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# Mapping of Potent and Specific Binding Motifs, GLOGEN and GVOGEA, for Integrin $\alpha1\beta1$ Using Collagen Toolkits II and III*[S]

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**Background:** The collagens contain G*x*OGE*x*″ integrin-binding motifs, whose identity and specificity are poorly defined.
**Results:** GLOGEN in collagen III is a high affinity ligand, and GVOGEA in collagen II is a specific ligand for $\alpha1\beta1$.
**Conclusion:** Collagen Toolkits enable such sites to be identified and compared.
**Significance:** GLOGEN- and GVOGEA-containing peptides can be used to characterize the properties of $\alpha1\beta1$.

**Integrins are well characterized cell surface receptors for extracellular matrix proteins. Mapping integrin-binding sites within the fibrillar collagens identified GFOGER as a high affinity site recognized by $\alpha2\beta1$, but with lower affinity for $\alpha1\beta1$. Here, to identify specific ligands for $\alpha1\beta1$, we examined binding of the recombinant human $\alpha1$ I domain, the rat pheochromocytoma cell line (PC12), and the rat glioma Rugli cell line to our collagen Toolkit II and III peptides using solid-phase and real-time label-free adhesion assays. We observed $Mg^{2+}$-dependent binding of the $\alpha1$ I domain to the peptides in the following rank order: III-7 (GLOGEN), II-28 (GFOGER), II-7 and II-8 (GLOGER), II-18 (GAOGER), III-4 (GROGER). PC12 cells showed a similar profile. Using antibody blockade, we confirmed that binding of PC12 cells to peptide III-7 was mediated by integrin $\alpha1\beta1$. We also identified a new $\alpha1\beta1$-binding activity within peptide II-27. The sequence GVOGEA bound weakly to PC12 cells and strongly to activated Rugli cells or to an activated $\alpha1$ I domain, but not to the $\alpha2$ I domain or to C2C12 cells expressing $\alpha2\beta1$ or $\alpha11\beta1$. Thus, GVOGEA is specific for $\alpha1\beta1$. Although recognized by both $\alpha2\beta1$ and $\alpha11\beta1$, GLOGEN is a better ligand for $\alpha1\beta1$ compared with GFOGER. Finally, using biosensor assays, we show that although GLOGEN is able to compete for the $\alpha1$ I domain from collagen IV ($IC_{50}$ ~3 $\mu$M), GFOGER is much less potent ($IC_{50}$ ~90 $\mu$M), as shown previously. These data confirm the selectivity of GFOGER for $\alpha2\beta1$ and establish GLOGEN as a high affinity site for $\alpha1\beta1$.**

Integrins are glycoprotein cell adhesion molecules that transmit bidirectional signals across the plasma membrane and regulate many physiological functions, including cell differentiation, cell migration, and wound healing (1, 2). They interact either with proteins of the extracellular matrix, such as fibronectin and collagen, or with counter-receptors expressed on other cells, as occurs, for example, in the binding of the leukocyte integrins to intercellular and vascular cell adhesion molecules. Integrins are unique in that their adhesiveness can be dynamically regulated through a process termed affinity regulation (inside-out signaling). Thus, stimuli delivered by cell surface receptors, such as those for chemokines, cytokines, or foreign antigens, initiate intracellular signals that impinge on integrin cytoplasmic domains and alter adhesiveness for extracellular ligands. Moreover, ligand binding transduces signals from the integrin extracellular domain to the cytoplasm in the classical direction (outside-in signaling).

Integrins contain two noncovalently associated $\alpha$- and $\beta$-subunits, each with a large extracellular domain, a single transmembrane domain, and, typically, a short cytoplasmic domain. The extracellular segment of the $\alpha$- and $\beta$-subunits has up to 1104 and 778 residues, respectively, with the N-terminal portions of each subunit combining to form a globular ligand-binding "head" connected to the membrane by a long (170 Å) stalk. In mammals, 24 integrins have been identified to date, resulting from different pairings among 18 $\alpha$- and 8 $\beta$-subunits (3). Half of the integrin $\alpha$-subunits (including the collagen-binding subset of $\beta1$ integrins) contain a domain of ~200 amino acids known as the inserted (I) domain (or the von Willebrand factor A domain). This I domain is the major ligand-binding site in all $\alpha$I-containing integrins (4, 5), including the collagen-binding integrins, $\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$, and $\alpha11\beta1$ (3), which all interact with native triple-helical collagen through this domain.

Ligand binding to the integrin I domain requires a divalent cation coordinated within an acidic pocket known as the metal ion-dependent adhesion site (MIDAS).[2] The ligand invariably uses a solvent-exposed aspartate or glutamate carboxylate anion to bind the MIDAS cation. Glutamate is the major MIDAS-reactive residue within the fibrillar collagens, and a series of G*x*OGER motifs have been identified and are discussed further below. Two conformations of the $\alpha2$ I domain were revealed by comparing the crystal structures of the free and ligated domains (6, 7), indicating the presence of two activation states for $\alpha2\beta1$ (8).

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⌘ *Author's Choice*—Final version full access.
[S] This article contains supplemental Fig. 1.
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[2] The abbreviations used are: MIDAS, metal ion-dependent adhesion site; SPBA, solid-phase binding assay; PMA, phorbol 12-myristate 13-acetate.

α1β1 (initially termed very late antigen-1) was identified on the surface of T cells from the joints of patients with rheumatoid arthritis (9). α1β1 is highly expressed in vascular and visceral smooth muscle, hepatic stellate cells (10), pericytes (11), mesangial cells (12, 13), bone marrow mesenchymal stem cells (14, 15), chondrocytes (16), neuronal cells (17), the microvascular endothelium, and many white blood cells (18, 19). During development, there is abundant and dynamic expression of α1β1 in the embryonic tissues. α1β1 expression has been shown to be regulated by cytokines, such as TGFβ, interleukin-1β, TNFα, and interferon-γ, whereas PDGF causes down-regulation in fibroblast cells (20–22). Dysregulation of very late antigen-1 has been shown in melanoma, leiomyosarcoma, and bronchoalveolar carcinoma (23–27).

Based on sequence similarity of the α-subunits, collagen-binding integrins can be subdivided into two pairs: 1) α1β1 and α2β1 and 2) α10β1 and α11β1. Nevertheless, binding studies have indicated that α1β1 and α10β1 preferentially bind collagen types IV and VI over fibrillar collagens, whereas α2β1 and α11β1 preferentially bind fibrillar collagens over the network-forming collagens (28, 29).

A binding site for α1β1 was identified in collagen IV, comprising Asp-441 of the α1(IV) chain and Arg-458 of the α2(IV) chain (30, 31). Biophysical studies have identified the most likely register of the chains in collagen IV at this point as α1;α1;α2 (32), and modeling of this peptide onto the α1 I domain structure supports Asp-441 being able to coordinate divalent cation in its MIDAS (33). However, a crystal structure of the complex is lacking.

A process of mapping integrin-binding sites within the fibrillar collagens was initiated by Barnes *et al.* (34), culminating in the identification of GFOGER as the high affinity site within collagen I recognized by α2β1 (35, 36). Other related motifs were identified at conserved locations in the fibrillar collagens (37, 38). Among G*x*OGER motifs, affinity for α2β1 decreased in the order *x* = F, L, M, and A, and similar but non-canonical motifs GLSGER, GQRGER, and GASGER were of intermediate or low affinity, which increased upon cell activation. Notably, GLOGER had relatively higher affinity for the α1 I domain than for the α2 I domain.

We subsequently developed a systematic approach to receptor mapping by synthesizing the entire triple-helical domain of collagens II and III as a set of overlapping homotrimeric peptides (termed the Collagen II and III Toolkits) (39). Study of α2β1 binding to collagen III led to the first observation of a non-GER integrin-binding motif, GLOGEN, within the fibrillar collagens that was unique to collagen III (39). Very recently, a homolog, GFPGEN, was expressed in *Streptococcus* and described as being α1β1-selective (40). Here, we used our Toolkit approach to identify high affinity sites for α1β1 in both Collagen II and III Toolkits using recombinant I domains and integrin-expressing cell lines, and we have identified native collagen sites that are specific or selective for α1β1.

## EXPERIMENTAL PROCEDURES

*Reagents*—Unless stated otherwise, all reagents were from Sigma. Collagen fibers were a gift from Ethicon Corp. (Somerville, NJ), and other monomeric pepsin-digested collagens were from Sigma: human I (C-7774), III (C-4407), IV (C-5533), and V (C-3657) and bovine II (C-1188).

*Peptides*—Toolkit peptides (as C-terminal amides) were synthesized on TentaGel R Ram resin using an Applied Biosystems Pioneer peptide synthesizer as described previously (39). Shorter peptides were made using the same Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) chemistry in a CEM Liberty microwave synthesizer. In either case, fractions containing homogeneous product were identified by analytical HPLC on an ACE Phenyl-300 (5 mm) column, characterized by MALDI-TOF mass spectrometry, pooled, and freeze-dried.

*Antibodies*—Horseradish peroxidase-conjugated mouse anti-GST antibody was from GE Healthcare (catalog no. RPN1236). Mouse anti-α2 I domain monoclonal antibody was from Serotec (product code MCA743), and HRP-conjugated goat anti-mouse IgG was from Dako (catalog no. P0447). Hamster monoclonal antibody to mouse/rat α1 (Ha31/8), and monoclonal antibodies to rat α2 (Ha1/29), mouse α2 (HMα2), and mouse β1 (9EG7) were from Pharmingen.

*Plasmids*—The recombinant human α1 and α2 I domain-encoding plasmids (pGEX-2T-α1-I and pGEX-2T-α2-I) (41) were a generous gift from Dr. D. Tuckwell (F2G Ltd., Manchester, United Kingdom). For binding kinetics, the α1 I domain ORF was cloned in the bacterial expression vector pDEST-N110 (a kind gift of Dr. M. Dyson) as described (42).

*I Domain Expression and Purification*—To express wild-type α1 and α2 I domains, a 100-ml overnight culture of transformants (Origami strain) was used to inoculate 1 liter of Luria broth containing 50 μg/ml ampicillin. The culture was grown for 2 h at 37 °C and then induced at room temperature for 4 h with isopropyl β-D-thiogalactopyranoside (0.1 mM; catalog no. MB1008, Melford Laboratories Ltd., Suffolk, United Kingdom). Cells were harvested by centrifugation at 4500 × *g* for 20 min, and pellets were resuspended in 10 ml of Dulbecco's phosphate-buffered saline containing 1 tablet of protease inhibitor mixture (Roche Applied Science) and 50 mg of lysozyme (Fluka). Suspensions were sonicated, and Triton X-100 was adjusted to 1% (v/v). Suspensions were incubated at room temperature for 15 min on a roller mixer and centrifuged at 18,000 × *g* for 20 min, and supernatants were pooled. The lysate was passed down a glutathione-agarose column equilibrated in TBS (20 mM Tris-HCl (pH 7.5) and 150 mM NaCl); the column was washed with 10 volumes of TBS containing 1 M NaCl and 1% (v/v) Triton X-100, and the GST-I domain fusion proteins were eluted with 10 mM glutathione reduced in 50 mM Tris-HCl (pH 8.0). The proteins were then dialyzed against TBS and concentrated using a Microcon-3 system (Amicon, Gloucestershire, United Kingdom). The I domains were checked for purity and degradation by 10% SDS-PAGE and Western blotting. Nitrocellulose blots were probed with horseradish peroxidase-conjugated anti-GST polyclonal antibody (GE Healthcare).

*I Domain Binding Assay*—I domain adhesion was determined colorimetrically as described (here termed solid-phase binding assay (SPBA)) (43). Peptides were coated at 10 μg/ml for 1 h at 22 °C on Immulon 2HB 96-well plates (Thermo Scientific) and blocked for 1 h with 200 μl of TBS containing 50 mg/ml BSA. Wells were washed four times with 200 μl of adhe-

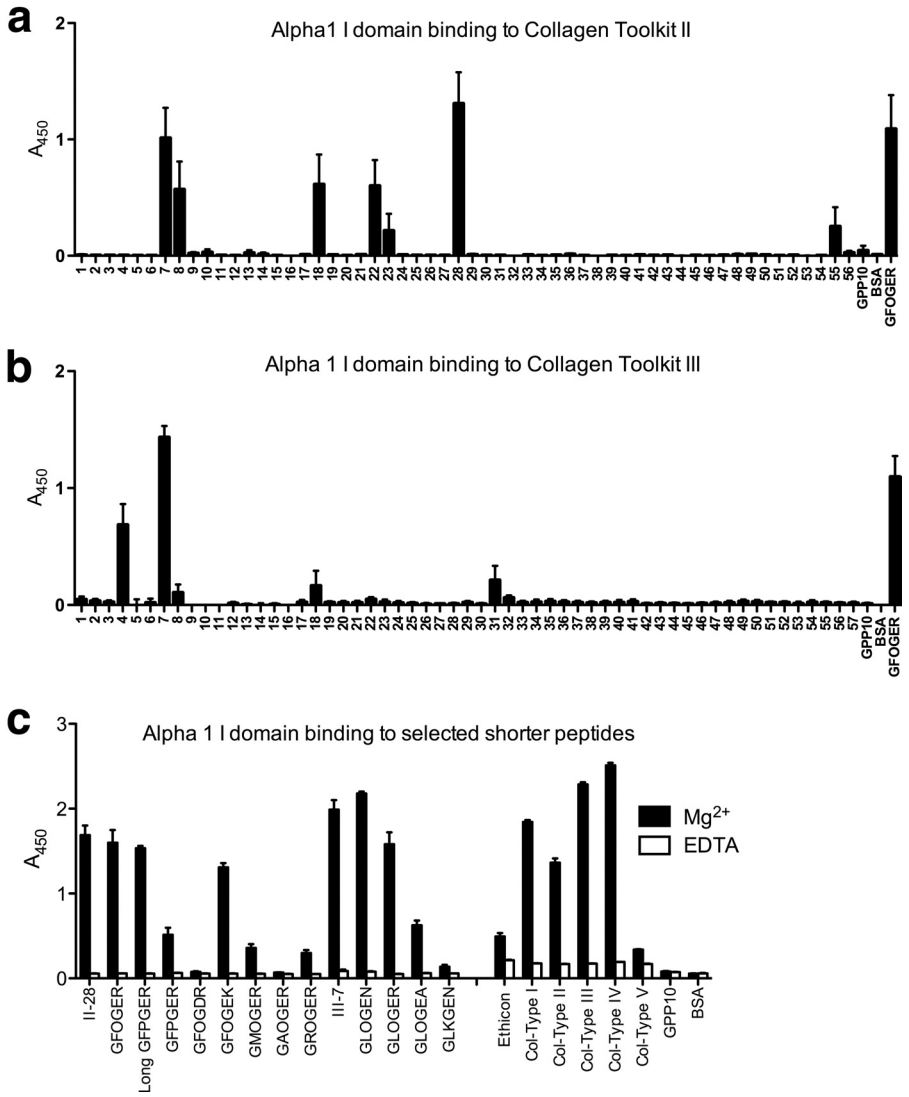


FIGURE 1. **Binding of wild-type α1 I domain to Toolkits II and III and to selected shorter triple-helical peptides.** The recombinant wild-type α1 I domain was incubated in wells of Immulon 2HB 96-well plates coated with peptides, and adhesion was measured in the presence of either 2 mM $Mg^{2+}$ (*black bars*) or 2 mM EDTA (*white bars*) as described under "Experimental Procedures." BSA, GPP10, and GFOGER served as control surface coatings. Experiments were performed in triplicate using 0.1 μg of protein/well. *a*, adhesion to Toolkit II; *b*, adhesion to Toolkit III; *c*, adhesion to selected shorter peptides, formatted with the indicated sequence flanked by $(GPP)_5$ host peptides. Long GFPGER has the same sequence as II-28 but with "O" in its GFOGER motif replaced with "P." Data are the mean ± S.E. of at least six independent experiments. *Col*, collagen.

sion buffer (TBS with 1 mg/ml BSA) before the addition of 100 μl of adhesion buffer containing 1 μg/ml recombinant GST-I domains in the presence of either 2 mM $MgCl_2$ or EDTA for 1 h at room temperature. Wells were washed five times with 200 μl of adhesion buffer containing $MgCl_2$ or EDTA before the addition of 100 μl of adhesion buffer containing HRP-conjugated anti-GST antibody at 1:10,000 dilution for 1 h at room temperature. After washing, color was developed using a TMB Substrate Kit (Pierce) according to the manufacturer's instructions. In some experiments, mouse anti-α2 I domain monoclonal antibody (38) was used at 1:2000 dilution and incubated at room temperature for 45 min, followed by detection using HRP-conjugated anti-mouse IgG at 1:5000 dilution.

*Cell Culture and Adhesion*—The rat PC12 cell line was obtained from the European Collection of Animal Cell Cultures (Porton Down, United Kingdom). Parental mouse myoblast C2C12 cells, which lack endogenous collagen-binding integrins, were stably transfected with human α2 cDNA or human α11 cDNA in the pBJ-1 vector as described previously (44). The derivative clones, C2C12-$\alpha2^+$ (constitutively expressing human α2β1) and C2C12-$\alpha11^+$ (constitutively expressing human α11β1), were maintained in RPMI 1640 medium containing 10% fetal bovine serum, 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml amphotericin. Cells were harvested by centrifugation, washed, and suspended at $0.5 \times 10^6$/ml in TBS supplemented with 5.5 mM glucose and either 2 mM EDTA or $MgCl_2$. SPBA was performed as follows: 100 μl of the cell suspension was added to peptide-coated wells (43) at 20 °C for 60 min. Cell adhesion was determined colorimetrically after lysis using a lactate dehydrogenase-based cell detection kit (Roche Diagnostics) according to the supplier's instructions.

*Real-time Cell Adhesion Using xCELLigence*—The extent of integrin-expressing cell binding to collagen peptides was quan-

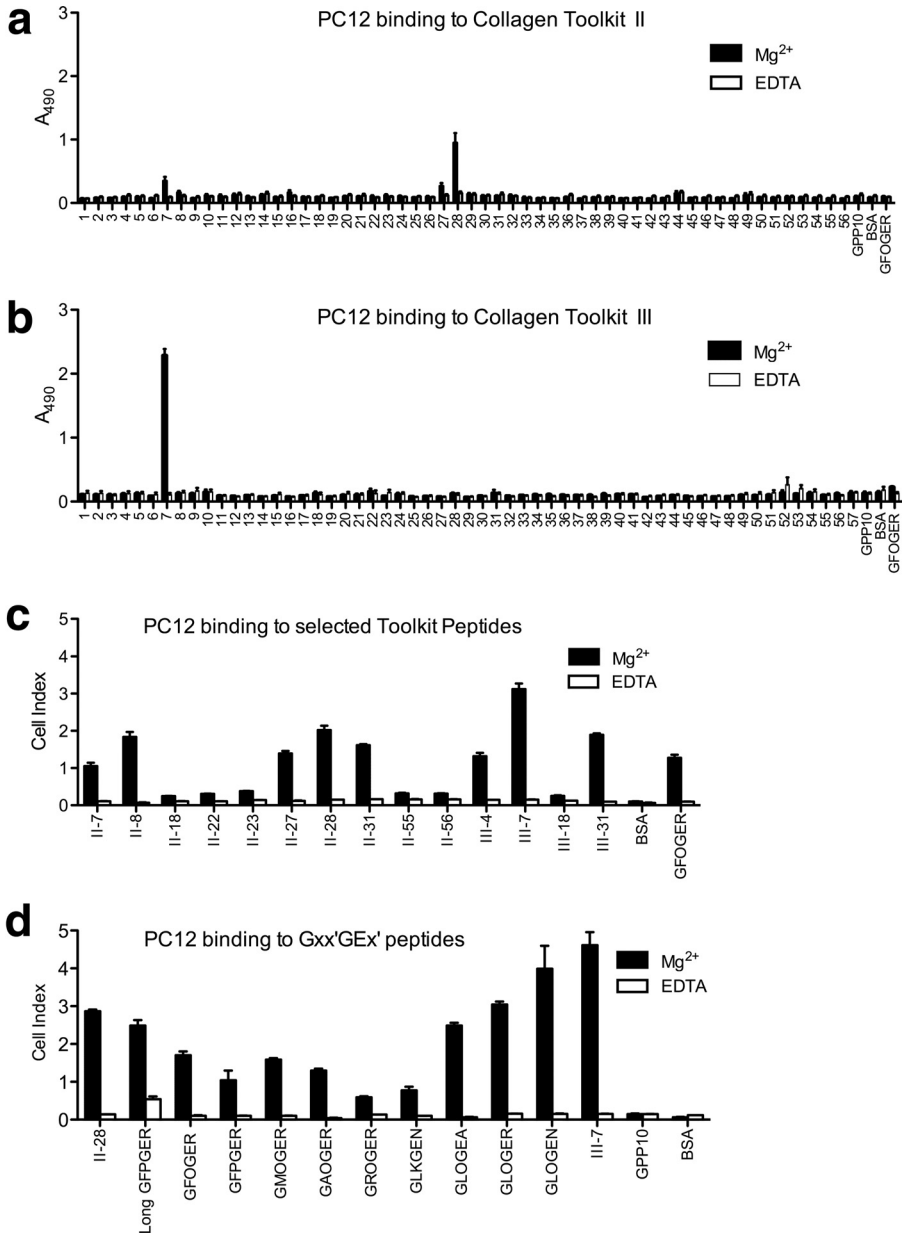


FIGURE 2. **Binding of PC12 cells to Toolkits II and III and selected peptides.** Immulon 2HB 96-well plates or E-Plates™ for the xCELLigence instrument were coated with the indicated peptides as described under "Experimental Procedures." *a* and *b*, PC12 cells were seeded at $10^5$/well, and SPBA was performed after 1 h as described using the colorimetric lactate dehydrogenase assay kit. *c* and *d*, PC12 cells were seeded onto E-Plates at $2.5 \times 10^4$ cells/well, and the cell index was measured continually. PC12 cell binding to Toolkits II (*a*) and III (*b*) is shown. PC12 cell adhesion to the indicated Toolkit peptides (*c*) and to selected shorter integrin-binding peptides (*d*) is shown as end point data after 2 h of adhesion. All experiments were performed in triplicate in the presence of either 2 mM $Mg^{2+}$ or EDTA as indicated. Data are the mean ± S.E. of three independent experiments.

titated using the impedance-based xCELLigence system (Roche Applied Science), which allows label-free dynamic monitoring of cell events in real time. The instrument expresses cell adhesion in cell index units, defined as $(R_n - R_b)/15$, where $R_n$ is the impedance of the well when it contains cells, and $R_b$ is the background impedance of the well containing medium alone.

Briefly, PC12 cells were seeded at 25,000 cells/well in a precoated E-Plate™ (Roche Applied Science) using the same conditions as described for SPBA. For antibody inhibition, cells were preincubated with the indicated amounts of anti-α1 I domain monoclonal antibody for 30 min and then added to collagen peptide-coated wells of E-Plates. The extent of cell adhesion was measured as changes in impedance monitored every minute for 2 h, as we have used previously (45).

*Competitive Binding of α1 and α2 I Domains to Peptide Coatings*—The adsorption of recombinant wild-type I domains onto peptide- or collagen-coated surfaces was measured in the presence of varying concentrations of either GLOGEN or GFOGER using either conventional SPBA, as described above, or the SRU Biosystems BIND Explorer system. This biosensor quantitates the adsorption of biomolecules by measuring the resulting shift in peak wavelength of light reflected from the base of a well. The shift in peak wavelength value (ΔPWV) is directly proportional to the mass of the molecule bound. The

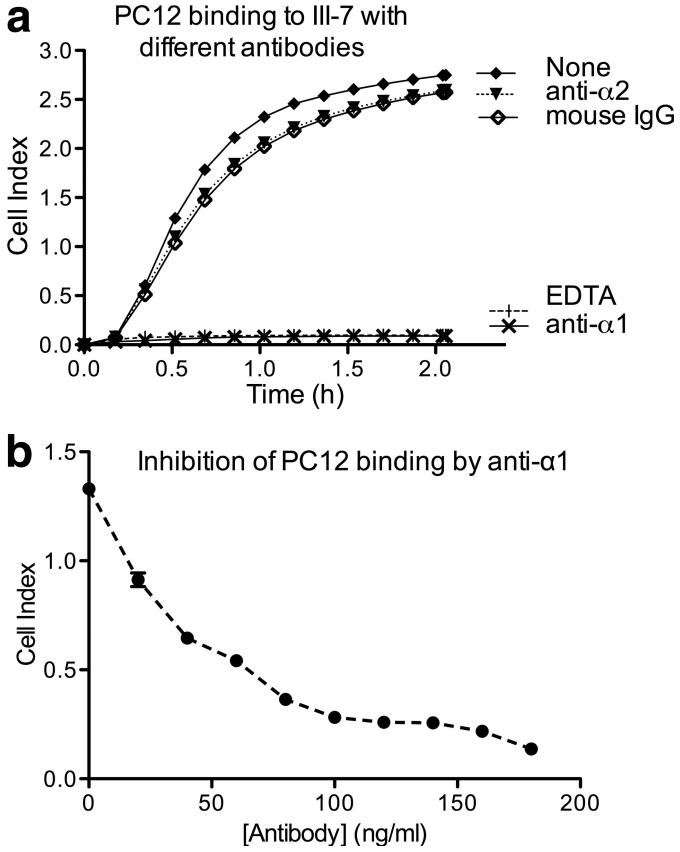


FIGURE 3. **Effect of anti-integrin antibodies on PC12 cell binding to peptide III-7.** Cells were incubated with anti-α1 or anti-α2 antibodies or controls as indicated for 30 min. $Mg^{2+}$-dependent label-free binding of PC12 cells was then measured as described under "Experimental Procedures" and in the legend to Fig. 2 (*c* and *d*). *a*, time course of adhesion measured at 10-min intervals over 2 h expressed as the mean cell index ± S.E. of three experiments. *b*, inhibition of PC12 cell adhesion to peptide III-7 in the presence of increasing levels of anti-α1 antibody shown as end point determinations after 2 h. Data are the mean cell index ± S.E. of five independent experiments. Where not visible, errors lie within the dimensions of the symbols.

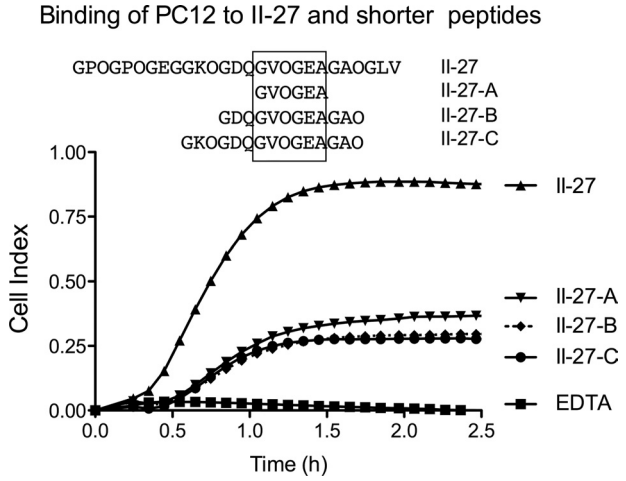


FIGURE 4. **Real-time binding of PC12 cells to II-27 and shorter II-27-derived peptides.** E-Plate™ wells were coated with the indicated peptides as described in the legend to Fig. 2 (*c* and *d*). The sequences of the peptides are shown above the graph. Cell adhesion was monitored for 2 h, and data were collected every minute. Every fifth data point is shown. Experiments were performed in triplicate in the presence of either 2 mM $Mg^{2+}$ or EDTA as indicated, and the mean cell index ± S.E. is shown from four independent experiments.

96-well TiO Bind biosensor plate was coated with peptide (GLOGEN, GFOGER, or GVOGEA; 50 μl at 20 μg/ml in acetate buffer (pH 5.0)). After coating, which was monitored until saturation, the surface was blocked with BSA (2 mg/ml) and then equilibrated in 50 mM Tris-HCl and 140 mM NaCl (pH 7.4) supplemented with 2 mM $MgCl_2$ and 0.5 mg/ml BSA. For the binding step, a fixed concentration of I domain (0.17 μM) was preincubated in solution with a varying amount of GFOGER or GLOGEN for 45 min and then added to the coated sensor, and the binding of the remaining free I domain was measured as ΔPWV. To estimate binding kinetics, peptide coatings were prepared as described above, increasing amounts of I domain (0 – 60 μM) were added, and ΔPWV was measured at saturation. Binding curves were analyzed with GraphPad Prism 5.0d using least-squares fit and constraining $B_{max}$ to the same value for each peptide.

## RESULTS

*Functional Analysis of α1 I Domain by SPBA*—The ligand-binding activity of the recombinant human GST-α1 I domain expressed in bacteria was examined using a conventional colorimetric 96-well SPBA. The I domain was applied to the Collagen II and III Toolkit peptides in the presence or absence of magnesium and detected using anti-GST antibody, and data are shown in Fig. 1. The background response is indicated by wells coated with GPP10 or BSA. A peptide containing the GFOGER motif was used as a positive control in these and all other experiments. The α1 I domain bound in a $Mg^{2+}$-dependent manner to the Toolkit peptides in the rank order II-28, II-7, II-8 = II-18 = II-22, II-55 = II-23 (Fig. 1*a*) and bound to III-7 and III-4 and weakly to III-31, III-18, and III-8 (Fig. 1*b*). Most of the reactive peptides contain a previously identified integrin-specific motif: GFOGER in II-28, GLOGER in II-7 and II-8, GAOGER in II-18, and GROGER in III-4. GLKGEN and GLOGEN are the two motifs found in peptide III-7, the best binder in Toolkit III, which supports better adhesion to the α1 I domain compared with GFOGER (Fig. 1*b*).

*Binding of α1 I Domain to Short Triple-helical Motifs*—To determine the potential adhesiveness of each of the GLKGEN and GLOGEN motifs, two short triple-helical peptides were tested alongside other known G*xx′*GE*x″*-containing peptides and III-7. Fig. 1*c* shows the results, which indicate that binding of the α1 I domain to III-7 is mediated by its GLOGEN motif only. This peptide supported more adhesion than any other motif tested, including GFOGER and GLOGER. In addition, the α1 I domain bound weakly to GLOGEA, GFPGER, GMOGER, and GROGER, all high or moderate affinity motifs for the α2 I domain (39, 46), but not to GAOGER, GLKGEN, or GFOGDR. For comparison, collagens were used in these experiments, and collagen IV proved to be the best substrate, with collagens I, II, and III supporting good adhesion, and collagen V, which lacks GLOGEN or other high affinity motifs, being only slightly more adhesive than negative controls.

*Binding of PC12 Cells to Toolkits II and III*—The PC12 cell line expresses α1β1 as its sole collagen-binding integrin (47, 48) and was therefore used to investigate the binding of α1β1 to collagen in a cellular context. Adhesion to Toolkit II and III peptides was performed first using SPBA, with lactate dehydro-

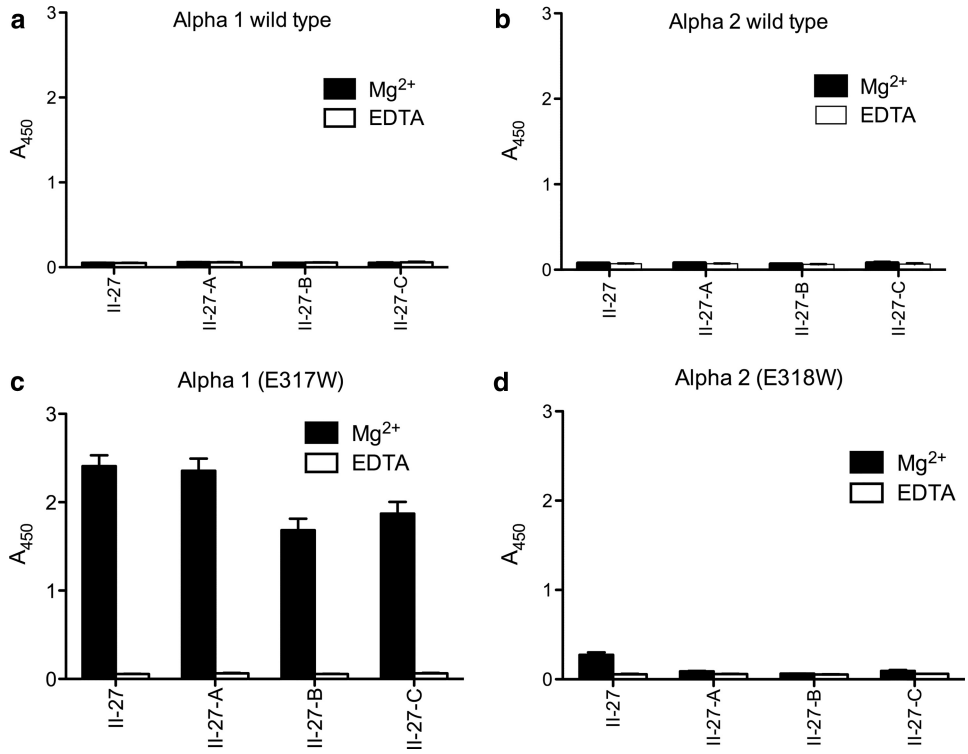


FIGURE 5. **Binding of wild-type and active α1 and α2 I domains to peptide II-27.** Experiments were performed as described in the legend to Fig. 1 using 0.1 μg of protein/well. Solid-phase adhesion of I domains to peptide II-27 or its shorter derivatives (II-27-A, II-27-B, and II-27-C) is shown. *a*, adhesion of the wild-type α1 I domain. *b*, adhesion of the wild-type α2 I domain. *c*, adhesion of the constitutively active E317W α1 I domain. *d*, adhesion of the constitutively active E318W α2 I domain. Data are the mean ± S.E. of four independent experiments, each performed in triplicate.

genase activity reporting the presence of adherent cells as described (45). PC12 cells bound in a $Mg^{2+}$-dependent manner to peptide II-28 and less well to peptides II-7 and II-27 (Fig. 2*a*). Tested in matched experiments with Toolkit III, PC12 cells showed very strong binding to peptide III-7 (Fig. 2*b*).

Second, we used the impedance-based xCELLigence system, which allows sensitive label-free real-time monitoring of cell adhesion (49, 50), to investigate selected established and potential integrin-binding Toolkit peptides. In rank order, III-7 (containing GLOGEN) showed the highest level of binding to PC12 cells, followed by II-28 (containing GFOGER), III-31, II-8, II-31, II-27, III-4, and II-7 (Fig. 2*c*). Using the short peptide set together with III-7 and II-28 as controls, III-7 supported the highest binding, followed by GLOGEN, GLOGER, II-28, and GLOGEA. Moderate binding to GFOGER, GMOGER, and GAOGER was observed, whereas GLKGEN (also present in III-7 alongside GLOGEN) and GROGER showed only slight activity. GFPGER, presented as a short peptide within $(GPP)_5$ hosts, supported weak binding (lower compared with GFOGER), whereas in the context of more primary sequence (long GFPGER), it was almost as active as its hydroxylated equivalent, II-28 (Fig. 2*d*). It should be noted that this real-time technique includes no physical perturbation such as wash steps and reports cell adhesion with great sensitivity. In these experiments, particular motifs tended to show greater adhesion when presented in the context of longer stretches of native sequence, as in Toolkit peptides, and the recombinant I domain bound to a wider range of peptides than the PC12 cells. This applied to GFPGER (supplemental Fig. 1), as well as to GFOGER.

*Inhibition of PC12 Cell Binding to III-7 Using Anti-α1 Antibody*—Adhesion of PC12 cells to peptide III-7, detected using the xCELLigence platform, was inhibited to a similar level upon EDTA treatment or preincubation of cells with anti-α1 inhibitory antibody, but not with inhibitory anti-α2 antibody or control IgG, each used at 10 μg/ml (Fig. 3). Fig. 3*a* shows that preincubation with anti-α2 antibody or mouse IgG did not affect the time dependence of cell binding to peptide III-7 and Fig. 3*b* that anti-α1 inhibitory antibody showed specific dose-dependent inhibition of PC12 cell binding to peptide III-7, with $IC_{50} \sim 40$ ng/ml and near-complete blockade at 180 ng/ml.

*Peptide II-27 and GVOGEA-binding Motif*—Peptide II-27 bound PC12 cells moderately well using real-time assay (Fig. 2*c*) but contains no known integrin-binding site. However, a candidate sequence is GVOGEA, conforming to the general G*x*OGE*x*″ motif. To assess the role of GVOGEA, three peptides containing GVOGEA within sequence derived from II-27 (to provide a larger local context) were synthesized within the host peptide backbone, $(GPP)_5$, and named II-27-A, II-27-B, and II-27-C (Fig. 4). PC12 cells bound to peptides II-27-A, II-27-B, and II-27-C to ~40% of the level attained with II-27, whereas no adhesion was observed in the presence of EDTA. In contrast, the wild-type α1 and α2 I domains did not bind to peptide II-27 or its short derivatives, II-27-A, II-27-B, and II-27-C (Fig. 5, *a* and *b*).

*Active Forms of Integrin α1 Bind to GVOGEA*—To investigate the sequence GVOGEA further, we used constitutively active constructs of the α1 I domain (E317W) (51) and of the α2 I domain (E318W) (52). E317W but not E318W bound strongly

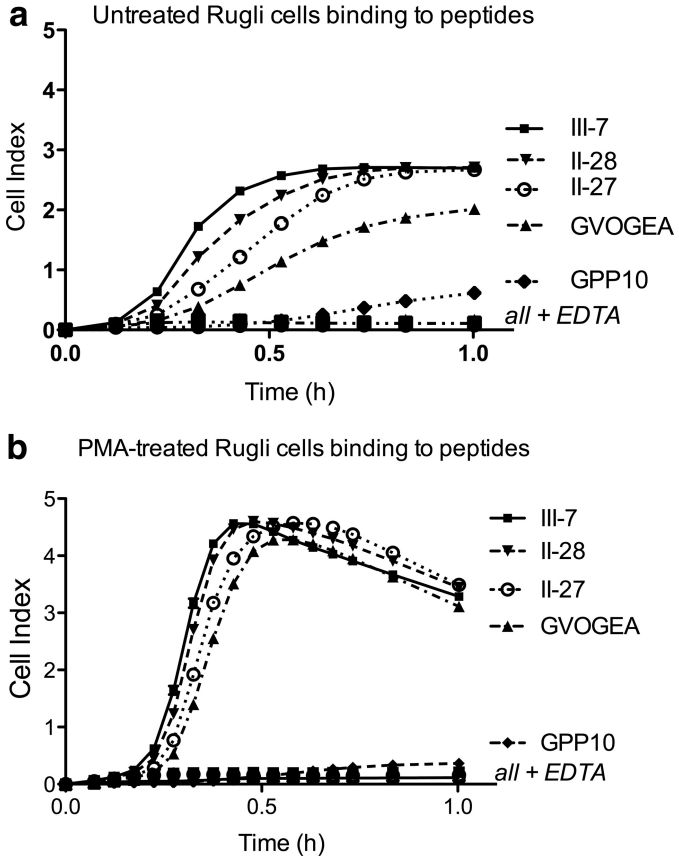


FIGURE 6. **Binding of Rugli cells to selected Toolkit peptides before and after activation by PMA.** Rugli cells were applied to peptide-coated E-Plates™ prepared as described under "Experimental Procedures," and the cell index was recorded at 1-min intervals. For clarity, fewer time points are shown in each graph. The mean cell index ± S.D. is shown from one representative experiment, performed in triplicate, from three similar data sets. The peptides used are indicated. Traces close to the base line include adhesion to the GPP10 control and to the indicated peptides in the presence of EDTA. *a*, adhesion of control Rugli cells. *b*, adhesion of Rugli cells activated, in parallel with controls, for 30 min with 1 μM PMA.

to II-27 and to each of its short derivatives (Fig. 5, *c* and *d*). Although substitution of $Mg^{2+}$ with $Mn^{2+}$, widely used to enhance integrin-binding activity, increased PC12 cell binding to II-27 (65%), GFOGER (40%), and III-7 (26%), measured with real-time binding assay, it had little effect (13%) on PC12 cell adhesion to GVOGEA (data not shown). However, preincubation of another α1β1-expressing cell line, the rat glioma Rugli cell, with 1 μM phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, raised its adhesion to GVOGEA to the same level as to the parent peptide, II-27 (Fig. 6, *a* and *b*). To highlight the selectivity of particular motifs for different integrins, we used the mouse myoblast C2C12 cell line in its parental form, lacking collagen-binding integrins (44), and its derivatives, C2C12-$\alpha2^+$ (expressing human α2β1) and C2C12-$\alpha11^+$ (expressing human α11β1). None of these cells showed any specific binding to II-27 or GVOGEA (or to II-27-B and II-27-C; data not shown) but showed better binding to II-28 than to III-7. In contrast, PC12 cells bound most strongly to III-7 and then to II-27 and II-28 (Fig. 7). Note that the parental C2C12 cells and transfected counterparts bound more to the control peptide (GPP10) than to II-27 or GVOGEA.

*Inhibition of α1 I Domain Binding to Collagen IV by GLOGEN*—α1β1 is known to have high affinity for collagen IV (30). To evaluate the relative affinity of α1β1 for GLOGEN and GFOGER, we used peptide to compete for the α1 I domain from collagen IV, measured using SPBA. The α1 I domain was preincubated with increasing doses of either GLOGEN or the control peptide (GPP10) before addition to collagen IV-coated plates. Fig. 8*a* shows the specific inhibition of α1 binding to collagen IV by the peptide, with $IC_{50} = 2.8$ μM (30 μg/ml). For comparison, GFOGER showed a lesser ability ($IC_{50} \sim 90$ μM, ~1000 μg/ml) to compete with collagen IV for the α1 I domain.

Using the SRU Biosystems Explorer platform, we measured the affinity of the wild-type α1 I domain for immobilized peptides (Fig. 8*b*). Saturation was observed for GLOGEN ($K_d$ = 13 ± 1.9 μM), but adsorption to GFOGER and GVOGEA was not saturated at a protein concentration up to 60 μM. Estimates of $K_d$ of 53 ± 6 and 140 ± 37 μM were obtained for GFOGER and GVOGEA, respectively. All values differed ($p < 0.0001$). In a competitive binding experiment, GLOGEN proved readily able to displace the α1 I domain from coatings of GFOGER or of itself (supplemental Fig. 1, *a* and *b*), whereas GFOGER was inactive at up to 40 μg/ml. Conversely, GFOGER in solution was able to displace the α2 I domain from GFOGER coatings and was a potent competitor of α2 I domain binding to GLOGEN. GLOGEN had little effect on α2 I domain binding to GFOGER while being able to displace the α2 I domain from itself (supplemental Fig. 1, *c* and *d*).

## DISCUSSION

We set out to identify sites within the fibrillar collagens that might bind the resting forms of α1β1 on the cell surface and be selective for integrin α1β1 over α2β1. To this end, we applied recombinant I domains and suitable cells to the Collagen Toolkits and to selected shorter peptides. The use of the recombinant α1 I domain confirmed its ability to bind the expected Toolkit peptides II-28, II-7, II-8, and II-18, which contain known integrin-binding motifs GFOGER, GLOGER, and GAOGER. The α1 I domain also showed moderate reactivity with II-22, II-23, and II-55, all of which contain at least one candidate glutamate residue, but we have not pursued these further. Toolkit III contains just two peptides that bound the α1 I domain strongly, III-4 and III-7, containing GROGER and GLOGEN, respectively, already known as integrin-recognizing motifs (39, 46). We have shown here that GLKGEN, also present in III-7, has only slight integrin-binding activity. Comparing all of these binding motifs suggested that GLOGEN, in the setting of Toolkit peptide III-7 or alone within the $(GPP)_5$ hosts, has the greatest ability to bind the α1 I domain, with GLOGER being of similar affinity and GLOGEA binding weakly.

The recently introduced xCELLigence label-free detection system measures cell adhesion with no washing step, so weaker interactions can be revealed. The method yielded data confirming the ability of PC12 cells to bind the higher affinity Toolkit peptides, III-7, II-7, and II-28 but revealed substantial activity in II-8, II-27, II-31, III-4, and III-31 (all G*x*OGE*x*″-containing peptides), which was less obvious from SPBA.

Peptide II-27 proved to be a moderate ligand for α1β1 in PC12 and Rugli cells as determined using either SPBA or xCEL-

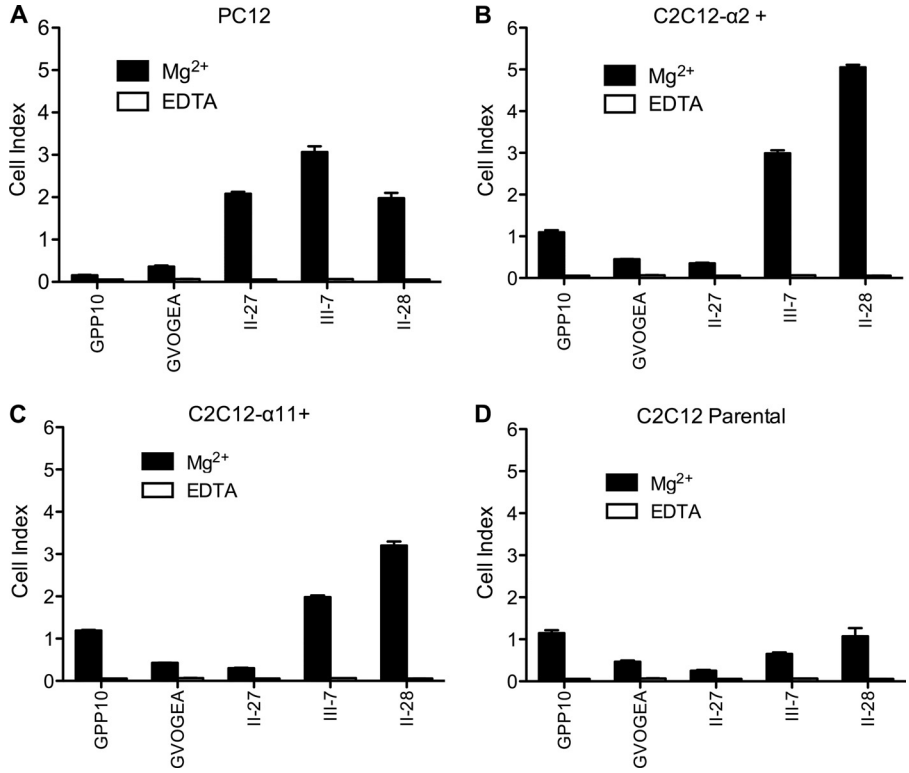


FIGURE 7. **Real-time adhesion of PC12, C2C12-α2$^+$, and C2C12-α11$^+$ cells to selected peptides.** The binding of integrin-expressing cells (25,000 cells/well) was measured using real-time adhesion assay as described under "Experimental Procedures" and in the legend to Fig. 2. Peptides III-7, II-27, II-28, and GVOGEA were used as adhesive coatings, with GPP10 as the control. The cell line and integrin expressed are indicated above each panel, which shows the mean cell index after 2 h ± S.E. from triplicate determinations from each of three experiments.

Ligence. It contains two candidate glutamate-containing motifs, GPOGEG and GVOGEA, in its sequence (GPO**GPOGEG**GKOGDQ**GVOGEA**GAOGV). GPOGEG was discounted because it also occurs in the inactive II-26 peptide. We synthesized the shorter peptides GVOGEA, GDQGVOGEAGAO, and GKOGDQGVOGEAGAO, all within GPP hosts. Each supported PC12 cell adhesion (measured using label-free detection) but at about one-third of the level supported by II-27 itself.

α1β1 has been shown to bind a sequence in collagen IV (31) containing an aspartate residue (Asp-441 in the α1 chain) considered to coordinate its MIDAS metal ion. This is consistent with the flatter surface of the α1 I domain compared with that of α2, which requires the longer glutamate side chain to reach the bound metal ion (7). However, because the binding of PC12 cells to GKOG**D**QGVOGEAGAO was no greater than to GVOGEA, we find no evidence that GKOGDQ can serve as an integrin recognition site, despite its similarity to GPOGDQ, which forms part of the α1β1-binding locus in collagen α1(IV). Docking studies suggest that Lys-456 in the corresponding GAKGRA sequence of collagen α2(IV) is essential, forming a salt bridge with the I domain surface (32). Consistent with this role for the residues surrounding the carboxylate anion of the ligand, homotrimeric GFOGDR proved inactive, suggesting that aspartate, even within an integrin-reactive motif, cannot in isolation substitute for glutamate. Investigating GVOGEA further, we found that the recombinant wild-type α1 I domain was unable to bind any GVOGEA-containing triple-helical peptide as measured using SPBA. Accordingly, we treated Rugli cells with PMA and found that their moderate affinity for the shorter GVOGEA-containing peptides increased to that for the parent peptide, II-27, confirming GVOGEA as a good ligand for α1β1 after activation of the integrin. Of note, treatment with PMA increased both the rate of onset of adhesion and the peak cell index (Fig. 6*b*). C2C12 cells stably expressing either α2β1 or α11β1 did not bind GVOGEA or its parent peptide (II-27), providing further evidence of α1β1 specificity.

GVOGEA in collagen II is unique among the human collagens, but two other related motifs, GVOGER (found in collagens α2(VIII) and α1(XXVIII)) and GVOGEK (in collagen α2(XI)) are promising ligands for α1β1 and, given the presence of the positively charged Arg or Lys residues, should be investigated in the context of other collagen-binding integrins. GVOGDL, occupying in collagen α1(I) the same position as GVOGER in collagen α1(II), is also worthy of investigation.

Taking all of our data together, we conclude that GLOGEN, the first G*xx*′GEN motif identified as an integrin ligand (39), is the most potent ligand for α1β1 discovered among the fibrillar collagen sequences to date. The exact motif occurs only in collagens III and XXII. Collagen III may form heterotypic fibrils with collagen I in skin and other tissues, and collagen XXII is a FACIT collagen (53). Both may therefore enhance the α1β1 reactivity of collagen fibers. Of other such motifs, GSOGEN occurs in collagen II (Toolkit peptides II-6 and II-7) but displays little activity. GSOGEN is conserved at the same locus in collagen α1(I), aligning with GAOGEN in the α2(I) chain. In general, where *x* = A, G*xx*′GE*x*″ motifs are poor integrin ligands (37, 38), consistent with the preference for long hydrophobic residues in this position that support extended contact with the I domain

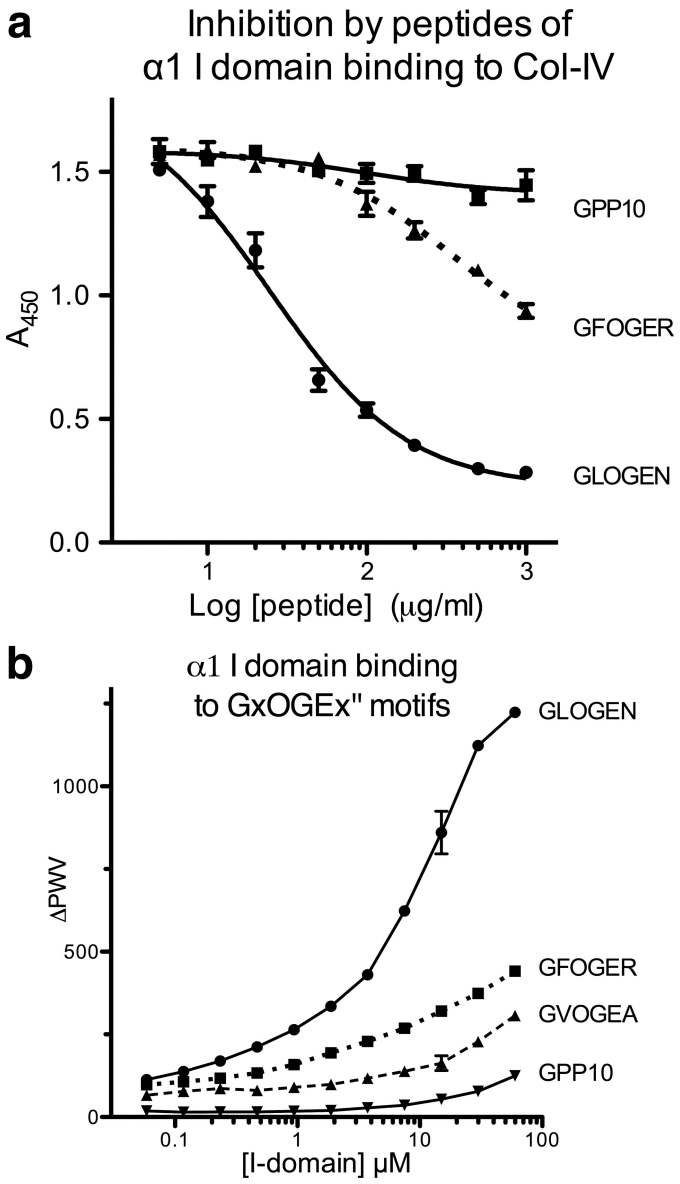


FIGURE 8. **Equilibrium binding of α1 I domain to collagenous substrates.** *a*, inhibition of binding of the wild-type α1 I domain to collagen IV (*Col-IV*) was determined as described in the legend to Fig. 1 in the presence of peptides. The I domain was preincubated with the indicated amount of GLOGEN, GFOGER, and GPP10. Data show the mean ± S.E. of three experiments. *b*, adhesion of the wild-type α1 I domain to GLOGEN, GFOGER, GVOGEA, and GPP10 was measured using the SRU Biosystems BIND Explorer as described under "Experimental Procedures", allowing $K_D$ to be estimated. Δ*PWV*, shift in peak wavelength value.

surface. Recently, a related but unnatural motif (GFPGEN) was expressed within *Streptococcus pyogenes* collagen-like molecules and found to be a selective ligand for α1β1 (40). Like GLOGEN, GFPGEN showed higher affinity for the α1 I domain compared with GFPGER, which we found previously to be a poor ligand for α1β1-expressing Rugli cells (54).

Finally, using competition assays, we have shown that the triple-helical peptide GLOGEN in solution is able to displace the α1 I domain from immobilized collagen IV with much higher affinity ($IC_{50}$ ~ 2.8 μM) compared with GFOGER ($IC_{50}$ ~ 90 μM). These data support the selective nature of the two integrins for our peptide ligands, as well as for the native collagens.

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## REFERENCES


1. Hynes, R. O. (2002) Integrins: bidirectional, allosteric signaling machines. *Cell* **110,** 673–687
2. Ginsberg, M. H., Partridge, A., and Shattil, S. J. (2005) Integrin regulation. *Curr. Opin. Cell Biol.* **17,** 509–516
3. Barczyk, M., Carracedo, S., and Gullberg, D. (2010) Integrins. *Cell Tissue Res.* **339,** 269–280
4. Michishita, M., Videm, V., and Arnaout, M. A. (1993) A novel divalent cation-binding site in the A domain of the β2 integrin CR3 (CD11b/CD18) is essential for ligand binding. *Cell* **72,** 857–867
5. Tuckwell, D., Calderwood, D. A., Green, L. J., and Humphries, M. J. (1995) Integrin α2 I domain is a binding site for collagens. *J. Cell Sci.* **108,** 1629–1637
6. Emsley, J., King, S. L., Bergelson, J. M., and Liddington, R. C. (1997) Crystal structure of the I domain from integrin α2β1. *J. Biol. Chem.* **272,** 28512–28517
7. Emsley, J., Knight, C. G., Farndale, R. W., Barnes, M. J., and Liddington, R. C. (2000) Structural basis of collagen recognition by integrin α2β1. *Cell* **101,** 47–56
8. Bella, J., and Berman, H. M. (2000) Integrin-collagen complex: a metal-glutamate handshake. *Structure* **8,** R121–R126
9. Hemler, M. E. (1990) VLA proteins in the integrin family: structures, functions, and their role in leukocytes. *Annu. Rev. Immunol.* **8,** 365–400
10. Racine-Samson, L., Rockey, D. C., and Bissell, D. M. (1997) The role of α1β1 integrin in wound contraction. A quantitative analysis of liver myofibroblasts *in vivo* and in primary culture. *J. Biol. Chem.* **272,** 30911–30917
11. Szulgit, G., Rudolph, R., Wandel, A., Tenenhaus, M., Panos, R., and Gardner, H. (2002) Alterations in fibroblast α1β1 integrin collagen receptor expression in keloids and hypertrophic scars. *J. Invest. Dermatol.* **118,** 409–415
12. Hemler, M. E., Sanchez-Madrid, F., Flotte, T. J., Krensky, A. M., Burakoff, S. J., Bhan, A. K., Springer, T. A., and Strominger, J. L. (1984) Glycoproteins of 210,000 and 130,000 m.w. on activated T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. *J. Immunol.* **132,** 3011–3018
13. Mendrick, D. L., Kelly, D. M., duMont, S. S., and Sandstrom, D. J. (1995) Glomerular epithelial and mesangial cells differentially modulate the binding specificities of VLA-1 and VLA-2. *Lab. Invest.* **72,** 367–375
14. Kuznetsov, S. A., Mankani, M. H., Gronthos, S., Satomura, K., Bianco, P., and Robey, P. G. (2001) Circulating skeletal stem cells. *J. Cell Biol.* **153,** 1133–1140
15. Deschaseaux, F., Gindraux, F., Saadi, R., Obert, L., Chalmers, D., and Herve, P. (2003) Direct selection of human bone marrow mesenchymal stem cells using an anti-CD49a antibody reveals their CD45med,low phenotype. *Br. J. Haematol.* **122,** 506–517
16. Loeser, R. F., Carlson, C. S., and McGee, M. P. (1995) Expression of β1 integrins by cultured articular chondrocytes and in osteoarthritic cartilage. *Exp. Cell Res.* **217,** 248–257
17. Stewart, H. J., Turner, D., Jessen, K. R., and Mirsky, R. (1997) Expression and regulation of α1β1 integrin in Schwann cells. *J. Neurobiol.* **33,** 914–928
18. Hemler, M. E., and Lobb, R. (1995) The leukocyte β1 integrins. *Curr. Opin. Hematol.* **2,** 61–67
19. de Fougerolles, A. R., Sprague, A. G., Nickerson-Nutter, C. L., Chi-Rosso, G., Rennert, P. D., Gardner, H., Gotwals, P. J., Lobb, R. R., and Koteliansky, V. E. (2000) Regulation of inflammation by collagen-binding integrins α1β1 and α2β1 in models of hypersensitivity and arthritis. *J. Clin. Invest.* **105,** 721–729
20. Heino, J., and Massagué, J. (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
21. Pirilä, L., and Heino, J. (1996) Altered integrin expression in rheumatoid

synovial lining type B cells: *in vitro* cytokine regulation of α1 β1, α6β1, and αvβ5 integrins. *J. Rheumatol.* **23,** 1691–1698
22. Gailit, J., Xu, J., Bueller, H., and Clark, R. A. (1996) Platelet-derived growth factor and inflammatory cytokines have differential effects on the expression of integrins α1β1 and α5β1 by human dermal fibroblasts *in vitro*. *J. Cell. Physiol.* **169,** 281–289
23. Schadendorf, D., Gawlik, C., Haney, U., Ostmeier, H., Suter, L., and Czarnetzki, B. M. (1993) Tumor progression and metastatic behavior *in vivo* correlates with integrin expression on melanocytic tumors. *J. Pathol.* **170,** 429–434
24. Schadendorf, D., Fichtner, I., Makki, A., Alijagic, S., Küpper, M., Mrowietz, U., and Henz, B. M. (1996) Metastatic potential of human melanoma cells in nude mice– characterization of phenotype, cytokine secretion, and tumor-associated antigens. *Br. J. Cancer* **74,** 194–199
25. Gilhar, A., Ullmann, Y., Kalish, R. S., Berkutski, T., Azizi, E., and Bank, I. (1997) Favorable melanoma prognosis associated with the expression of the tumor necrosis factor receptor and the α1β1 integrin: a preliminary report. *Melanoma Res.* **7,** 486–495
26. Mechtersheimer, G., Barth, T., Quentmeier, A., and Möller, P. (1994) Differential expression of β1 integrins in non-neoplastic smooth and striated muscle cells and in tumors derived from these cells. *Am. J. Pathol.* **144,** 1172–1182
27. Koukoulis, G. K., Warren, W. H., Virtanen, I., and Gould, V. E. (1997) Immunolocalization of integrins in the normal lung and in pulmonary carcinomas. *Hum. Pathol.* **28,** 1018–1025
28. Tulla, M., Pentikäinen, O. T., Viitasalo, T., Käpylä, J., Impola, U., Nykvist, P., Nissinen, L., Johnson, M. S., and Heino, J. (2001) Selective binding of collagen subtypes by integrin α1I, α2I, and α10I domains. *J. Biol. Chem.* **276,** 48206–48212
29. Zhang, W. M., Käpylä, J., Puranen, J. S., Knight, C. G., Tiger, C. F., Pentikäinen, O. T., Johnson, M. S., Farndale, R. W., Heino, J., and Gullberg, D. (2003) α11β1 integrin recognizes the GFOGER sequence in interstitial collagens. *J. Biol. Chem.* **278,** 7270–7277
30. Eble, J. A., Golbik, R., Mann, K., and Kühn, K. (1993) The α1β1 integrin recognition site of the basement membrane collagen molecule $(\alpha1(IV))_2\alpha2(IV)$. *EMBO J.* **12,** 4795–4802
31. Golbik, R., Eble, J. A., Ries, A., and Kühn, K. (2000) The spatial orientation of the essential amino acid residues arginine and aspartate within the α1β1 integrin recognition site of collagen IV has been resolved using fluorescence resonance energy transfer. *J. Mol. Biol.* **297,** 501–509
32. Saccà, B., Fiori, S., and Moroder, L. (2003) Studies of the local conformational properties of the cell adhesion domain of collagen type IV in synthetic heterotrimeric peptides. *Biochemistry* **42,** 3429–3436
33. Saccà, B., Sinner, E. K., Kaiser, J., Lübken, C., Eble, J. A., and Moroder, L. (2002) Binding and docking of synthetic heterotrimeric collagen type IV peptides with α1β1 integrin. *ChemBioChem.* **3,** 904–907
34. Fitzsimmons, C. M., Cawston, T. E., and Barnes, M. J. (1986) The platelet reactivity of collagen type I: evidence for multiple platelet-reactive sites in the type I collagen molecule. *Thromb. Haemost.* **56,** 95–99
35. Knight, C. G., Morton, L. F., Onley, D. J., Peachey, A. R., Messent, A. J., Smethurst, P. A., Tuckwell, D. S., Farndale, R. W., and Barnes, M. J. (1998) Identification in collagen type I of an integrin α2β1-binding site containing an essential GER sequence. *J. Biol. Chem.* **273,** 33287–33294
36. Knight, C. G., Morton, L. F., Peachey, A. R., Tuckwell, D. S., Farndale, R. W., and Barnes, M. J. (2000) The collagen-binding A domains of integrins α1β1 and α2β1 recognize the same specific amino acid sequence, GFOGER, in native (triple-helical) collagens. *J. Biol. Chem.* **275,** 35–40
37. Xu, Y., Gurusiddappa, S., Rich, R. L., Owens, R. T., Keene, D. R., Mayne, R., Höök, A., and Höök, M. (2000) Multiple binding sites in collagen type I for the integrins α1β1 and α2β1. *J. Biol. Chem.* **275,** 38981–38989
38. Siljander, P. R., Hamaia, S., Peachey, A. R., Slatter, D. A., Smethurst, P. A., Ouwehand, W. H., Knight, C. G., and Farndale, R. W. (2004) Integrin activation state determines selectivity for novel recognition sites in fibrillar collagens. *J. Biol. Chem.* **279,** 47763–47772
39. Raynal, N., Hamaia, S. W., Siljander, P. R., Maddox, B., Peachey, A. R., Fernandez, R., Foley, L. J., Slatter, D. A., Jarvis, G. E., and Farndale, R. W. (2006) Use of synthetic peptides to locate novel integrin α2β1-binding motifs in human collagen III. *J. Biol. Chem.* **281,** 3821–3831
40. Seo, N., Russell, B. H., Rivera, J. J., Liang, X., Xu, X., Afshar-Kharghan, V., and Höök, M. (2010) An engineered α1 integrin-binding collagenous sequence. *J. Biol. Chem.* **285,** 31046–31054
41. Calderwood, D. A., Tuckwell, D. S., Eble, J., Kühn, K., and Humphries, M. J. (1997) The integrin α1 A domain is a ligand-binding site for collagens and laminin. *J. Biol. Chem.* **272,** 12311–12317
42. Dyson, M. R., Shadbolt, S. P., Vincent, K. J., Perera, R. L., and McCafferty, J. (2004) Production of soluble mammalian proteins in *Escherichia coli*: identification of protein features that correlate with successful expression. *BMC Biotechnol.* **4,** 32
43. Onley, D. J., Knight, C. G., Tuckwell, D. S., Barnes, M. J., and Farndale, R. W. (2000) Micromolar $Ca^{2+}$ concentrations are essential for $Mg^{2+}$-dependent binding of collagen by the integrin α2β1 in human platelets. *J. Biol. Chem.* **275,** 24560–24564
44. Tiger, C. F., Fougerousse, F., Grundström, G., Velling, T., and Gullberg, D. (2001) α11β1 integrin is a receptor for interstitial collagens involved in cell migration and collagen reorganization on mesenchymal non-muscle cells. *Dev. Biol.* **237,** 116–129
45. Castillo-Briceño, P., Bihan, D., Nilges, M., Hamaia, S., Meseguer, J., García-Ayala, A., Farndale, R. W., and Mulero, V. (2011) A role for specific collagen motifs during wound healing and inflammatory response of fibroblasts in the teleost fish gilthead seabream. *Mol. Immunol.* **48,** 826–834
46. Kim, J. K., Xu, Y., Xu, X., Keene, D. R., Gurusiddappa, S., Liang, X., Wary, K. K., and Höök, M. (2005) A novel binding site in collagen type III for integrins α1β1 and α2β1. *J. Biol. Chem.* **280,** 32512–32520
47. Tawil, N. J., Houde, M., Blacher, R., Esch, F., Reichardt, L. F., Turner, D. C., and Carbonetto, S. (1990) α1β1 integrin heterodimer functions as a dual laminin/collagen receptor in neural cells. *Biochemistry* **29,** 6540–6544
48. Zhang, Z., Tarone, G., and Turner, D. C. (1993) Expression of integrin α1β1 is regulated by nerve growth factor and dexamethasone in PC12 cells. Functional consequences for adhesion and neurite outgrowth. *J. Biol. Chem.* **268,** 5557–5565
49. Lahti, M., Bligt, E., Niskanen, H., Parkash, V., Brandt, A. M., Jokinen, J., Patrikainen, P., Käpylä, J., Heino, J., and Salminen, T. A. (2011) Structure of collagen receptor integrin α1 I domain carrying the activating mutation E317A. *J. Biol. Chem.* **286,** 43343–43351
50. Jarvis, G. E., Bihan, D., Hamaia, S., Pugh, N., Ghevaert, C. J., Pearce, A. C., Hughes, C. E., Watson, S. P., Ware, J., Rudd, C. E., and Farndale, R. W. (2011) A role for adhesion and degranulation-promoting adapter proteins in collagen-induced platelet activation via integrin alpha(2)beta(1). *J. Thromb. Haemost.* **10,** 268–277
51. Tulla, M., Lahti, M., Puranen, J. S., Brandt, A. M., Käpylä, J., Domogatskaya, A., Salminen, T. A., Tryggvason, K., Johnson, M. S., and Heino, J. (2008) Effects of conformational activation of integrin α1I and α2I domains on selective recognition of laminin and collagen subtypes. *Exp. Cell Res.* **314,** 1734–1743
52. Aquilina, A., Korda, M., Bergelson, J. M., Humphries, M. J., Farndale, R. W., and Tuckwell, D. (2002) A novel gain-of-function mutation of the integrin α2 VWFA domain. *Eur. J. Biochem.* **269,** 1136–1144
53. Ricard-Blum, S. (2011) The collagen family. *Cold Spring Harb. Perspect. Biol.* **3,** a004978
54. Perret, S., Eble, J. A., Siljander, P. R., Merle, C., Farndale, R. W., Theisen, M., and Ruggiero, F. (2003) Prolyl hydroxylation of collagen type I is required for efficient binding to integrin α1β1 and platelet glycoprotein VI but not to α2β1. *J. Biol. Chem.* **278,** 29873–29879