimulated with lipopolysaccharide. Hybridomas were then selected by fusion with the SP2/Ag14 murine cell line using standard techniques (46), and 450 hybridomas were produced by fusion of mouse splenocytes with the Sp2/0-Ag14 cell line and SP2/0-Ag14 murine myeloma cell line were purchased from American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 plus 10% fetal calf serum.

Cells and Cell Culture—Human umbilical vein endothelial cells were harvested by collagenase (Sigma) treatment of umbilical cord veins. Cells were grown and maintained in RPMI 1640 (Flow Laboratories, McLean, VA) supplemented with 20% fetal calf serum (Life Technologies, Inc.), 0.3 mg/ml endothelial cell growth factor (Sigma) and 30 units/ml porcine mucous heparin sodium (Fisons, Thornleigh, New South Wales, Australia) in 5% CO2. The K562 human erythroleukemic cell line and SF2/0-Ag14 murine myeloma cell line were purchased from the American Type Culture Collection (Walkersville, MD), and maintained in RPMI 1640 plus 10% fetal calf serum.

Mononuclear cells were isolated from EDTA-anticoagulated blood drawn from laboratory pigs, sheep, rabbits, and rats by centrifugation over ficoll-hypaque (Pharmacia) for 400 x g for 30 min at 22 °C.

Flow Cytometry—Anticoagulated blood (0.5 x 106 to 1.0 x 106 white blood cells) or K562 cells (0.5 x 106 cells in phosphate-buffered saline) were incubated for 10 min at 22 °C with the appropriate mAb (10 μg/ml) and then washed twice with phosphate-buffered saline containing 1% fetal calf serum and resuspended in 200 μl of 1:200 dilution of fluorescein-conjugated IgG fraction of sheep antibody to mouse or rat IgG (depending on species of origin of primary antibody) (Silenus Laboratory, Victoria, Australia). After incubation for 10 min at 22 °C, red blood cells were lysed with lysis buffer (0.1% NaHCO3, and 0.0037% EDTA, w/v). Cells for analysis were washed twice with phosphate-buffered saline containing 1% fetal calf serum, fixed with 1% buffered formalin, and analyzed on a FACSscan cytometer (Becton Dickinson, Mountain View, CA). Flow cytometric analysis of mononuclear cells was performed by gating on the appropriate cell populations.

Production of Human Fibronectin—Fibronectin was isolated from fresh human cremated plasma by affinity chromatography on gelatin-Sepharose (Pharmacia), as described previously (12). The isolated fibronectin yielded a single band on SDS-polyacrylamide gel electrophoresis under nonreducing conditions and a closely spaced doublet of 215,000 and 230,000 daltons under reducing conditions, consistent with the reported properties of plasma fibronectin (47). The concentration of fibronectin was determined by a modified assay at 280 nm (1 mg/ml = 1 A 1.3 (12)).

Radiolabeling of Protein—The fibronectin was labeled with 125I by a modified chloramine-T procedure, as described previously (12). Greater than 90% of the radioactivity was precipitated by 10% trichloroacetic acid. Aliquots were stored at -70 °C following the addition of bovine serum albumin to 1% final concentration. The mAb 9EG7 was labeled with 125I as described previously (12), and aliquots stored at -70 °C.

Soluble Ligand Binding Assays—Binding assays using either 125I-fibronectin or 125I-9EG7 were performed as described previously (12). The standard buffer used in the assays was modified Tyrode’s buffer (150 mM NaCl, 2.6 mM KCl, 12 mM NaHCO3, 1 mM bovine serum albumin, 0.4 mM MgCl2, pH 7.4). The time was 30 min at 37 °C. Preliminary studies showed that maximal antibody and fibronectin binding occurred within this time frame. A typical binding assay, performed in a 1.5-ml microcentrifuge tube (Greiner International, Kremsnitter, Austria), was in a 200-μl volume composed of 120 μl of cells (2 x 106 per tube), 40 μl of radiolabeled protein, and 40 μl of stimulus (QE.2E5) and/or inhibitor (EDTA, excess cold protein, or mAb). Following the incubation, 50-μl aliquots were layered in triplicate onto 300 μl of 20% sucrose in the same buffer in microcentrifuge tubes (Eppendorf, Hamburg, Germany) and centrifuged for 3 min at 12,000 rpm in a Beckman 1 Microfuge (Beckman Instruments). The tips were amputated and counted, and the bound protein was calculated from the specific activity of the ligand (assuming a molecular weight for fibronectin of 440,000 and of 9EG7 of 150,000). The residual bound radioactivity in the presence of 5 mM EDTA (for fibronectin) or 50× excess cold 9EG7 was subtracted as nonspecific binding. The data was fitted to equilibrium binding models using the LIGAND program (48).

Assays of Cell Adhesion to Immobilized Fibronectin—Cell adhesion to immobilized fibronectin was quantified as described previously (12). Fibronectin (50 μg/well) at the concentrations indicated in the text was coated onto a 96-well Immulon II plate (DYNATECH Laboratories, Inc., Chantilly, VA) for 2 h at 37 °C, and then the wells were blocked with 1% bovine serum albumin in phosphate-buffered saline for 1 h at 37 °C. The buffer used in the adhesion assay was the same as in the binding assays. At the commencement of the assay, the cells (concentration, 1 x 106 K562 cells/ml or 3 x 106 mononuclear cells/ml) were added to the wells in 100-μl aliquots with appropriate concentrations of stimulus or inhibitor. Following a 30 min incubation at 37 °C, the nonadherent cells were washed off with three rounds of gentle pipetting. The residual adherent cells were checked by visual inspection and quantified with a colorimetric reaction using endogenous cellular acid phosphatase activity (49). Optical densities were determined using an enzyme-linked immunosorbant assay plate reader (TiterTek Multiskan MCC) with a 410 nm filter. Background values, determined in wells coated with 1% bovine serum albumin alone, were subtracted from each point. Adherence was expressed as a percentage of the number of cells originally added to each well and was determined from a standard curve generated using known numbers of cells.

Epitope Mapping of QE.2E5—Epitope mapping was performed using Chinese hamster ovary (CHO) cells expressing human/mouse chimeras of the integrin β1 chain, as we have previously described (40). The human β1 cDNA clone was cloned from a human endothelial Agt11 library with partial β1 cDNA as a probe. The partial mouse β1 cDNA was cloned from a mouse embryonic Agt11 cDNA library. Polymerase chain reaction was used to create novel restriction sites at the boundaries to facilitate gene fusion (Nsil, Stul1, and BgII sites were used for the h354/m, h425/m, and h587/m β1 chimeras, respectively). The cDNA fragments obtained by polymerase chain reaction were verified by DNA sequencing. Fused cDNAs were subcloned into the expression vector pBl1 and transfected into CHO cells by electroporation. Stable clones

2 R. Faull, unpublished studies.
were selected using G418 (Life Technologies, Inc.), and cells expressing the highest level of wild-type or chimeric β1 were selected by cell sorting using an anti-β1 mAb. Antibody reactivity with each cell clone was quantified by flow cytometry and expressed as % positive cells.

**RESULTS**

The **mAb QE.2E5 Binds to and Activates β1 Integrins**—The extensive studies described under “Materials and Methods” characterized QE.2E5 as a mAb directed against the human β1 chain. Further confirmation is shown in the first part of Table I, which includes flow cytometric data used in the mapping of the QE.2E5 epitope (see below). The antibody does not bind to untransfected CHO cells but binds to cells that have been transfected with cDNA of the human β1 chain. The binding of A1A5 to these cells confirms expression of the human β1 chain.

The functional effects of this anti-β1 mAb were then explored using assays of cell adhesion to immobilized fibronectin. Most of these experiments used the erythroleukemic cell line K562, which has the high affinity fibronectin receptor α5β1 (12) as its only β1 integrin. Preliminary studies showed that QE.2E5 bound to the α5β1 on K562 cells with a $K_d$ of approximately 4 nM and that the binding was neither temperature- nor cation-dependent. Saturation (~40,000 molecules/cell) was reached at a concentration of 20 nM. The same concentration of QE.2E5 significantly increased the adhesion of K562 cells to purified human fibronectin (Fig. 1), as did the anti-β1 mAb 8A2, which has been well characterized as an activating antibody (36, 12). The enhancing effect of both antibodies is clearest at lower concentrations of the immobilized fibronectin. The facts that (a) there was no additional effect with higher concentrations of QE.2E5, and (b) the concentration giving half maximal effect (~1 nM) (not shown) approximated the $K_d$ both suggested that direct binding of QE.2E5 to a receptor was necessary for receptor activation.

| Antibody | CHO human wt$^a$ | h587/m | h425/m | h354/m |
|----------|------------------|--------|--------|--------|
| QE.2E5   | 1.0              | 96.3   | 95.8   | 2.41   | 1.71   |
| A1A5     | 1.5              | 96.9   | 97.7   | 98.9   | 97.4   |
| mlG      | 0.9              | 3.4    | 1.4    | 2.4    | 1.7    |

$^a$ wt, wild type; mlG, control purified mouse IgG.

To test the hypothesis that QE.2E5 increases K562 cell adhesion to fibronectin by activating α5β1, the binding of $^{125}\text{I}$-labeled human fibronectin to K562 cells was measured using a soluble ligand binding assay. Preliminary studies confirmed the previous observation (12) that soluble fibronectin binds with low affinity to the “resting” α5β1 on these cells ($K_d$ ~1 μM) (Fig. 2A). In the presence of 20 nM of QE.2E5, considerably more fibronectin bound to the cells (Fig. 2A). The maximum number of fibronectin molecules bound (~80,000 per cell) suggested that each bivalent QE.2E5 bound approximately two receptors (~40,000 antibodies/cell). Scatchard analysis, facilitated by the LIGAND analysis program (48), confirmed that QE.2E5 induces significantly (>40-fold) higher affinity binding of soluble fibronectin ($K_d$ ~15 nM). Under the same conditions, 8A2 induced fibronectin binding of a similar affinity ($K_d$ ~17 nM) (not shown), which is comparable to the previously reported binding affinity of fibronectin in the presence of 8A2 (12). Blocking studies with the anti-α5β1 mAb PHM2 and anti-fibronectin mAb Fn1–11 (see “Materials and Methods”) confirmed that the fibronectin was binding to α5β1 (Fig. 2B).

Two further points were addressed in additional experiments. First, exchanging Ca$^{2+}$ for Mg$^{2+}$ in the buffer marginally reduced the basal binding of soluble fibronectin to the cells, but there was still a clear-cut increase in the presence of QE.2E5 or 8A2 (albeit less than with Mg$^{2+}$) (not shown). Substitution with Mn$^{2+}$ induced high basal binding of fibronectin, and it was not possible to adequately assess any additional effect of QE.2E5 or 8A2. This is consistent with the known activating effect of Mn$^{2+}$.

The question of whether activation of α5β1 by QE.2E5 occurred via intracellular signals (particularly by cross-linking of receptors with a bivalent antibody) would have been best addressed with Fab fragments of the antibody. However, successful production of these fragments was not possible despite repeated attempts. The results of an alternative approach are shown in Fig. 3. The K562 cells were metabolically poisoned by preincubation with sodium azide and 2-deoxyglucose, and their

---

**Fig. 1. QE.2E5 (20 nM) increases the α5β1-mediated adhesion of K562 cells to immobilized human fibronectin.** Fibronectin was purified and the adhesion assay was performed as described under “Materials and Methods.” The effect on adhesion of mAb 8A2 (10 nM) is also shown.
adhesion to immobilized fibronectin was compared to that of healthy cells in the presence of either 8A2 or QE.2E5. The “poisoned” cells failed to spread on fibronectin, and their basal adhesion was completely abolished (Fig. 3B). However, both 8A2 and QE.2E5 still induced a clear-cut increase in adhesion comparable to that seen with healthy cells that had been preincubated with the standard buffer (Fig. 3A). The activation induced by 8A2 is independent of intracellular signaling (12); this result suggests that the same applies to QE.2E5.

**QE.2E5 and 8A2 Have Different Effects on the Expression of the β1 Activation Epitope Recognized by the mAb 9EG7**—The preceding experiments show that QE.2E5 activates the integrin α5β1 and suggest significant similarities to the mAb 8A2. We explored this point further by comparing the effect of these antibodies on the expression of the β1 integrin activation epitope recognized by the rat mAb 9EG7 (45). In preliminary flow cytometry studies (not shown), the basal binding of 9EG7 to α5β1 on resting K562 cells was significantly less than that of the rat anti-human β1 mAb A11B2 (which binds to all β1 integrins expressed on the cell surface), whereas the addition of...
8A2 increased 9EG7 expression to levels comparable to A11B2. In contrast, QE.2E5 had no effect on the binding of 9EG7. In control experiments, neither 8A2 nor QE.2E5 cross-reacted with the anti-rat secondary antibody used, and 9EG7 had no effect on the binding of QE.2E5. We confirmed these preliminary results by examining the binding of 125I-labeled 9EG7 to K562 cells in a soluble ligand binding assay (Fig. 4). The low basal binding was significantly increased by 8A2, whereas QE.2E5 (even at a concentration of 50 nM) had no effect.

**QE.2E5 Recognizes and Activates β1 Integrins from Other Species—** Certain regions of integrins are highly conserved between species, which suggests that these regions have fundamental roles in integrin function. Using flow cytometry, we observed that QE.2E5 binds to rabbit, sheep, and pig but not rat mononuclear cells (Fig. 5). It immunoprecipitated a band of 130 kDa (reduced) and 115 kDa (nonreduced) (SDS-polyacrylamide gel electrophoresis) from 125I-labeled sheep mononuclear cells (not shown), consistent with the expected size of the β1 chain based on studies of human cells. The QE.2E5 epitope is functionally relevant on these cells, as it effectively stimulated the adhesion of both sheep and pig mononuclear cells to immobilized human fibronectin (Fig. 6), whereas 8A2, which does not cross-react with these cells, had no effect (not shown). The QE.2E5 also significantly increased the binding of 125I-labeled soluble fibronectin to pig mononuclear cells (not shown). In the presence of 100 nM of labeled fibronectin, only 47 ± 61 molecules/cell bound to the pig cells in the absence of any stimulus, whereas QE.2E5 induced the binding of 2910 ± 289 molecules/cell.

**The Epitope Recognized by QE.2E5 Is Remote from the Epitopes of Other Activating Anti-β1 mAbs—** The preceding results suggested that QE.2E5 recognized a functionally significant region of the β1 chain that was different from that recognized by 8A2. The following experiments were designed to map the site of this highly conserved epitope, using flow cytometric analysis of CHO cell lines stably expressing high levels of chimeric (mouse/human) integrin β1 chains (40). The chimeras used are shown diagramatically in Fig. 7. The cell lines express wild-type human β1 (which complexes with hamster α chains to be expressed on the cell surface as intact heterodimers) or chimeric receptors containing decreasing amounts of human β1 residues (587, 425, and 354 amino acids). The anti-β1 mAb used for comparison in this study was A1A5 (41), which has activating properties and recognizes an epitope in the same region as 8A2 (residues 207–218) (40). Both mAbs bound to CHO cells transfected with wild-type human β1 integrin cDNA, but neither bound to untransfected CHO cells (Table I). Each also bound to the cells expressing the chimeric receptors containing the first 587 human amino acid residues, but QE.2E5 alone failed to recognize the chimera consisting of human residues 1–425 and the remaining residues corresponding to the murine sequence. This suggests that QE.2E5 binds to a site within the region bordered by residues 426 and 587, which is remote from both the predicted ligand binding site and the binding sites of other function-modifying anti-β1 mAb. A1A5,
as predicted, as well as other anti-β1 mAbs (8A2, antibody 13 (50)), bound to all of the chimeras that were used in this experiment.

**DISCUSSION**

We draw the following conclusions from these experiments: (a) the mAb QE.2E5 recognizes an epitope on the integrin β1 chain and activates β1 integrins; (b) the epitope recognized by QE.2E5 is highly conserved, and it lies in a region remote from both the mapped ligand binding site in the integrin β1 chain and the epitopes recognized by other function-modifying anti-β1 mAb; and (c) QE.2E5 does not induce an activation epitope on the integrin β1 chain, whereas this epitope is induced by another well characterized activating mAb (8A2).

QE.2E5 induces the integrin α5β1 to bind soluble fibronectin with an affinity ($K_d = 15 \text{ nM}$) that is similar to that induced by 8A2 ($K_d = 17 \text{ nM}$), and they have similar effects on the α5β1-dependent adhesion of K562 cells to immobilized fibronectin. Despite these functional similarities, epitope mapping places the binding site of QE.2E5 distant from that of 8A2 and at least 194 residues away from the predicted ligand binding site (Fig. 8). The QE.2E5 epitope is also conserved in a number of other species. The region containing the QE.2E5 epitope overlaps considerably with the cysteine-rich repeats (residues 443–600) which are a consistent feature of all integrin β chains (2). The repeats are thought to be internally disulfide bonded, and the fact that the QE.2E5 epitope is lost upon reduction suggests that it is dependent on one or more of these bonds (51). Electron microscopy and computer-assisted structure prediction localize these repeats to the β1 chain tail, which is estimated to be about 2 nm thick and 18–20 nm long (11). The entire α5β1 structure was estimated to be 28 nm long in the same study. Our study does not directly address the spatial separation between the QE.2E5 epitope and the site of interaction with ligand, and conceivably, tertiary folding of the molecule could bring these two regions into close apposition. A crude estimation of the distance can be made based on the study of the activating anti-β3 antibody L1BS2 (39), which maps to a region of the β3 chain that corresponds to an area quite close to the site of the QE.2E5 epitope in the β1 chain (residues 602–691 in β3 for L1BS2 correspond to residues 612–708 in β1). In that study, electron microscopic images of complexes of fibrinogen, αIIbβ3, and L1BS2 revealed that the epitope and the ligand binding site are separated by about 16 nm. Repeating the same study using QE.2E5, fibronectin, and α5β1 would be necessary to accurately measure the distance, but this is indirect support for our proposal that the two regions are separated in space. We hypothesize that the activating change in the conformation of the ligand binding site induced by QE.2E5 is propagated a considerable distance along the length of the receptor and may mimic the predicted propagated conformational change during physiological receptor activation.

The epitopes of three other mAb have been mapped to within the cysteine-rich repeats of the β1 chain (40, 52). One study mapped the mAb K20, which has been reported to have negative signaling effects (53, 54). Two anti-chicken β1 mAbs were mapped to this area in another study (52). The first, TASC, has been reported to have a mixture of adhesion-inhibiting (to vitronectin) and adhesion-promoting (to laminin and collagen) properties (55). QE.2E5 does not cross-react with chicken cells, and so presumably they do not recognize identical epitopes. The other antibody, G, has been reported to disrupt αβ heterodimer formation and hence interferes with receptor function (56). A functionally significant mutation in the inte-

---

**Fig. 7.** Diagrammatic representation of the human/mouse integrin β1 chimeras used for epitope mapping. Chimeric cDNA was produced and transfected into CHO cells and stable cell lines were generated as described under “Materials and Methods.”

**Fig. 6.** QE.2E5 increases the adhesion of sheep and pig mononuclear cells to immobilized fibronectin. Assays of the adhesion of sheep (a) and pig (b) cells to fibronectin were performed as described under “Materials and Methods.”

---

3 Y. Takada, unpublished observation.
**Figure 8. Diagrammatic representation of predicted ligand binding sites in the integrin β1 chain and the sites of epitopes of QE.2E5 and other functionally active mAb against the β1 chain.** The ligand binding sites (residues 117–182 and 220–231) correspond to the putative ligand binding sequences mapped for the integrin β3 chain. Residues 207–218 contain the epitopes for several previously mapped activating and inhibiting anti-β1 mAb (40). The site of the highly conserved cysteine-rich repeats is also shown.

**REFERENCES**

1. Hynes, R. O. (1987) J. Biol. Chem. 262, 155–162
2. Arroyo, A. G., Garcia-Pardo, A., and Sanchez-Madrid, F. (1993) J. Biol. Chem. 268, 8963–8968
3. Nermut, M., Green, N., Eason, P., Yamada, S. S., and Yamada, K. M. (1988) EMBO J. 7, 4093–4099
4. Faull, R. J., Kewach, N. L., Harlan, J. M., and Ginsberg, M. H. (1993) J. Cell Biol. 121, 155–162
5. Lollo, B., Chan, W. K. H., Hansson, E. M., Moy, T. Y., and Brian, A. A. (1993) J. Biol. Chem. 268, 21693–21700
6. Alt, J. D., Bader, R., Mannucci, P. M., and Edgington, T. S. (1987) J. Cell Biol. 107, 1893–1900
7. Bennett, S. J., and Vilaire, G. (1979) J. Cell. Biol. 74, 1393–1401
8. Bennett, S. J., Hoxie, J. A., Leitman, S. F., Vilaire, G., and Cines, D. B. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 3417–3421
9. Marguerie, G. A., Plow, E. F., and Edgington, T. S. (1979) J. Biol. Chem. 254, 5357–5363
10. Marguerie, G. A., Edgington, T. S., and Plow, E. F. (1980) J. Biol. Chem. 255, 151–161
11. Plow, E. F., and Ginsberg, M. H. (1981) J. Biol. Chem. 256, 9477–9482
12. D'Souza, S. E., Ginsberg, M. H., Burke, T. A., Lam, S. C. T., and Plow, E. F. (1988) Science 242, 91–93
13. Arroyo, A. G., Garcia-Pardo, A., and Sanchez-Madrid, F. (1993) EMBO J. 12, 3101–3108
14. Lollo, B. A., Chan, K. W. H., Plow, E. F., and Shattil, S. J. (1992) J. Biol. Chem. 267, 7345–7352
15. Takada, Y., Lofo, J. C., Yu, X., Glass, A., Ruggeri, Z. M., Shattil, S. J., Plow, E. F., and Ginsberg, M. H. (1990) Cell Regul. 1, 883–893
16. Shattil, S. J., Hoxie, J. A., Cunningham, M., and Brass, L. F. (1985) J. Biol. Chem. 260, 1107–1114
17. Blunt, S. B. (1989) J. Cell Biol. 103, 451–456
18. Lofo, J. C., O'Toole, T. E., Plow, E. F., Glass, A., Frelinger, A. L., and Ginsberg, M. H. (1990) Science 249, 915–918
19. Bajt, M. L., Ginsberg, M. H., Frelinger, A. L., Berndt, M. C., and Lofo, J. C. (1992) J. Biol. Chem. 267, 3789–3794
20. D'Souza, S. E., Ginsberg, M. H., Burke, T. A., Lam, S. C. T., and Plow, E. F. (1990) J. Biol. Chem. 265, 3440–3446
21. Takada, Y., Yanane, J., Mandelman, D., Puoz, W., and Ginsberg, M. H. (1992) J. Biol. Chem. 267, 1193–921
22. Chen, Y. P., Djafar, I., Pidhirny, D., Steinier, B., Cieutat, A. M., Caen, J. P., and Rosa, J. P. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 10169–10173
23. O'Toole, T. E., Katagiri, Y., Faull, R. J., Peter, K., Tamura, R., Quanta, V., Lofo, J. C., Shattil, S. J., and Ginsberg, M. H. (1994) J. Cell Biol. 124, 1487–1493
24. Frelinger, A. L., Lam, S. C. T., Plow, E. F., Smith, M. A., Roberts, J., Huijbers, R. J. F., Weder, P., van de Kastelee, W., Meijler, C. J. M., and Figdor, C. G. (1992) J. Cell. Biol. 117, 461–470
25. Du, X., Gu, M., Weisel, J. W., Nagazawami, C., Bennett, J. S., Boudvith, R., and Ginsberg, M. H. (1993) J. Biol. Chem. 268, 9515–9520
26. Frelinger, A. L., Du, X., Plow, E. F., and Ginsberg, M. H. (1991) J. Biol. Chem. 266, 17186–17111
27. Kouna, W. C., Wall, C. D., White, M. M., Fox, C. F., and Jennings, L. K. (1990) J. Biol. Chem. 265, 20594–20601
28. Kovach, N. L., Carlso, T. M., Yee, E., and Harlan, J. M. (1992) J. Cell Biol. 116, 569–590
29. Arroyo, A. G., Sanchez-Mateos, P., Campanero, M. R., Fabra, I., Dignani, M., and Sanchez-Madrid, F. (1992) J. Cell Biol. 117, 659–670
30. van de Wiel-van Keulen, E., van Koyk, Y., de Boer, A. J., Heijnen, R. J. F., Weder, P., van de Kastelee, W., Meijler, C. J. M., and Figdor, C. G. (1992) J. Cell Biol. 117, 461–470
31. Du, X., Gu, M., Weisel, J. W., Nagazawami, C., Bennett, J. S., Boudvith, R., and Ginsberg, M. H. (1993) J. Biol. Chem. 268, 9515–9520
32. Frelinger, A. L., Du, X., Plow, E. F., and Ginsberg, M. H. (1991) J. Biol. Chem. 266, 17186–17111
33. Frelinger, A. L., Cohen, I., Plow, E. F., Smith, M. A., Roberts, J., Huijbers, R. J. F., Weder, P., van de Kastelee, W., Meijler, C. J. M., and Figdor, C. G. (1992) J. Cell Biol. 117, 461–470
34. Beck, G. J., Hancock, W. C., Kraft, N., Lanyon, H. C., and Atkins, R. C. (1981) Pathology 13, 699–718
35. D'Souza, S. E., Ginsberg, M. H., Burke, T. A., Lam, S. C. T., and Plow, E. F. (1990) J. Biol. Chem. 265, 1415–1421
36. Weisel, J. W., Nagazawami, C., Vilaire, G., and Bennett, J. S. (1992) J. Biol. Chem. 267, 11637–11643
37. Carrell, N. A., Fitzgerald, L. A., Steiner, B., Erickson, H. P., and Phillips, D. R. (1985) J. Biol. Chem. 260, 1743–1749
38. Arroyo, A. G., Garcia-Pardo, A., and Sanchez-Madrid, F. (1992) J. Cell Biol. 116, 2175–2184
39. Becker, G. J., Hancock, W. C., Kraft, N., Lanyon, H. C., and Atkins, R. C. (1981) Pathology 13, 699–718
25106

**Novel Activating Anti-β1 Integrin Antibody**

48. Munson, P. J., and Rodbard, D. (1980) *Anal. Biochem.* **107**, 220–239
49. Prater, C. A., Plotkin, J., Jaye, D., and Frazier, W. A. (1991) *J. Cell Biol.* **112**, 1031–1040
50. Akiyama, S. K., Yamada, S. S., Chen, W. T., and Yamada, K. M. (1989) *J. Cell Biol.* **108**, 863–875
51. Calvete, J. J., Henschen, A., and Gonzalez-Rodriguez, J. (1991) *Biochem. J.* **274**, 63–71
52. Shih, D.-T., Edelman, J. M., Horwitz, A. F., Grunwald, G. B., and Buck, C. A. (1993) *J. Cell Biol.* **122**, 1361–1371
53. Groux, H., Huet, S., Valentin, H., Pham, D., and Bernard, A. (1989) *Nature* **339**, 152–154
54. Ticchioni, M., Aussel, C., Breittmayer, J.-P., Manic, S., Pelassy, C., and Bernard, A. (1991) *J. Immunol.* **151**, 119–127
55. Neugebauer, K. M., and Reichardt, L. F. (1991) *Nature* **350**, 68–71
56. Buck, C. A., Shea, E., Duggin, K., and Horwitz, A. (1986) *J. Cell Biol.* **103**, 2421–2428
57. Arnaout, M. A., Dana, N., Gupta, S. K., Tenen, D. G., and Fathallah, D. M. (1990) *J. Clin. Invest.* **85**, 977–981
58. Bazzoni, G., Shih, D.-T., Buck, C. A., and Hemler, M. E. (1995) *J. Biol. Chem.* **270**, 25570–25577