Regulation of $\alpha_2\beta_1$-mediated Fibroblast Migration on Type I Collagen by Shifts in the Concentrations of Extracellular Mg^{2+} and Ca^{2+}

John J. Grzesiak, George E. Davis, Daniel Kirchhofer, and Michael D. Pierschbacher

La Jolla Cancer Research Foundation, 10901 North Torrey Pines Road, La Jolla, California 92037

Abstract. Extracellular Ca^{2+} can reverse the Mg^{2+}-dependent, $\alpha_2\beta_1$-mediated adhesion of WI38 human fibroblasts to type I collagen substrates. Affinity chromatography data also demonstrate that Ca^{2+} can specifically elute the fibroblast $\alpha_2\beta_1$ integrin bound to type I collagen-Sepharose in Mg^{2+}. In modified Boyden chamber migration assays, Mg^{2+} alone supports the $\alpha_2\beta_1$-mediated migration of fibroblasts on type I collagen substrates, while Ca^{2+} does not. However, a twofold enhancement in migration was observed when combinations of the two cations were used, with optimal migration observed when the Mg^{2+}/Ca^{2+} ratio was higher than one. Inhibitory mAbs directed against various integrin subunits demonstrate that these observed cation effects appear to be mediated primarily by $\alpha_2\beta_1$. These data, together with reports that under certain physiological conditions significant fluctuations in the concentrations of extracellular Ca^{2+} and Mg^{2+} can take place in vivo, suggest that the ratio between these two cations is involved in the up- and downregulation of integrin function, and thus, may influence cell migratory behavior.

The integrins are a family of heterodimeric cell surface receptors that function to mediate the binding of cells to extracellular matrix components as well as, in some cases, to other cells (Hemler, 1990; Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Springer, 1990). The regulation of integrin function is still unclear, but existing data indicate that the binding specificity and activity might be controlled by various means, including phosphorylation (Chatila et al., 1989; Dahl and Grabel, 1989; Freed et al., 1989; Hara and Fu, 1986; Hirst et al., 1986; Parise et al., 1990; Shaw et al., 1990), RNA splicing (Bray et al., 1990; Brown et al., 1989; van Kuppevelt et al., 1989), the membrane lipid environment (Cheresh et al., 1987; Conforti et al., 1990; Santoro, 1989; Stallcup et al., 1989), and by other posttranslational modifications (Cierniewski et al., 1989; Loftus et al., 1988). Growth factors have been shown to regulate integrin expression (Heino and Massague 1989; Heino et al., 1989; Ignotz et al., 1989; Defilippi et al., 1991). The binding function of some integrins can also be activated or increased by agonists, such as ADP and thrombin for GPIIb-IIIa or ADP and phorbol esters for CD11b/CD18 (Altieri and Edgington, 1988; Marguerie and Plow, 1983; Phillips et al., 1988; Wright and Meyer, 1986).

A common characteristic of all integrins is the requirement for divalent cations, such as Ca^{2+} and Mg^{2+}, to maintain binding function (Dransfield and Hogg, 1989; Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Fujimura and Phillips, 1983; Fitzgerald and Phillips, 1985). These cations presumably exert their effects by binding to the three to five putative cation-binding domains located on all integrin α subunits (Argraves et al., 1987), and possibly by interacting directly with β subunits as well (Loftus et al., 1990). In examining the role that Ca^{2+} and Mg^{2+} may play in the regulation of the binding function of two Arg-Gly-Asp (RGD)[1]-dependent integrins, $\alpha_v\beta_1$ (Vogel et al., 1990; Bodary and McLean, 1990) and $\alpha_v\beta_3$ (Pytela et al., 1985), both of which share the same α subunit, we have recently shown that while $\alpha_v\beta_3$ binds to ligand in either Ca^{2+} or Mg^{2+}, $\alpha_v\beta_1$ binds only in Mg^{2+} and not in Ca^{2+} (Kirchhofer et al., 1991). These cation effects were demonstrated in cell adhesion experiments, and with isolated receptors using affinity chromatography and receptor-binding liposome assays. We also showed that in the presence of Mg^{2+}, Ca^{2+} had opposite effects on these receptors in that it inhibited the ligand binding of $\alpha_v\beta_1$ but enhanced the binding of $\alpha_v\beta_3$. These results suggested to us a potential regulatory role for Ca^{2+} in integrin-mediated cell adhesion and supported the possible involvement of the β subunit in cation binding.

It has been shown that a collagen receptor on platelets,

G. E. Davis' present address is Department of Pathology and Laboratory Medicine, Texas A & M University College of Medicine, 208 Medical Sciences Building, College Station, Texas 77843-1114.

D. Kirchhofer's present address is F. Hoffman-LaRoche Ltd., Pharma Division, Preclinical Research, 4002 Basle, Switzerland.

1. *Abbreviation used in this paper*: RGD, Arg-Gly-Asp.

$\alpha_2\beta_1$, exhibits the same Ca^{2+}-inhibitable binding characteristics as $\alpha_v\beta_1$ (Staatz et al., 1989), and that the Mg^{2+}-dependent, $\alpha_2\beta_1$-mediated adhesion of platelets to collagen is also inhibited by Ca^{2+} (Santoro, 1986). One of the integrins mediating adhesion to collagen on fibroblasts is also an $\alpha_2\beta_1$ very similar to that found on platelets (Kunicki et al., 1988), though, depending on the cell type on which it is expressed, this receptor may display altered ligand specificity (Languino et al., 1989; Kirchhofer et al., 1990; Elices and Hemler, 1989; Lotz et al., 1990).

In this study we have examined the potential regulatory role of extracellular Ca^{2+} and Mg^{2+} on the $\alpha_2\beta_1$-mediated migration of fibroblasts on type I collagen. The results presented here suggest that integrin-mediated fibroblast migration on type I collagen can be regulated, at least in part, by small changes in divalent cation concentration. We demonstrate that fibroblastic cells which express both $\alpha_2\beta_1$ and $\alpha_v\beta_3$ exhibit migration in the presence of Mg^{2+} on type I collagen but not on the control, vitronectin, while in the presence of Ca^{2+}, the cells migrate on vitronectin but not on type I collagen. Furthermore, we demonstrate that migration on collagen is significantly enhanced when both cations are present, but is optimal when the ratio of Mg^{2+}/Ca^{2+} concentrations is slightly higher than one. mAbs directed against the α_2 and β_1 integrin subunits inhibit this migration on type I collagen, suggesting that these observed cation effects are mediated through $\alpha_2\beta_1$. These data suggest a possible physiological role for the divalent cations, Mg^{2+} and Ca^{2+}, in the modulation of integrin-mediated cell migration.

Materials and Methods

Proteins

Bovine type I collagen was from Collaborative Research Inc. (Bedford, MA). Human vitronectin was purified from plasma by mAb affinity chromatography (Hayman et al., 1983; Suzuki et al., 1984).

Cells

WI38 human lung fibroblasts were obtained from the American Type Culture Collection (Rockville, MD). The cells were cultured in DME (Gibco Laboratories, Irvine, CA) supplemented with 10% FCS (Tissue Culture Biologicals, Tulare, CA) at 37°C.

Antibodies

mAbs P1H5 and P1E6 against α_2, P1B5 against α_3 (Wayner and Carter, 1987), 147 against α_v (Pytela et al., 1985), AIIB2 against β_1, and BIIG2 against α_5 (Hall et al., 1990), control mAb 8E6 against vitronectin (Hayman et al., 1983), and polyclonal antiserum to $\alpha_v\beta_3$ (Freed et al., 1989) have been described.

Cell Attachment Assays

The WI38 cell attachment assays were performed as previously described (Ruoslahti et al., 1982). Briefly, 3×10^4 WI38 cells in 100 μl of TBS containing 2.5 mg/ml BSA were added to each well of microtiter plates (Titertek, Flow Laboratories, McLean, VA) which were previously coated with 10 μg/ml of bovine type I collagen or vitronectin. $CaCl_2$ or $MgCl_2$ were added to each well at constant concentration (2.5 mM) and mixed with the cells. After 45 min of incubation at 37°C the alternate cation was added to the wells and incubated for another 45 min at 37°C. The unattached cells were washed away and attached cells were fixed with 3% paraformaldehyde in PBS. The cells were then stained with 0.5% toluidine blue in 3.7% formaldehyde, solubilized with 2% SDS, and the absorbance at 600 nm was measured in a vertical pathway spectrophotometer.

Affinity Chromatography

WI38 cells were detached with 4 mM EDTA and washed with PBS. The final cell pellet (0.6 ml) was resuspended in PBS and labeled with 3 mCi of [^{125}I]sodium iodide according to the lactoperoxidase method (Lebien et al., 1982). The reaction was stopped with DME containing 0.02% sodium azide, and the cells were washed with cold PBS. The cells were extracted at 4°C for 20 min in TBS containing 100 mM octyl glucopyranoside (Calbiochem Corp., LaJolla, CA) and 2 mM PMSF (Sigma Chemical Co., St. Louis, MO). The lysate was centrifuged at 12,000 g for 20 min. The supernatant was supplemented with 3 mM $MgCl_2$ and applied onto bovine type I collagen-Sepharose prepared by coupling the protein to cyanogen bromide-activated Sepharose 4B according to the manufacturer's instructions (Sigma Chemical Co.). After 2 h of incubation, the column was washed with 20 bed volumes of TBS containing 50 mM octyl glucopyranoside and 3 mM $MgCl_2$. The column was eluted with 3 mM $CaCl_2$ and subsequently with 10 mM EDTA in TBS containing 50 mM octyl glucopyranoside and the fractions analyzed by 7.5% SDS-PAGE under nonreducing conditions and autoradiography.

Immunoprecipitation

To perform immunoprecipitations, mAbs were absorbed overnight at 4°C onto anti-mouse IgG-agarose (Sigma Chemical Co.). The beads were washed with TBS containing 50 mM octyl glucopyranoside, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 1 mM PMSF (wash buffer) and added to receptor fractions. After incubation for 6 h at 4°C the beads were washed five times with the wash buffer described above, added to SDS-PAGE sample buffer, boiled, and the eluted material was analyzed by SDS-PAGE on 7.5% gels under nonreducing conditions. The gels were dried and exposed to x-ray film (X-OMAT AR; Eastman Kodak Co., Rochester, NY).

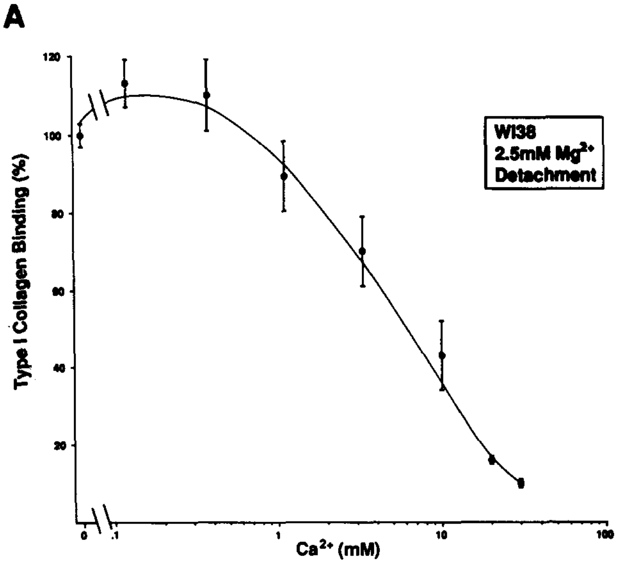
Migration Assays

Migration assays were conducted using the modified Boyden chamber as previously described (Banai et al., 1990). Briefly, the chamber consists of two compartments separated by a filter, and migration is measured by counting the number of cells crossing the membrane through pores of defined size. Lower chambers were filled with modified, serum-free DME without Ca^{2+}, Mg^{2+}, or PO_4^{3-} (Gibco Laboratories, Grand Island, NY). Various $CaCl_2$, $MgCl_2$, and/or EGTA concentrations were then added along with 20 ng/ml PDGF (Gibco Laboratories). 10-μm-pore polycarbonate membrane filters (Poretics Corp., Livermore, CA) that had been previously coated with 10 μg/ml of bovine type I collagen or vitronectin were then placed on top of the lower chambers, and the upper chambers were secured in place. Upper chambers were filled with 3.0×10^4 WI38 cells per chamber in the same media described above without PDGF plus the various $CaCl_2$, $MgCl_2$, and/or EGTA concentrations consistent with those in the lower chamber. In some cases, purified anti-integrin antibodies were also added to the upper chambers. Lower chamber final volumes were 27 μl and the upper chambers were 50 μl. The entire apparatus was then incubated for 3 h at 37°C. After the incubation period, the upper chamber was removed and the filter was fixed in 3% paraformaldehyde and stained with 0.5% toluidine blue in 3.7% formaldehyde. Excess stain was washed away with water, the attached cells on the upper side of the filter were removed and the migrated cells on the underside were quantitated by counting two high-powered fields (at a magnification of 200) per well.

Results

Ca^{2+} Reverses the Mg^{2+}-dependent, $\alpha_2\beta_1$-mediated Attachment of Fibroblasts to Type I Collagen Substrates

The integrin profile of WI38 human fibroblasts has been shown by immunoprecipitation to include $\alpha_2\beta_1$ (Vogel et al., 1990). When WI38 cells were tested for their attachment activity to bovine type I collagen in the presence of increasing concentrations of either Mg^{2+} or Ca^{2+}, we found that they adhered well in the presence of Mg^{2+} alone but not at all in the presence of Ca^{2+} alone (not shown). mAb P1E6, directed against the α_2 integrin subunit, inhibited this Mg^{2+}-dependent attachment in a concentration-dependent manner



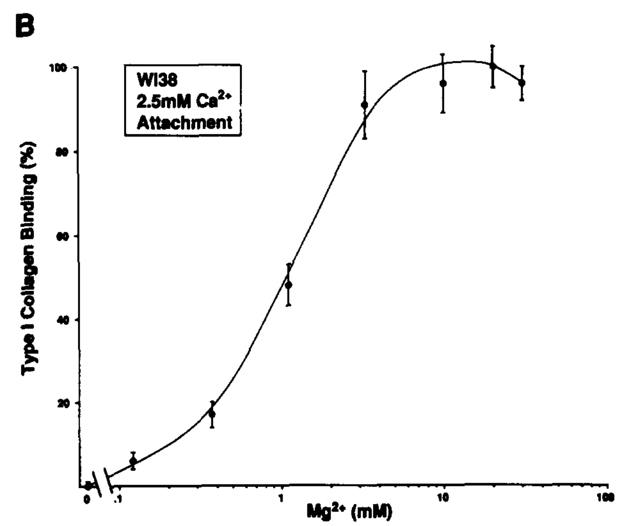


Figure 1. The effects of Ca^{2+} and Mg^{2+} on the cell detachment (*A*) or attachment (*B*) of cells to surfaces coated with bovine type I collagen after initial incubation with the alternate cation. 3.0×10^4 WI38 cells were added to each well of microtiter plates coated with 10 μg/ml bovine type I collagen and incubated for 45 min at 37°C in the presence of either 2.5 mM Mg^{2+} or 2.5 mM Ca^{2+}. After the initial incubation a titration of the alternate cation was added to the wells and reincubated for another 45 min. Attached cells were fixed with 3% paraformaldehyde and stained with 0.5% toluidine blue in 37% formaldehyde. The cells were then solubilized with 2% SDS and quantitated by measuring the absorbance at 600 nm. The results are the mean $\pm$ SD of three experiments done in triplicate. In detachment experiments (*A*), 100% adhesion was defined as the adhesion of WI38 cells in 2.5 mM Mg^{2+} alone without any Ca^{2+} added. The mean absorbance at 600 nm was 0.385 $\pm$ SD. For attachment experiments (*B*), 100% was observed at 20 mM Mg^{2+} after initial incubation in the presence of 2.5 mM Ca^{2+}. The mean absorbance at 600 nm was 0.439 $\pm$ SD. Nonspecific binding to control wells coated with 5 mg/ml BSA (2% of maximum) has been subtracted from each value.

(not shown). It is noteworthy that maximal attachment of WI38 cells to both type I collagen and vitronectin was observed in the range of 5–10 mM cation. And, like the $\alpha_v\beta_1$-mediated attachment of IMR 32 cells to RGD substrates, and the $\alpha_2\beta_1$-mediated binding of platelets to collagen (Kirchhofer et al., 1991; Santoro, 1986; Staatz et al., 1989), the attachment of WI38 cells to type I collagen substrates in the presence of 2.5 mM Mg^{2+}, was inhibited as the Ca^{2+} concentration increased. Similar results were obtained in receptor-loaded liposome binding assays with $\alpha_2\beta_1$ integrin purified from human platelets (data not shown). Because the Mg^{2+}-dependent WI38 cell adhesion to type I collagen via $\alpha_2\beta_1$ was inhibited by Ca^{2+}, we asked whether the addition of Ca^{2+} could reverse this adhesion. As is shown in Fig. 1 *A*, fibroblasts previously attached in Mg^{2+} (2.5 mM) could be detached by the addition of Ca^{2+}, and the extent of this detachment was directly proportional to the increase in Ca^{2+} concentration. To test whether this effect of Ca^{2+} was detrimental to the cells we conducted the reverse experiment where cells were first incubated in the presence of Ca^{2+} (2.5 mM) and subsequently exposed to a Mg^{2+} titration. While no attachment was observed on type I collagen in the presence of Ca^{2+} alone, subsequent addition of increasing concentrations of Mg^{2+} yielded a proportional increase in attachment (Fig. 1 *B*).

Ca^{2+} Specifically Elutes the $\alpha_2\beta_1$ Integrin from Bovine Type I Collagen–Sepharose

To study this apparent reversibility in cation-dependent adhesion further, surface ^{125}I-labeled WI38 membrane extracts supplemented with 3 mM Mg^{2+} were chromatographed over bovine type I collagen–Sepharose and the column was subsequently eluted with 3 mM Ca^{2+}. As shown in Fig. 2 *A*, all of the bound integrin was eluted from the column with Ca^{2+}, and subsequent elution with 10 mM EDTA released no additional receptor. Fig. 2 *B* shows by immunoprecipitation that the integrin eluted was indeed $\alpha_2\beta_1$. After extensive dialysis back into 3 mM Mg^{2+}, this purified receptor rebound to the column and was again eluted with Ca^{2+} (not shown). In a reverse experiment, where WI38 extracts supplemented with 3 mM Ca^{2+} were chromatographed over GRGDSPK-Sepharose, $\alpha_v\beta_3$ bound to the column and could not be eluted with 3 mM Mg^{2+}. Elution could only be achieved with EDTA (not shown).

To confirm that the elution of $\alpha_2\beta_1$ was due to the addition of Ca^{2+} and not the removal of Mn^{2+}, we conducted two additional experiments. Mg^{2+}-supplemented WI38 extracts were loaded onto a type I collagen–Sepharose column and eluted first with Mn^{2+}, then Ca^{2+}. Again, all of the integrin was released with Ca^{2+} while none was eluted with Mn^{2+} (data not shown). We also loaded WI38 extracts supplemented with 1 mM Mg^{2+} onto a type I collagen–Sepharose column and eluted with 4 mM Mg^{2+}, 3 mM Mg^{2+} plus 1 mM Ca^{2+}, and finally EDTA. The change to 4 mM Mg^{2+} caused no integrin to be released. However, when 3 mM Mg^{2+} plus 1 mM Ca^{2+} was introduced, the integrin was eluted, clearly indicating that Ca^{2+} reverses the Mg^{2+}-dependent binding of $\alpha_2\beta_1$ to type I collagen (not shown). It is noteworthy that the detachment by Ca^{2+} of cells previously bound in Mg^{2+} (Fig. 1 *A*) correlates well with this affinity chromatography result in that Ca^{2+} appears to weaken the Mg^{2+}-dependent integrin–ligand interactions. In other exper-

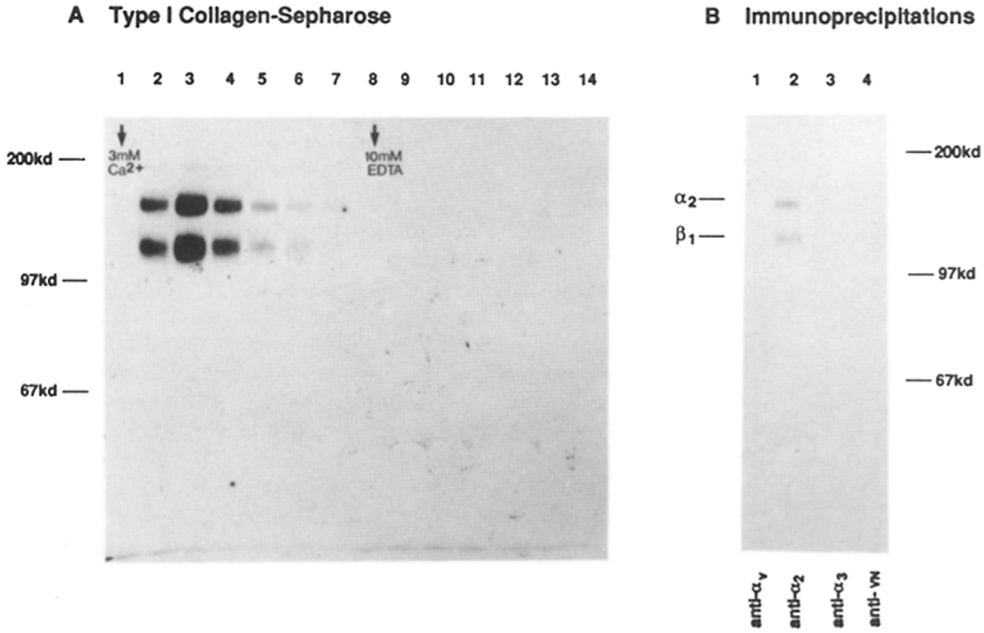


Figure 2. Affinity chromatography of extracts of ^{125}I–surface-labeled WI38 cells on bovine type I collagen-Sepharose. Extracts of surface-iodinated WI38 cells were supplemented with 3 mM $MgCl_2$ and applied onto a 1.0 ml collagen–Sepharose column. After washing with wash buffer (see Materials and Methods) containing 3 mM Mg^{2+}, the column was eluted with 3 mM Ca^{2+} followed by 10 mM EDTA (*A*). The eluted fractions were analyzed on a 7.5% SDS-polyacrylamide gel under nonreducing conditions followed by autoradiography. Eluted material from Lanes *2–4* was used in immunoprecipitation experiments using mAbs P1H5 against α_2, P1B5 against α_3, 147 against α_v, and 8E6 against vitronectin (*B*). The immunoprecipitates were separated by SDS-PAGE (7.5%) under nonreducing conditions, and protein bands were visualized by autoradiography. The molecular mass markers were myosin (200 kD), phosphorylase b (97 kD), and BSA (67 kD). The positions of the subunits α_2 and β_1 are indicated.

iments not reported here, we also found that Ca^{2+} can elute both $\alpha_5\beta_1$ from fibronectin–Sepharose when this integrin has been bound to the column from Mg^{2+} supplemented WI38 extracts, and $\alpha_v\beta_1$ from RGD-Sepharose bound from Mg^{2+}-supplemented IMR32 extracts.

Elevated Extracellular Mg^{2+} in the Presence of Ca^{2+} Enhances $\alpha_2\beta_1$ Integrin-Mediated Fibroblast Migration on Type I Collagen

The attachment/detachment data together with the affinity chromatography results suggested that Ca^{2+} and Mg^{2+} could be involved in the modulation of integrin function, such as in mediating a cellular process like migration. In modified Boyden chamber migration assays, we found that while WI38 fibroblasts were migratory on type I collagen in Mg^{2+} and not in Ca^{2+} (Fig. 3 *A*), a twofold enhancement of migration was observed when these cations were used in combination, with maximum migration observed when Mg^{2+}/Ca^{2+} ratios were higher than one (Fig. 4, *A* and *B*). Specifically, Fig. 4 *B* shows that in the presence of 1.5 mM Mg^{2+} without Ca^{2+}, migration is about half that observed when optimal combinations of the two cations are used. As a control, WI38 cells migrated on vitronectin substrates in the presence of Ca^{2+} alone but, surprisingly, not in Mg^{2+} (Fig. 3 *B*), indicating that the β_3 integrin has different cation requirements for the promotion of migration versus adhesion (Kirchhofer et al., 1991). It should be noted that maximal migratory activity is achieved at significantly lower combined divalent cation concentrations (<4 mM) than maximal adhesion (5–10 mM) in a single cation. Fig. 5 shows a relative comparison between the maximum fibroblast migration observed on type I collagen substrates in Ca^{2+} alone (Fig. 5 *A*), Mg^{2+} alone (Fig. 5 *B*), and in combination with slight excess of Mg^{2+} (Fig. 5 *C*).

Because migration was enhanced when the two cations were present together, we questioned whether the migration observed in the presence of Mg^{2+} alone is dependent on the efflux of Ca^{2+} from the cell. This seems unlikely because no migration was observed without the addition of cations, even after a 3 h incubation on either type I collagen or the control ligand, vitronectin, which exhibits migratory activity in Ca^{2+} alone (Fig. 3 *B*). Moreover, the addition of 5 mM EGTA to wells containing 3 mM Mg^{2+} reduced fibroblast migration on type I collagen substrates by only ~25% of that observed in the presence of 3 mM Mg^{2+} alone, but completely abolished migration on vitronectin substrates in the presence of 3 mM Ca^{2+} (data not shown). Some nonspecific chelation of Mg^{2+} by EGTA is the likely explanation for the observed reduction in migration on collagen.

Antibodies Directed Against the Integrin α_2 and β_1 Subunits Inhibit Fibroblast Migration on Type I Collagen

mAbs directed against various integrin subunits were tested for inhibitory activity of migration under the optimal conditions of 2.5 mM Mg^{2+}/1.5 mM Ca^{2+} (Fig. 4 *A*). As shown in Fig. 6, P1H5 and P1E6, directed against the α_2 subunit, and AIIB2, directed against the β_1 subunit, essentially completely inhibited WI38 fibroblast migration on type I collagen. No inhibition was observed in the presence of equivalent concentrations of P1B5 directed against the α_3 subunit, BIIG2 directed against the α_5 subunit or a polyclonal antiserum directed against $\alpha_v\beta_3$. In control migration studies on vitronectin, laminin, and fibronectin, we determined that the antibodies showing no inhibition on type I collagen were indeed functional blockers on their respective ligand (not shown). Thus, it appears that the integrin $\alpha_2\beta_1$ is the target of the divalent cation effects described in this study.

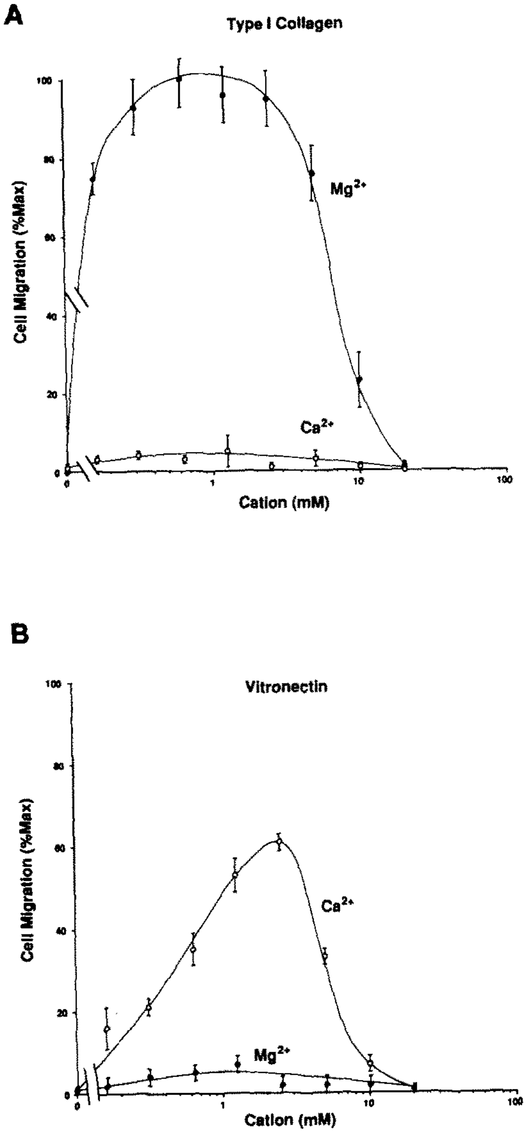


Figure 3. Effects of Ca^{2+} and Mg^{2+} on fibroblast migration through ligand coated filters. Using modified Boyden chambers, 3.0×10^4 WI38 cells were added to each upper chamber and allowed to migrate, through filters of defined pore size (10 μm), towards PDGF in the lower chamber for 3 h at 37°C in the presence of various concentrations of Ca^{2+} (○) or Mg^{2+} (●). The filters were coated with 10 μg/ml bovine type I collagen (*A*) or vitronectin (*B*). After incubation, filters were fixed in methanol and stained with Diff-Quik Solution II (Scientific Products, McGaw Park, IL). After rinsing, adherent cells were removed from the upper side of the filter and migrated cells on the underside were counted by taking the mean of two high-powered fields (magnification of 200) per well using an inverted, light microscope (model CK2; Olympus Corp., Lake Success, NY). The results represent the mean ± SD of three experiments done in triplicate. Migration on type I collagen and the control, vitronectin, are compared with each other and all values are expressed as a percentage of maximum migration (68 cells ± SD/high-powered field), which was observed on type I collagen. No migration was observed in the absence of divalent cations.

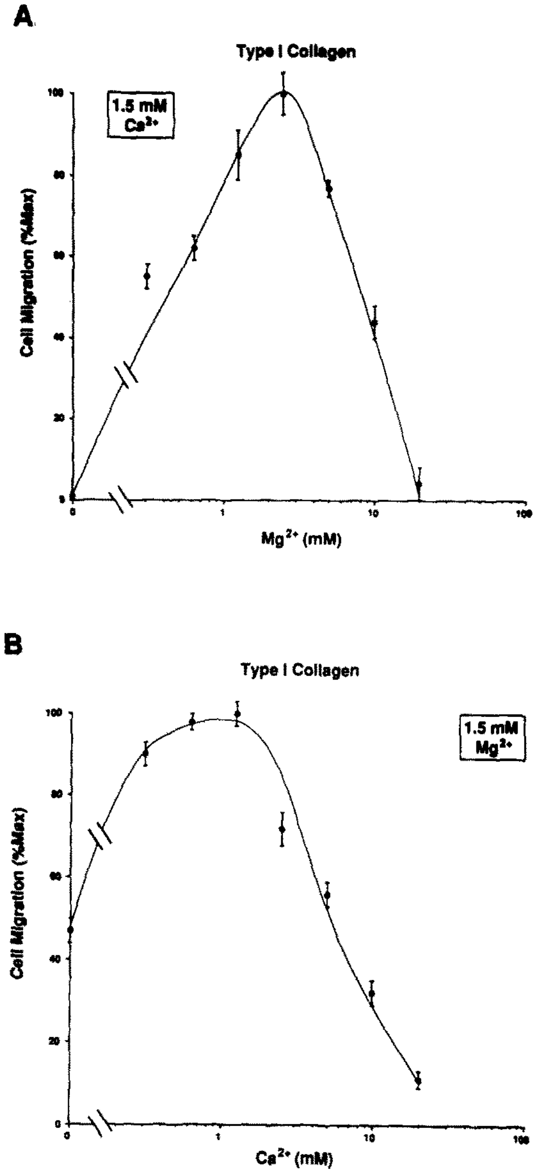


Figure 4. Effects of Ca^{2+} and Mg^{2+} in combination on fibroblast migration on type I collagen. Migration was determined and quantitated as described in Fig. 3 with 1.5 mM Ca^{2+} (*A*) or Mg^{2+} (*B*) plus a titration of the alternate cation on type I collagen–(10 μg/ml) coated filters. The results represent the mean ± SD of three experiments done in triplicate. 100% (mean = 145 cells ± SD/high-powered field) was observed at 2.5 mM Mg^{2+}/1.5 mM Ca^{2+} (*A*), and at 1.5 mM Mg^{2+}/1.25 mM Ca^{2+} (*B*). No migration was observed in the absence of divalent cations.

Discussion

Evidence derived from studies of integrin–ligand interactions using integrin-mediated cell adhesion, affinity chromatography, and modified Boyden chamber migration assays demonstrate that Ca^{2+} can reverse the Mg^{2+}-dependent, $\alpha_2\beta_1$-mediated adhesion and dramatically affect the migration of fibroblasts to type I collagen substrates. That WI38 cells were still capable of Mg^{2+}-dependent adhesion after preincubation with Ca^{2+} alone suggests that exposure to Ca^{2+} is not detrimental to the cell or receptor. We also demonstrate that Ca^{2+} can specifically elute the fibroblast $\alpha_2\beta_1$ integrin from a type I collagen-Sepharose column when it is bound in the presence of Mg^{2+}. Interestingly, while WI38 cells migrate on type I collagen in the presence

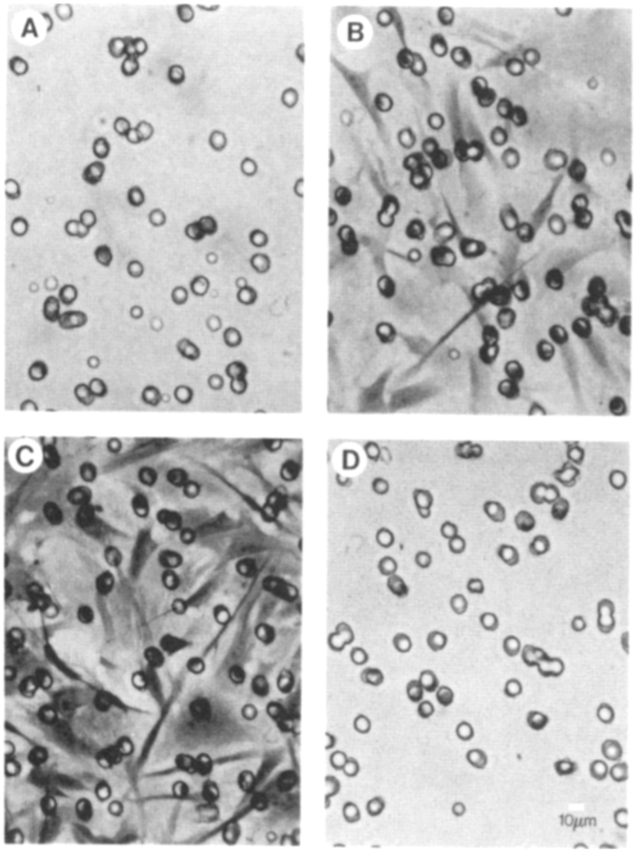


Figure 5. Comparison of maximum migration of WI38 fibroblasts on type I collagen under various cationic conditions. Light micrographs shown are representative examples of maximum migration in modified Boyden Chamber assays (see Material and Methods and Figs. 3 and 4) on type I collagen-(10 μg/ml) coated filters under various divalent cation conditions. Ca^{2+} alone (1.25 mM) (*A*), Mg^{2+} alone (1.25 mM) (*B*), Mg^{2+}/Ca^{2+} combination (2.5 mM/1.5 mM) (*C*), and no cations (*D*). Micrographs shown are at a magnification of 200 using an inverted light microscope.

of Mg^{2+} alone and not in Ca^{2+} alone, a combination of Mg^{2+} and Ca^{2+} with Mg^{2+} in a slight excess caused a twofold enhancement of migration. Taken together, these results suggest that the relative concentrations of extracellular Mg^{2+} and Ca^{2+} could be involved in the regulation of the function of this integrin and could influence the migratory behavior of fibroblasts. It has also been proposed that $\alpha_3\beta_1$ can be regulated by a shift in divalent cation concentration (Elices et al., 1991). These data, together with our results demonstrating that Ca^{2+} can also elute $\alpha_5\beta_1$ and $\alpha_v\beta_1$ bound to ligand in Mg^{2+} (unpublished observations), may reflect a general inhibitory effect of Ca^{2+} on the function of β_1 integrins and indicate that Mg^{2+}/Ca^{2+} ratios may critically affect the binding function of β_1 integrins.

In recent studies using $\alpha_v\beta_1$ and $\alpha_v\beta_3$, two integrins sharing a common α subunit, we were surprised to find that the two receptors function in different extracellular cation environments (Kirchhofer et al., 1991). We and others (Edwards et al., 1988; Loftus et al., 1990; Dransfield et al., 1990) have proposed that residues on the β subunit might participate with the proposed cation binding sequences on the α subunit to provide the sixth coordination site (−Z) for divalent cation binding. Recent crystallographic studies of the cation-binding loops (EF loops or hands) in parvalbumin, demonstrate that cations with an ionic radius closest to 1 Å are favored thermodynamically. Those with smaller ionic radii are more constraining, and thus require more energy. Of all the physiological cations, Ca^{2+} comes closest with an ionic radius of 0.94 Å, and this accounts for its demonstrated higher affinity binding in these cation-binding loops (Lehky et al., 1977; Pechére, 1977; Wnuk et al., 1982). Mg^{2+}, however, with an ionic radius of 0.65 Å, also binds in these loops and crystallographic data show that the coordination spheres of parvalbumin that contain these cation-binding domains contract and become more constrained in the presence of Mg^{2+} when compared with Ca^{2+} (Declercq et al., 1991). If one assumes that the same cation-dependent conformational changes occur in the cation-binding domains of integrins, and involvement of the β subunit in at least one of these domains is critical for ligand binding, a mechanism based on fluctuations in the relative concentrations of Mg^{2+} and Ca^{2+}, resulting in changes in the affinity of integrins, could explain how their function is up- and downregulated during a cellular process such as migration.

Under normal physiological conditions, the extracellular environment has about a 1.5 mM higher concentration of Ca^{2+} than Mg^{2+} (Olinger, 1989). Our in vitro results suggest that fibroblasts are capable of a certain level of $\alpha_2\beta_1$-mediated adhesion (Fig. 1, *A* and *B*) and migration (Fig. 4, *A* and *B*) on type I collagen under these conditions. However, our data also suggest that the potential exists for a twofold increase in this activity. It is not obvious whether sufficient Mg^{2+} could be recruited to affect the extracellular Mg^{2+} concentration. Interestingly, the concentration of intracellular Mg^{2+} in the typical mammalian cell is reported to be between 15 and 30 mM while intracellular Ca^{2+} is only about 1–2 mM (Polimeni and Page, 1973; Henrotte, 1988; Caddell and Reed, 1989; Alberts et al., 1989). In the case of tissue injury, for example, it is possible that a local increase in the extracellular Mg^{2+} levels might occur as the damaged tissue releases its cellular contents. One could speculate that such an increase in extracellular Mg^{2+} might stimulate strong platelet adhesion to collagen through $\alpha_2\beta_1$ (Santoro, 1986). A Mg^{2+} gradient, set up locally from the site of injury, along with growth factors released from the platelet, may then provide the stimulus and directional signaling necessary to mobilize fibroblasts and other cells required for a successful wound healing response. This mechanism appears plausible in light of the results showing that even subtle changes in this cation ratio can dramatically affect a cell's ability to migrate on type I collagen.

Another type of regulation which could influence an integrin-mediated wound migratory response might be factors that deplete extracellular Ca^{2+} levels. Ca^{2+}-binding proteins released from platelets or other cells might serve locally as Ca^{2+} chelators. It is well known that transmembrane Ca^{2+} fluxes via voltage-gated Ca^{2+} channels in the plasma membrane are associated with amoeboid cell movement (Cooper and Schliwa, 1988). A recent report (Fujimoto et al., 1991) suggests that a GPIIb-IIIa complex-associated Ca^{2+} channel facilitates extracellular Ca^{2+} influx across the plasma membrane of platelets after thrombin stimulation. Whether resulting from increases in extracellular Mg^{2+}, de-

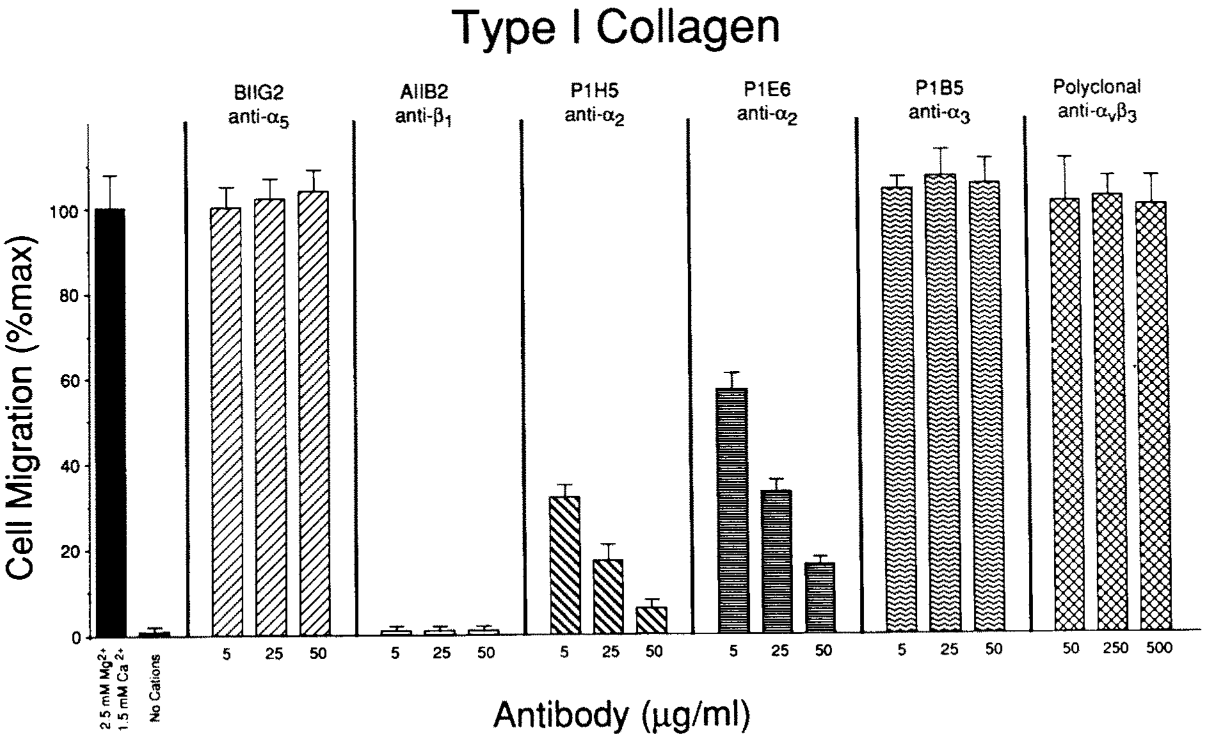


Figure 6. Effects of inhibitory anti-integrin monoclonal/polyclonal antibodies on WI38 fibroblast migration on type I collagen. Migration was determined and quantitated as described in Fig. 3 in the presence of 2.5 mM Mg^{2+}/1.5 mM Ca^{2+} and the indicated purified antibody concentrations. 100% (mean = 150 cells ± SD/high-powered field) is defined as that observed with 2.5 mM Mg^{2+}/1.5 mM Ca^{2+} without any antibody. The results represent the mean ± SD of two experiments done in duplicate. No migration was observed in the absence of divalent cations.

creases in extracellular Ca^{2+}, or a combination of both, it seems possible that alterations of the extracellular cation environment can occur, and that these changes can influence the behavior of cells during an integrin-mediated migratory response.

Indirect support for such speculation has been documented where, in cases of trauma, such as severe burns, spinal cord injury, and myocardial ischemia, there is an increased requirement for extracellular Mg^{2+} during recovery from these injuries, and internal mechanisms are established in an attempt to maintain elevated extracellular Mg^{2+} levels even at the expense of healthy adjacent tissue (Hearse et al., 1978; Cunningham et al., 1987; Demediuk et al., 1990). A report illustrating that capillary endothelial cell proliferation and migration, both important for revascularization after trauma, are enhanced in the presence of elevated extracellular Mg^{2+} (Banai et al., 1990) suggests that these extracellular Mg^{2+} increases may provide a physiological stimulus for endothelial cell migration. Our data demonstrate that the same appears to be true for fibroblasts which are a predominant cell type responsible for re-establishing the extracellular matrix in wound areas (Clark, 1990). Support for this hypothesis is also suggested in a report demonstrating that increased extracellular Ca^{2+} significantly inhibits both keratinocyte chemotaxis and adhesion on type I collagen. Using an in vivo rat model, they demonstrated further that topical Ca^{2+} significantly delays wound contraction characteristic of a chronic or impaired wound, though the correlation to integrin function was not made (Sank et al., 1989). However, recent inhibitory mAb studies suggest that $\alpha_2\beta_1$ appears to be the integrin responsible for migration of the human keratinocyte cell line HaCaT on type I collagen (Scharffetter-Kochanek et al., 1992), and this integrin may account for the cation effects observed in the system described above. Finally, the leukocyte β_2 integrins appear to undergo conformational changes which stimulate their ligand binding functions (Springer, 1990). In one of these studies, a mAb was defined which reacted with all three β_2 integrins only after leukocyte activation and only in the presence of Mg^{2+} (Dransfield and Hogg, 1989). This work raised the possibility that activation may result in integrin conformational changes which allow Mg^{2+} binding or that Mg^{2+} binding is required for the conformational changes to occur.

Together, these findings suggest that during injury a resulting shift in the relative concentrations of extracellular Ca^{2+} and Mg^{2+} favoring Mg^{2+} could facilitate and/or enhance a potential integrin-mediated wound migratory response. As the injury is repaired and the extracellular environment is normalized, the entire mechanism could downregulate, and the Mg^{2+}-dependent integrins would return to their normal physiological state. Such sensitivity to even subtle changes in the ratio between these two extracellular divalent cations may act to control the migration of cells such as fibroblasts, keratinocytes, leukocytes, and endothelial cells, all of which play crucial roles in a successful wound healing response.

We would like to thank Drs. M. Johansson, E. Pasquale, E. Ruoslahti, B. Tomasini-Johansson, and B. Vogel for helpful comments on the manuscript. AIIB2 and BIIG2 ascites were the generous gift of Dr. C. Damsky. P1H5 was the generous gift of Dr. E. Wayner.

This work was supported by Grants HL38417 from the National Heart Lung and Blood Institute, and Cancer Center Support Grant CA30199 from the National Cancer Institute, National Institutes of Health.

Received for publication 9 October 1991 and in revised form 18 March 1992.

References

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, editors. 1989. *In* Molecular Biology of the Cell. Garland Publishing, Inc., New York. 301.

Altieri, D. C., and T. S. Edgington. 1988. A monoclonal antibody reacting with distinct adhesion molecules defines a transition in the functional state of the receptor CD11b/CD18 (Mac-1). *J. Immunol.* 141:2656–2660.

Argraves, W. S., S. Suzuki, H. Arai, K. Thompson, M. D. Pierschbacher, and E. Ruoslahti. 1987. Amino acid sequence of the human fibronectin receptor. *J. Cell Biol.* 105:1183–1190.

Banai, S., L. Haggroth, S. E. Epstein, and W. Casscells. 1990. Influence of extracellular magnesium on capillary endothelial cell proliferation and migration. *Circ. Res.* 67:645–650.

Bodary, S. C., and J. W. McLean. 1990. The integrin β_1 subunit associates with the vitronectin receptor α_v subunit to form a novel vitronectin receptor in a human embryonic kidney cell line. *J. Biol. Chem.* 265:5938–5941.

Bray, P. F., C. S.-I. Leung, and M. A. Shuman. 1990. Human platelets and megakaryocytes contain alternatively spliced glycoprotein IIb mRNAs. *J. Biol. Chem.* 265:9587–9590.

Brown, N. H., D. L. King, M. Wilcox, and F. C. Kafatos. 1989. Developmentally regulated alternative splicing of Drosophila integrin PS2 α transcripts. *Cell.* 59:185–195.

Caddell, J. L., and G. F. Reed. 1989. Validity of the parenteral magnesium load test for mature mammals. *Magnesium.* 8(2):65–70.

Chatila, T. A., R. S. Geha, and M. A. Arnaout. 1989. Constitutive and stimulus-induced phosphorylation of CD11/CD18 leukocyte adhesion molecules. *J. Cell Biol.* 109:3435–3444.

Cheresh, D. A., R. Pytela, M. D. Pierschbacher, F. G. Klier, E. Ruoslahti, and R. A. Reisfeld. 1987. An Arg-Gly-Asp-directed receptor on the surface of human melanoma cells exists in a divalent cation-dependent functional complex with the disialoganglioside GD2. *J. Cell Biol.* 105:1163–1173.

Cierniewski, C. S., J. Krzeslowska, Z. Pawlowka, H. Witas, and M. Meyer. 1989. Palmitylation of the glycoprotein IIb-IIIa complex in human blood platelets. *J. Biol. Chem.* 264:12158–12164.

Clark, A. F. 1990. Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. *J. Invest. Dermatol.* 94:128s–134s.

Conforti, G., A. Zanetti, I. Pasquali-Ronchetti, D. Quaglino, Jr., P. Neyroz, and E. Dejana. 1990. Modulation of vitronectin receptor binding by membrane lipid composition. *J. Biol. Chem.* 265:4011–4019.

Cooper, M. S., and M. Schliwa. 1988. Ca-channels and amoeboid cell movement. *In* Signal Transduction in Cytoplasmic Organization and Cell Motility. P. Satir, J. S. Condelis, and E. Lazarides, editors. Alan R. Liss, Inc., New York. 271–278.

Cunningham, J. J., R. D. Anbar, and J. D. Crawford. 1987. Hypomagnesemia: a multifactorial complication of treatment of patients with severe burn trauma. *J. Parenter. Enteral Nutr.* 11(4):364–367.

Dahl, S. C., and L. B. Grabel. 1989. Integrin phosphorylation is modulated during the differentiation of F-9 teratocarcinoma stem cells. *J. Cell Biol.* 108:183–190.

Declercq, J., B. Tinant, J. Parello, and J. Rambaud. 1991. Ionic interactions with parvalbumins. Crystal structure determination of pike 4.10 parvalbumin in four different ionic environments. *J. Mol. Biol.* 220:1017–1039.

Defilippi, P., G. Truffa, G. Stefanuto, F. Altruda, L. Silengo, and G. Tarone. 1991. Tumor necrosis factor α and interferon γ modulate the expression of the vitronectin receptor (integrin β_3) in human endothelial cells. *J. Biol. Chem.* 266:7638–7645.

Demediuk, P., M. Lemke, and A. I. Faden. 1990. Spinal cord edema and changes in tissue content of Na^+, K^+, and Mg^{2+} after impact trauma in rats. *Adv. Neurol.* 52:225–232.

Dransfield, I., and N. Hogg. 1989. Regulated expression of Mg^{2+} binding epitope on leukocyte integrin α subunits. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3759–3765.

Dransfield, I., A-M. Buckle, and N. Hogg. 1990. Early events of the immune response mediated by leukocyte integrins. *Immunol. Rev.* 114:29–44.

Edwards, J. G., H. Hameed, and G. Campbell. 1988. Induction of fibroblast spreading by Mn^{2+}: a possible role for unusual binding sites for divalent cations in receptors for proteins containing Arg-Gly-Asp. *J. Cell Sci.* 89: 507–513.

Elices, M. J., and M. E. Hemler. 1989. The human integrin VLA-2 is a collagen receptor on some cells and a collagen/laminin receptor on others. *Proc. Natl. Acad. Sci. USA.* 86:9906–9910.

Elices, M. J., L. A. Urry, and M. E. Hemler. 1991. Receptor functions for the integrin VLA-3: Fibronectin, Collagen, and Laminin binding are differentially influenced by ARG-GLY-ASP peptide and by divalent cations. *J. Cell Biol.* 112:169–181.

Fitzgerald, L. A, and D. R. Phillips. 1985. Calcium regulation of the platelet membrane glycoprotein IIb-IIIa complex. *J. Biol. Chem.* 260:11366–11374.

Freed, E., J. Gailit, P. van der Geer, E. Ruoslahti, and T. Hunter. 1989. A novel integrin β subunit is associated with the vitronectin receptor α subunit (α_v) in a human osteosarcoma cell line and is a substrate for protein kinase C. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:2955–2965.

Fujimoto, T., K. Fujimura, and A. Kuramoto. 1991. Electrophysiological evidence that glycoprotein IIb-IIIa complex is involved in calcium channel activation on human platelet plasma membrane. *J. Biol. Chem.* 266:16370–16375.

Fujimura, K., and D. R. Phillips. 1983. Calcium cation regulation of glycoprotein IIb-IIIa complex formation in platelet plasma membranes. *J. Biol. Chem.* 258:10247–10252.

Hall, D. E., L. F. Reichardt, E. Crowley, B. Holley, H. Moezzi, A. Sonnenberg, and C. H. Damsky. 1990. The α_1/β_1 and α_6/β_1 integrin heterodimers mediate cell attachment to distinct sites on laminin. *J. Cell Biol.* 110:2175–2184.

Hara, T., and S. M. Fu. 1986. Phosphorylation of α,β subunits of 180/100 Kd polypeptides (LFA-1) and related antigens. *In* Leukocyte typing II: human myeloid and hematopoietic cells. Vol. 3. E. L. Reinherz, B. F. Haynes, L. M. Nadler, and I. D. Bernstein, editors. Springer-Verlag, New York. 77–84.

Hayman, E. G., M. D. Pierschbacher, Y. Ohgren, and E. Ruoslahti. 1983. Serum spreading factor (vitronectin) is present at the cell surface and in tissues. *Proc. Natl. Acad. Sci. USA.* 80:4003–4007.

Hearse, D. J., D. A. Stewart, and M. V. Braimbridge. 1978. Myocardial protection during ischemic cardiac arrest: The importance of magnesium in cardioplegic infusates. *J. Thorac. Cardiovasc. Surg.* 75:877–885.

Heino, J., and J. Massague. 1989. Transforming growth factor-β switches the pattern of integrins expressed in MG-63 human osteosarcoma cells and causes a selective loss of cell adhesion to laminin. *J. Biol. Chem.* 264:21806–21811.

Heino, J., R. A. Ignotz, M. E. Hemler, C. Crouse, and J. Massague. 1989. Regulation of cell adhesion receptors by transforming growth factor-β. *J. Biol. Chem.* 264:380–388.

Hemler, M. E. 1990. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. *Annu. Rev. Immunol.* 8:365–400.

Henrotte, J. G. 1988. Genetic regulation of blood and tissue magnesium content in mammals. *Magnesium.* 7(5–6):306–314.

Hirst, R., A. Horwitz, C. Buck, and L. Rohrschneider. 1986. Phosphorylation of the fibronectin receptor complex in cells transformed by oncogenes that encode tyrosine kinases. *Proc. Natl. Acad. Sci. USA.* 83:6470–6474.

Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48: 549–554.

Ignotz, R. A., J. Heino, and J. Massague. 1989. Regulation of cell adhesion receptors by transforming growth factor-β. *J. Biol. Chem.* 264:389–392.

Kirchhofer, D., L. R. Languino, E. Ruoslahti, and M. D. Pierschbacher. 1990. $\alpha_2\beta_1$ integrins from different cell types show different binding specificities. *J. Biol. Chem.* 265:615–618.

Kirchhofer, D., J. Grzesiak, and M. D. Pierschbacher. 1991. Calcium as a potential physiological regulator of integrin-mediated cell adhesion. *J. Biol. Chem.* 266:4471–4477.

Kretsinger, R. G. 1976. Calcium-binding proteins. *In* Annual Review in Biochemistry. Vol. 45. E. S. Snell, P. D. Boyer, A. Meister, and C. C. Richardson, editors. Annual Reviews, Inc., Palo Alto, CA. 239–266.

Kunicki, T., D. Nugent, S. Staats, R. Orchekowski, E. Wayner, and W. Carter. 1988. The human fibroblast class II extracellular matrix receptor mediates platelet adhesion to collagen and is identical to the platelet glycoprotein Ia-IIa complex. *J. Biol. Chem.* 263:4516–4519.

Languino, L. R., K. R. Gehlsen, E. Wayner, W. G. Carter, E. Engvall, and E. Ruoslahti. 1989. Endothelial cells use $\alpha_2\beta_1$ integrin as a laminin receptor. *J. Cell Biol.* 109:2455–2462.

Lebien, T. W., D. R. Boue, J. G. Bradley, and J. H. Kersey. 1982. Antibody affinity may influence antigenic modulation of the common acute lymphoblastic leukemia antigen in vitro. *J. Immunol.* 129:2287–2292.

Lehky, P., M. Comte, E. H. Fischer, and E. A. Stein. 1977. A new solid-phase chelator with high affinity and selectivity for calcium: parvalbumin-polyacrylamide. *Anal. Biochem.* 82:158–169.

Loftus, J. C., E. F. Plow, L. K. Jennings, and M. H. Ginsberg. 1988. Alternative proteolytic processing of platelet membrane glycoprotein IIb. *J. Biol. Chem.* 263:11025–11028.

Loftus, J. C., T. E. O'Toole, E. F. Plow, A. Glass, A. L. Frelinger III, and M. H. Ginsberg. 1990. A β_3 integrin mutation abolishes ligand binding and alters divalent cation dependent conformation. *Science (Wash. DC).* 249: 915–918.

Lotz, M. M., C. A. Korzelius, and A. M. Mercurio. 1990. Human colon carcinoma cells use multiple receptors to adhere to laminin: involvement of $\alpha_6\beta_4$ and $\alpha_2\beta_1$ integrins. *Cell Regul.* 1:249–257.

Marguerie, G. A., and E. F. Plow. 1983. The fibrinogen-dependent pathway of platelet aggregation. *Ann. NY Acad. Sci.* 408:556–567.

Olinger, M. L. 1989. Disorders of calcium and magnesium metabolism. *Emerg. Med. Clin. North Am.* 7(4):795–822.

Parise, L. V., A. B. Criss, L. Nannizzi, and M. R. Wardell. 1990. Glycoprotein IIIa is phosphorylated in intact human platelets. *Blood.* 75:2263–2268.

Pechére, J. F. 1977. The significance of Parvalbumin among muscular calciproteins. *In* Calcium-Binding Proteins and Calcium. R. H. Wasserman, R. Corradino, E. Carafoli, R. H. Kretsinger, D. H. MacLennan, and F. L. Siegel, editors. Elsevier/North Holland, New York. 213–221.

Phillips, D. R., I. F. Charo, L. V. Parise, and L. A. Fitzgerald. 1988. The platelet membrane glycoprotein IIb-IIIa complex. *Blood.* 71:831–843.

Polimeni, P. I., and E. Page. 1973. Magnesium in heart muscle. *Circ. Res.* 33: 367–374.

Pytela, R., M. D. Pierschbacher, and E. Ruoslahti. 1985. Identification and isolation of a 140 kd cell surface receptor with properties expected of a fibronectin receptor. *Cell.* 40:191–198.

Ruoslahti, E., and M. D. Pierschbacher. 1987. New perspectives in cell adhesion: RGD and integrins. *Science (Wash. DC).* 238:491–497.

Ruoslahti, E., E. G. Hayman, M. D. Pierschbacher, and E. Engvall. 1982. Fibronectin: purification, immunochemical properties, and biological activities. Vol. 82. *In* Methods of Enzymology. S. P. Colowick and N. O. Kaplan, editors. Academic Press Inc. 803–831.

Sank, A., M. Chi, T. Shima, R. Reich, and G. R. Martin. 1989. Increased calcium levels alter cellular and molecular events in wound healing. *Surgery (St. Louis)* 106:1141–1148.

Santoro, S. A. 1986. Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen. *Cell.* 46:913–920.

Santoro, S. A. 1989. Inhibition of platelet adhesion to fibronectin, fibrinogen, and von Willebrand factor substrates by complex gangliosides. *Blood.* 73: 484–489.

Scharffetter-Kochanek, K., C. E. Klein, G. Heinen, C. Mauch, T. Schaefer, B. C. Adelmann-Grill, G. Goerz, N. E. Fusenig, T. M. Krieg, and G. Plewig. 1992. Migration of a human keratinocyte cell line (HACAT) to interstitial collagen type I is mediated by the $\alpha_2\beta_1$-integrin receptor. *J. Invest. Dermatol.* 98:3–11.

Shaw, L. M., J. M. Messier, and A. M. Mercurio. 1990. The activation dependent adhesion of macrophages to laminin involves cytoskeletal anchoring and phosphorylation of the $\alpha_6\beta_1$ integrin. *J. Cell Biol.* 110:2167–2174.

Springer, T. A. 1990. Adhesion receptors of the immune system. *Nature (Lond.).* 346:425–434.

Staatz, W. D., S. M. Rajpara, E. A. Wayner, W. G. Carter, and S. A. Santoro. 1989. The membrane glycoprotein Ia-IIa (VLA-2) complex mediates the Mg^{2+}-dependent adhesion of platelets to collagen. *J. Cell Biol.* 108:1917–1924.

Stallcup, W. B., R. Pytela, and E. Ruoslahti. 1989. A neuroectoderm-associated ganglioside participates in fibronectin receptor-mediated adhesion of germinal cells to fibronectin. *Dev. Biol.* 132:212–229.

Suzuki, S., M.D. Pierschbacher, E. G. Hayman, K. Nguyen, Y. Ohgren, and E. Ruoslahti. 1984. Domain structure of vitronectin. *J. Biol. Chem.* 259: 15307–15314.

van Kuppevelt, T. H. M. S. M., L. R. Languino, J. O. Gailit, S. Suzuki, and E. Ruoslahti. 1989. An alternative cytoplasmic domain of the integrin β_3 subunit. *Proc. Natl. Acad. Sci. USA.* 86:5415–5418.

Vogel, B. E., G. Tarone, F. G. Giancotti, J. Gailit, and E. Ruoslahti. 1990. A novel fibronectin receptor with an unexpected subunit composition ($\alpha_v\beta_1$). *J. Biol. Chem.* 265:5934–5937.

Wayner, E. A., and W. G. Carter. 1987. Identification of multiple cell adhesion receptors for collagen and fibronectin in human fibrosarcoma cells possessing unique α and common β subunits. *J. Cell Biol.* 105:1873–1884.

Wnuk, W., J. A. Cox, and E. A. Stein. 1982. Parvalbumin and other soluble high-affinity calcium-binding proteins from muscles. *In* Calcium and Cell Function. Vol II. W. Y. Cheung, editor. Academic Press, New York. 243–278.

Wright, S. D., and B. C. Meyer. 1986. Phorbol esters cause sequential activation and deactivation of complement receptors on polymorphonuclear leukocytes. *J. Immunol.* 136:1759–1764.