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Folding and Function of I Domain-deleted Mac-1 and Lymphocyte Function-associated Antigen-1*

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In those integrins that contain it, the I domain is a major ligand recognition site. The I domain is inserted between β-sheets 2 and 3 of the predicted β-propeller domain of the integrin α subunit. We deleted the I domain from the integrin α_M and α_L subunits to give I-less Mac-1 and lymphocyte function-associated antigen-1 (LFA-1), respectively. The I-less α_M and α_L subunits were expressed in association with the wild-type β_2 subunit on the surface of transfected cells and bound to all the monoclonal antibodies mapped to the putative β-propeller and C-terminal regions of the α_M and α_L subunits, suggesting that the folding of these domains is independent of the I domain. I-less Mac-1 bound to the ligands iC3b and factor X, but this binding was reduced compared with wild-type Mac-1. In contrast, I-less Mac-1 did not bind to fibrinogen or denatured bovine serum albumin. Binding to iC3b and factor X by I-less Mac-1 was inhibited by the function-blocking antibody CBRM1/32, which binds to the β-propeller domain of the α_M subunit. I-less LFA-1 did not bind its ligands intercellular adhesion molecule-1 and -3. Thus, the I domain is not essential for the folding, heterodimer formation, and surface expression of Mac-1 and LFA-1 and is required for binding to some ligands, but not others.

Integrins are a family of cell-surface molecules that play an important role in cell-cell and cell-matrix interactions (1). These molecules are heterodimers composed of an α subunit and a β subunit (2). The β_2 subfamily of integrins contains four members that have a common β_2 subunit associated with distinct α subunits. The β_2 subfamily includes the integrins Mac-1 (CD11b/CD18, α_M/β_2), LFA-1 [1] (CD11a/CD18, α_L/β_2), p150,95 (CD11c/CD18, α_X/β_2), and α_D/β_2 (3, 4). They are expressed on all leukocytes and play a critical role in immune and inflammatory responses (4). Human patients with a defective β_2 subunit have a disease known as leukocyte adhesion deficiency characterized by the inability of phagocytic cells to bind and to migrate across the endothelium (5, 6). This results in severe bacterial and fungal infections in these patients, indicating the crucial role of these integrins in normal immune responses.

Mac-1 is predominantly expressed on myeloid and natural killer cells, where it mediates numerous physiological functions, including phagocytosis of foreign particles (7), transmigration and adhesion of leukocytes to the endothelium (8), chemotaxis (9), and activation of neutrophils and monocytes (10). To mediate these physiological functions, Mac-1 binds to a wide repertoire of ligands, including ICAM-1 (8, 11), fibrinogen (12), iC3b (13), factor X (14), denatured proteins (15), neutrophil inhibitory factor (16), lipopolysaccharide (17), and zymosan (18). LFA-1 is expressed on T-cells and mediates the interaction with Ig superfamily members ICAM-1, ICAM-2, and ICAM-3 (19–21). LFA-1 plays a critical role in adhesion of T-cells once the T-cell receptor has been activated (22).

The extracellular regions of the Mac-1 and LFA-1 α subunits have several distinct domains. The N-terminal region of the integrin α subunits contains seven repeats of 60 amino acids each (23). These repeats have been predicted to fold into a β-propeller domain similar to the β-propeller seen in the G-protein β subunit (24). Studies with monoclonal antibodies showed that the predicted β-propeller in the α subunit folds correctly only with the association of the β_2 subunit (25, 26). All the β_2 integrin α subunits have an inserted domain known as the I domain between β-sheets 2 and 3 in the predicted β-propeller of the α subunit (24). The I domain has ~200 amino acids that can fold independently of the other regions of the α subunit (27, 28). The structures of I domains from Mac-1, LFA-1, $\alpha_2\beta_1$, and $\alpha_1\beta_1$ have been solved, and they have a dinucleotide-binding fold with a unique cation coordination site known as the metal ion-dependent adhesion site (29–34). This site has been shown to coordinate both Mg^{2+} and Mn^{2+} ions (30). Recent studies with mAbs have shown that the I domain and most of the region C-terminal to the β-propeller fold independently of the β_2 subunit (26). The C-terminal region of the α_M subunit has been predicted to fold into multiple β-sandwich domains (26). The β_2 subunit contains an "I domain-like" region in the N-terminal region. Electron micrographs of integrins show that the N-terminal regions of the α and β subunits fold into a globular head connected to the membrane by a long stalk-like region composed of the C-terminal regions (35–37).

Both mutagenesis and mAb mapping/blocking studies have identified the I domain as the major ligand-binding site in integrins that contain I domains (28, 38–41). Recombinant I domain from Mac-1 can bind ICAM-1, and fibrinogen but does not bind factor X (28). Although most function-blocking mAbs to Mac-1 bind to the I domain, mAb CBRM1/32 blocks binding to ligands by binding to the upper surface of the β-propeller domain (40, 42). In the case of LFA-1, both the isolated I domain (43) and peptides from the β-propeller domain encompassing putative cation-binding motifs (44) have been reported to bind to ICAM-1. Mutagenesis studies showed that the I

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[1] The abbreviations used are: LFA-1, lymphocyte function-associated antigen-1; ICAM, intercellular adhesion molecule; mAb, monoclonal antibody; BSA, bovine serum albumin.

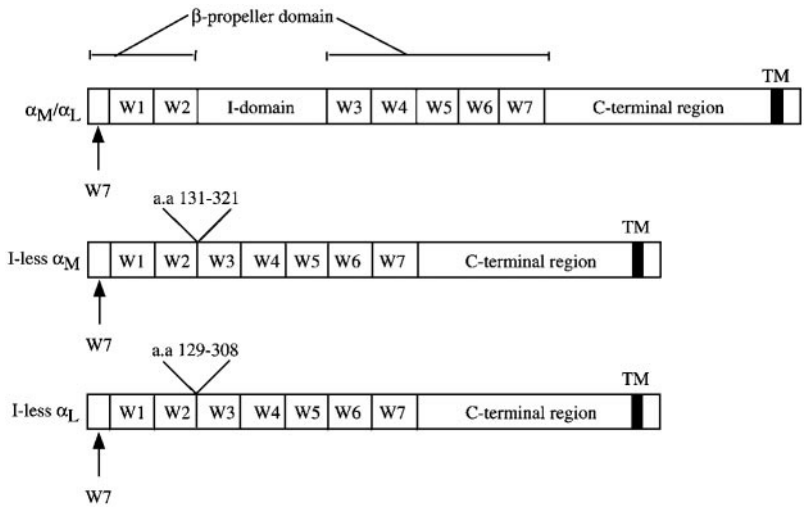

FIG. 1. **Schematic diagram of the wild-type and I-less α_L and α_M subunits.** The *W*s represent the β-sheets in the predicted β-propeller domain. The I domain is inserted between β-sheets 2 and 3 in the predicted β-propeller of both Mac-1 and LFA-1 α subunits. *TM* indicates the transmembrane region. In the I-less α_M subunit, Glu^{131}–Gly^{321} is deleted. In the I-less α_L subunit, Asn^{129}–Val^{308} is deleted.

domain-like region of the β_2 subunit is also involved in ligand binding of both Mac-1 and LFA-1 (45). These studies point to the fact that sequences outside the I domain might be involved in ligand binding.

In this study, we examined the effect of the removal of the I domain on the structure and function of Mac-1 and LFA-1. We demonstrate that the folding of the β-propeller domain and other α subunit regions is independent of the I domain. The I domain is not essential for the formation of α/β heterodimers for both Mac-1 and LFA-1. I-less Mac-1 can bind at reduced levels to some Mac-1 ligands, including factor X and iC3b, but does not bind to the ligands fibrinogen and denatured BSA. Binding to iC3b and factor X is blocked by mAb to the β-propeller domain. In contrast, I-less LFA-1 cannot bind to its ligands ICAM-1 and ICAM-3. This study demonstrates a role for the α subunit β-propeller domain in the binding of some ligands by Mac-1 and underlines the complexity of ligand binding by integrins.

MATERIALS AND METHODS

Cell Lines—Human embryonic kidney 293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and 50 μg/ml gentamycin. K562 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and 50 μg/ml gentamycin.

Monoclonal Antibodies—The sources for the murine mAbs against α_L and β_2 (25, 46) and α_M (26) were previously described.

cDNA Constructs—The human wild-type α_M subunit cDNA was subcloned in the expression vector pcDNA3.1$^+$ as described previously (42). A DNA fragment encoding Glu^{131}–Gly^{321} was deleted from the full-length α_M subunit (47) by overlap polymerase chain reaction (48, 49), and I-less α_M was cloned into the vector pcDNA3.1$^+$. Similarly, the DNA fragment encoding Asn^{129}–Val^{308} was deleted from the α_L subunit cDNA in plasmid AprM8 (50) by overlap polymerase chain reaction.

Transient and Stable Transfections—Transient transfection of 293T cells was as described previously (51, 52). Briefly, 7.5 μg each of α_M and β_2 subunit cDNAs were transfected into 293T cells in 6-cm plates. Cells were detached 48 h post-transfection with phosphate-buffered saline containing 5 mM EDTA for adhesion assays and flow cytometry. Stable expression in K562 cells was as described previously (53). Briefly, the I-less mutant cDNAs of α_M and α_L were subcloned into the pEFpuro vector and cotransfected with the wild-type β_2 subunit into K562 cells by electroporation. Transfectants were selected in medium supplemented with 4 μg/ml puromycin. Clones of K562 transfectants that expressed similar levels of surface wild-type and I-less integrins were selected for further study.

Flow Cytometry—Flow cytometry was performed on K562 and 293T cell transfectants as described previously (53).

Radiolabeling and Immunoprecipitation—For metabolic labeling, 293T cells were plated in six-well tissue culture dishes and transfected with the appropriate cDNA constructs; 24 h later, they were washed twice with methionine- and cysteine-free RPMI 1640 medium and labeled with 0.5 mCi of [^{35}S]methionine and [^{35}S]cysteine in 2 ml of methionine- and cysteine-free RPMI 1640 medium containing 10% fetal bovine serum for 16 h. Cell lysates were prepared and subjected to immunoprecipitation as described previously (26), except that the cell lysates were precleared with protein A-agarose bound to a nonspecific myeloma IgG1. In addition, 1 mM Mg^{2+} and 0.15 mM Ca^{2+} were included in all the buffers.

Adhesion Assays—For the Mac-1 adhesion assay, 50 μl of purified iC3b (2 μg/ml; Life Technologies, Inc.), 50 μl of factor X (80 milliunits/ml; Sigma), 50 μl of fibrinogen (1 mg/ml; Sigma), or 50 μl of BSA (1 mg/ml) denatured by heating at 95 °C for 5 min was coated onto plastic in each well of a 96-well microtiter plate (ICN Biomedicals, Inc., Aurora, OH) by incubation overnight at 4 °C. Nonspecific binding sites were blocked with 400 μl of blocking buffer (0.05% polyvinylpyrrolidone in phosphate-buffered saline) for 60 min at 37 °C. K562 cells expressing wild-type or I-less Mac-1 were labeled with 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester as described previously (54) and resuspended in L-15 medium and 0.5% human serum albumin. For cation treatment, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein-labeled cells were washed twice with Hanks' balanced salt solution lacking calcium and magnesium ions (Sigma) and resuspended in the same buffer with the appropriate cations as indicated. Cell suspensions (5×10^4 cells in 50 μl) were added to the ligand-coated wells containing 50 μl of stimulatory and/or inhibitory mAb at a concentration of 20 μg/ml (10 μg/ml final concentration) or buffer alone. Cells were centrifuged at 200 $\times$ *g* for 5 min at room temperature and incubated for 60 min at 37 °C. Unbound cells were removed by a microplate autowasher (Bio-Tek Instruments, Winooski, VT) using six washes with Hanks' balanced salt solution. The wash program dispensed 250 μl of buffer and a 100-μl volume remained after aspiration under a pressure of 2 p.s.i. Fluorescence intensity of the bound cells and the total input cells was measured on a fluorescence concentration analyzer (IDEXX, Westbrook, ME). The bound cells were expressed as a percentage of the total input cells in each sample well.

For the LFA-1 adhesion assay, the LFA-1 ligands ICAM-1 and ICAM-3 were purified from human tonsil lysate using R6.5 and IC3/1 antibody affinity columns (19, 55), respectively, and diluted to 5 μg/ml in 20 mM Tris (pH 9), 150 mM NaCl, 2 mM Mg^{2+}, and 0.01% octyl glucoside. The LFA-1 adhesion assay conditions were identical to those used for Mac-1 adhesion assays, except that the nonspecific sites were blocked by 2% BSA.

RESULTS

Cell-surface Expression of I-less Mac-1 and LFA-1—To examine the role of the I domain in the folding and function of Mac-1 and LFA-1, the I domain was deleted from the α subunit of these integrins. The length of the deleted region was determined based on the predicted length of the I domain as defined by x-ray crystal structures (29, 31). The I-less α_M subunit lacked Glu^{131}–Gly^{321}, and the I-less α_L subunit lacked Asn^{129}–Val^{308} (Fig. 1). The N and C termini of the I domain are close to one another; and therefore, no linker segment was added. To study the assembly and cell-surface expression of I-less Mac-1,

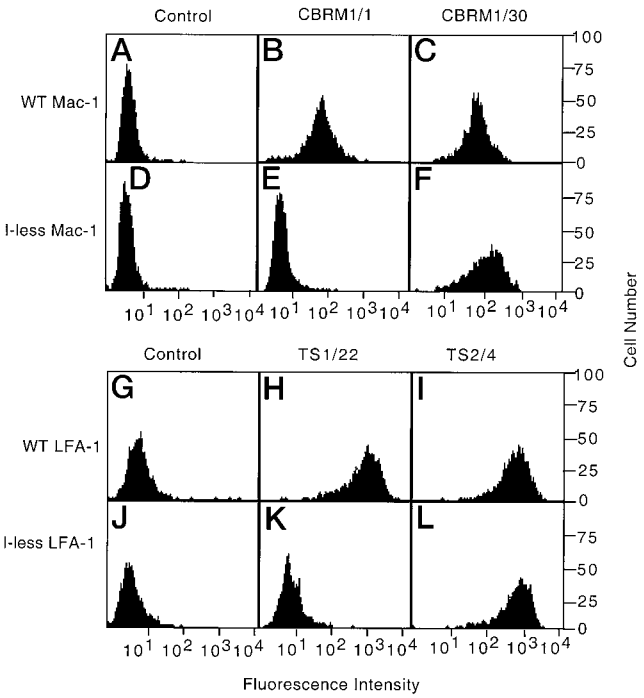

FIG. 2. **Heterodimer formation of wild-type and I-less Mac-1.** Shown are the results from immunofluorescent flow cytometry of wild-type (*WT*) or I-less Mac-1 and LFA-1 stably expressed on K562 cells. *A–F*, K562 cells that express wild-type or I-less Mac-1 were stained with a nonbinding control (X63 IgG; *A* and *D*), mAb CBRM 1/1 to the α_M subunit I domain (*B* and *E*), or mAb CBRM1/30 to the α_M subunit C-terminal segment (*C* and *F*). *G–L*, K562 cells that express wild-type or I-less LFA-1 were stained with a nonbinding control (X63 IgG; *G* and *J*), mAb TS1/22 to the α_L subunit I domain (*H* and *K*), or mAb TS2/4 to the α_L subunit β-propeller domain (*I* and *L*).

stable transfectants expressing I-less and wild-type Mac-1 were generated in K562 cells. The expression of Mac-1 was determined by immunofluorescent flow cytometry. The antibody CBRM1/30, which binds to α_M only when it is associated with β_2 (26), bound equally well to both I-less and wild-type Mac-1 (Fig. 2, *C* and *F*). The antibody CBRM1/1 to the Mac-1 I domain bound to wild-type Mac-1, but not to I-less Mac-1, confirming that the I domain was deleted (Fig. 2, *B* and *E*). Similarly, the I-less α_L subunit was coexpressed with the wild-type β_2 subunit in K562 cells. mAb TS2/4, which reacts with the α_L subunit β-propeller domain only when complexed with the β_2 subunit (25), reacted equally well with the transfectants expressing I-less and wild-type LFA-1 (Fig. 2, *I* and *L*). mAbs to the I domain did not bind to the I-less mutant, confirming the deletion of the I domain (Fig. 2, *H* and *K*). These results show that the I domain deletion did not affect the assembly and cell-surface expression of α_L/β_2 and α_M/β_2 heterodimers.

To confirm $\alpha\beta$ complex formation, transiently transfected 293T cells were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine and subjected to immunoprecipitation. The I-less and wild-type α_M subunits were immunoprecipitated in association with the β_2 subunit by both mAb CBRM1/30 to α_M and mAb CBR LFA1/2 to β_2 (Fig. 3). The I-less α_M subunit was lower in molecular mass than the wild-type α_M subunit, as expected. Furthermore, the relative amounts of α and β subunits were similar in wild-type and I-less Mac-1 immunoprecipitates.

Folding of the I-less Mac-1 and LFA-1 Heterodimers—To determine whether the I domain is required for the folding of other domains in Mac-1 and LFA-1, we tested the binding of a panel of mAbs previously mapped to different domains/regions of Mac-1 and LFA-1 (25, 26, 46). Antibodies that bind to the β-propeller domain and the C-terminal region of the α_M subunit bound equally well to wild-type and I-less α_M/β_2 (Table I). The antibody TS1/18 to the β_2 subunit I-like domain also bound wild-type and I-less Mac-1 equally well (Table I).

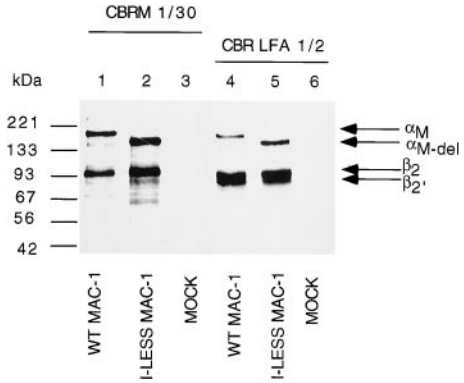

FIG. 3. **Immunoprecipitation of wild-type and I-less Mac-1.** 293T cells transiently transfected with wild-type (*WT*) or I-less Mac-1 were labeled with [^{35}S]methionine and cysteine. Cell lysates were subjected to immunoprecipitation with mAb CBRM1/30 to the α_M subunit or mAb CBR LFA1/2 to the β_2 subunit, followed by SDS-7.5% polyacrylamide gel electrophoresis and fluorography. α_M, full-length α_M; α_M-*del*, I-less α_M; β_2, mature β_2; $\beta_{2'}$, precursor β_2

The folding of the different domains of the I-less α_L/β_2 complex was similarly studied using a panel of antibodies. mAbs to the α_L β-propeller domain and C-terminal region reacted equally well with wild-type and I-less LFA-1. In addition, a panel of mAbs that bind to the β_2 subunit I-like domain and C-terminal cysteine-rich region bound to I-less and wild-type LFA-1 to comparable levels (Table II). Thus, the folding of other domains of Mac-1 and LFA-1 is independent of the I domain.

Ligand Binding Specificity of I-less Mac-1—To study the role of the I domain in Mac-1 function, we tested the binding of wild-type and I-less Mac-1 to various Mac-1 ligands, including iC3b, factor X, fibrinogen, and denatured BSA. Both wild-type and I-less Mac-1 transfectants bound to iC3b in the presence of the activating antibody CBR LFA1/2 (Fig. 4*A*). The binding of I-less Mac-1 transfectants was reduced by 70% compared with wild-type Mac-1 transfectants, showing that the I domain is required for maximal binding of Mac-1 to iC3b. The binding of I-less Mac-1 transfectants to iC3b was completely inhibited by the function-blocking β-propeller antibody CBRM1/32 and was unaffected by mAb CBRM1/1 to the I domain (Fig. 4*A*). The binding of wild-type transfectants was blocked by both mAb CBRM1/32 to the β-propeller and mAb CBRM1/1 to the I domain, consistent with previous results (40) and with an important contribution by both domains to iC3b binding.

The binding of wild-type Mac-1 to iC3b is dependent on divalent cations. Similarly, I-less Mac-1 bound to iC3b in the presence of Mn^{2+} or Mg^{2+}, but not in the presence of EDTA (Fig. 4*B*), showing that divalent cations are essential for the binding of I-less Mac-1 to iC3b.

Wild-type and I-less Mac-1 on K562 cells bound to immobilized factor X, although, the binding of I-less Mac-1 was lower than that of wild-type Mac-1 (Fig. 4). The binding of I-less Mac-1 was inhibited by mAb CBRM1/32 to the β-propeller domain, but not by an I domain mAb. Binding by wild-type Mac-1 was inhibited by mAb to both the I domain and the β-propeller domain (Fig. 5).

In contrast to results with iC3b and factor X, binding to fibrinogen and denatured BSA was dependent on the Mac-1 I domain. I-less Mac-1 showed little or no binding to fibrinogen (Fig. 6*A*) and no binding to denatured BSA (Fig. 6*B*), whereas wild-type Mac-1 showed robust binding. In agreement with this and in contrast to the results with iC3b and factor X, mAb CBRM1/32 to the β-propeller domain gave only partial inhibi-

TABLE I
Reactivity of antibodies to wild-type and I-less Mac-1 determined by immunofluorescent flow cytometry

Stably transfected K562 cells or transiently transfected 293T cells expressing wild-type (WT) or I-less Mac-1 were stained with the indicated antibodies. Results were similar for the two cell types. Negative (−) and positive (+) staining refer to staining similar to control and CBRM1/1 staining of Mac-1 in Fig. 2.

mAb	Epitope	WT Mac-1	I-less Mac-1
CBRM1/1	I domain	+	−
CBRM1/2	I domain	+	−
CBRM1/29	I domain	+	−
CBRM1/32	β-Propeller	+	+
CBRM1/10	C terminus	+	+
CBRM1/16	C terminus	+	+
CBRM1/23	C terminus	+	+
CBRM1/30	C terminus	+	+
TS1/18	I-like domain	+	+

TABLE II
Reactivity of antibodies to wild-type and I-less LFA-1 determined by immunofluorescent flow cytometry

Stably transfected K562 cells and transiently transfected 293T cells expressing wild type (WT) or I-less LFA-1 were stained with the indicated antibodies. Results were similar for the two cell types. Negative (−) and positive (+) staining refer to staining similar to control and TS2/4 staining of LFA-1 in Fig. 2.

mAb	Epitope	WT LFA-1	I-less LFA-1
TS1/22	I domain	+	−
TS2/6	I domain	+	−
MAY.035	I domain	+	−
CBR LFA1/1	I domain/β-propeller	+	−
CBR LFA1/10	β-Propeller	+	+
TS2/4	β-Propeller	+	+
S6F1	β-Propeller	+	+
G25.2	β-Propeller/C terminus	+	+
NKI-L16	β-Propeller/C terminus	+	+
CBR LFA1/3	β-Propeller/C terminus	+	+
6.5E	I-like domain	+	+
MAY.017	I-like domain	+	+
TS1/18	I-like domain	+	+
YFC51	I-like domain	+	+
CBR LFA1/2	β_2 Cys-rich region	+	+
CBR LFA1/7	β_2 Cys-rich region	+	+

tion of binding to fibrinogen and heat-denatured BSA (Fig. 6, *A* and *B*). The I domain mAb CBRM1/1 gave complete inhibition of binding to fibrinogen and denatured BSA.

I-less LFA-1 Mutant Does Not Bind to LFA-1 Ligands—To test whether LFA-1 lacking the I domain can bind to ligands, stable K562 transfectants were examined for their ability to bind to purified ICAM-1 and ICAM-3. Without activation, wild-type LFA-1 on the surface of K562 cells showed little binding to either ICAM-1 or ICAM-3, as previously reported (53, 56). Activating mAbs differentially activated LFA-1 binding to ICAM-1 and ICAM-3. Whereas all three of the activating antibodies CBR LFA1/2, Kim185, and Kim127 increased wild-type LFA-1 binding to ICAM-1 (Fig. 7*A*), only Kim127 enhanced LFA-1 binding to ICAM-3 (Fig. 7*B*). However, the I-less LFA-1 mutant did not bind to ICAM-1 or ICAM-3 in the presence of activating mAbs. We further examined ligand binding by I-less LFA-1 in 293T cell transfectants, in which β_2 integrins are constitutively active. In the absence of activation, wild-type LFA-1 showed strong specific binding to ICAM-1, but little binding to ICAM-3; binding to ICAM-3 was greatly enhanced by the activating antibodies CBR LFA1/2, Kim185, and Kim127 (data not shown). In contrast to wild-type LFA-1, I-less LFA-1 expressed on 293T cells did not bind to ICAM-1 or ICAM-3 in the presence or absence of activating mAbs (data not shown). Thus, the I domain is essential for LFA-1 binding to its ligands ICAM-1 and ICAM-3.

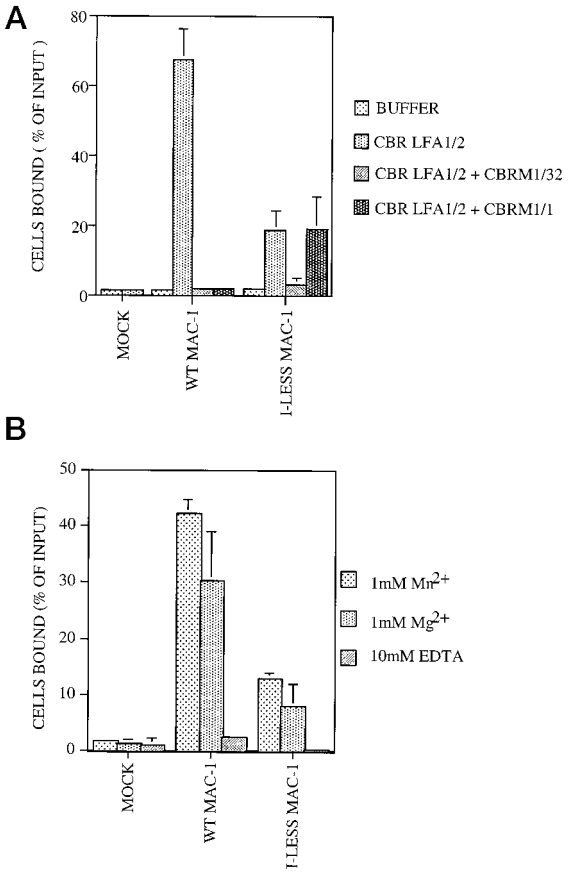

FIG. 4. **Binding of K562 cells that express wild-type or I-less Mac-1 to purified iC3b.** K562 cells expressing wild-type (*WT*) or I-less Mac-1 or transfected with vector alone were fluorescently labeled and added to microtiter wells coated with purified iC3b. *A*, the activating β_2 mAb CBR LFA1/2, inhibitory mAb CBRM1/32 to the β-propeller domain, or inhibitory mAb CBRM1/1 to the I domain was added as indicated. Data are means ± S.D. of triplicate samples and are representative of three independent experiments. *B*, binding to iC3b was in the presence of 1 mM Mg^{2+}, 1 mM Ca^{2+}, or 10 mM EDTA. Data are means ± S.D. of triplicate samples.

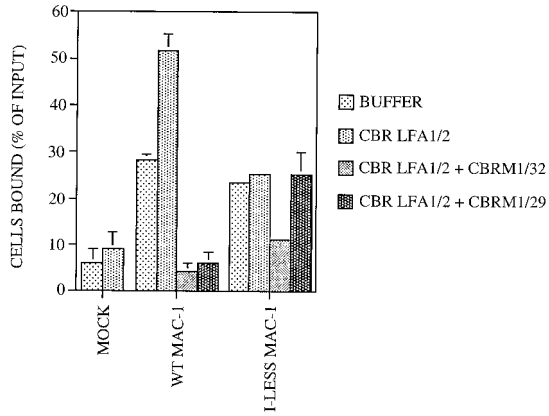

FIG. 5. **Binding of wild-type and I-less Mac-1 on K562 cells to immobilized factor X.** Factor X was immobilized onto plastic in a microtiter plate. Fluorescently labeled K562 cells expressing wild-type or I-less Mac-1 or transfected with vector alone were incubated with immobilized factor X in the presence of the indicated mAbs. Data are means ± S.D. of triplicate samples. mAbs are described in the Fig. 4 legend, except CBRM1/29, an inhibitory mAb to the I domain.

DISCUSSION

We examined the role of the I domain in the folding, heterodimer formation, and ligand binding of the β_2 integrins Mac-1 and LFA-1. In the absence of the I domain, the LFA-1

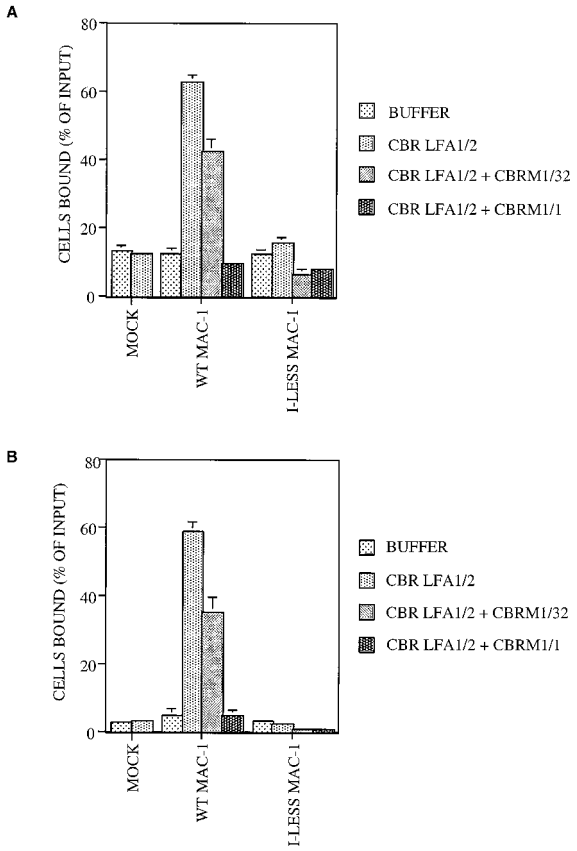

FIG. 6. **Binding of cells expressing wild-type or I-less Mac-1 to immobilized fibrinogen and denatured BSA.** Microtiter plates were coated with fibrinogen (*A*) or denatured BSA (*B*). Fluorescently labeled K562 cells expressing wild-type (*WT*) or I-less Mac-1 were added to these microtiter plates and incubated in the absence or presence of the indicated mAbs (see Fig. 4 legend). Data are means ± S.D. of triplicate samples and are representative of three independent experiments.

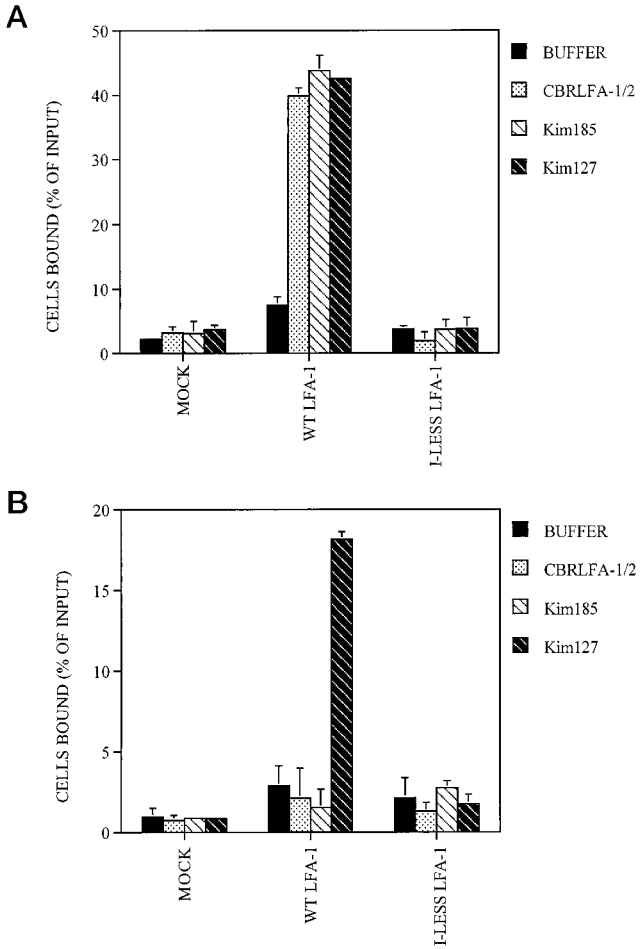

FIG. 7. **Binding of K562 cells expressing wild-type and I-less LFA-1 to ICAM-1 and ICAM-3.** Fluorescently labeled K562 cell transfectants were incubated in microtiter plates coated with purified ICAM-1 (*A*) or ICAM-3 (*B*) in the presence of the indicated mAbs. Results are means ± S.D. of triplicate samples and are representative of three independent experiments. *WT*, wild-type.

and Mac-1 α and β subunits were well expressed on the surface of transfected cells and formed heterodimers as shown by reactivity with mAbs to the α subunit β-propeller domain and the β subunit I-like domain that require α and β subunit association for reactivity (25, 26, 46). Furthermore, association of the α and β subunits was demonstrated by coprecipitation with mAb directed against the α_M and β_2 subunits. The reactivity of multiple mAbs that recognize distinct epitopes in the β-propeller domain was of particular interest because the I domain is inserted between β-sheets 2 and 3 of the predicted β-propeller domain. The integrity of the β-propeller domain after deletion of the I domain is consistent with its predicted structure. Binding of mAbs that map to multiple segments C-terminal to the β-propeller domain in the Mac-1 α subunit was also unaffected by I domain deletion. Furthermore, binding of mAbs that recognize multiple epitopes in the β_2 subunit I-like domain and cysteine-rich region was unaffected by I domain deletion. These results demonstrate that other domains in integrins fold independently of the I domain and complement previous results that the I domain folds independently of other integrin domains (25, 27, 28).

Our studies demonstrate that multiple sites in Mac-1 contribute to ligand binding and that the importance of the I domain relative to other integrin domains is dependent on the ligand. We found that I-less Mac-1 can bind to iC3b, but the binding was reduced compared with wild-type Mac-1. Previous studies showed that iC3b can bind recombinant I domain (28). Mutagenesis results indicate that the I-like domain of the β_2 subunit is also required for binding to iC3b (45). Based on these data and our results, we hypothesize that there are ligand-binding sites both within and outside the I domain in Mac-1. Simultaneous binding to both of these sites in Mac-1 may generate strong binding to iC3b.

Mac-1 can also bind to factor X in the absence of the I domain, although as with iC3b, the binding of I-less Mac-1 to factor X is much lower than that of wild-type Mac-1. Our results are consistent with the previous finding that recombinant I domain does not bind factor X (28) in that at least one factor X-binding site is located outside the I domain. Although inhibition with mAb suggests that the I domain is important for binding to factor X, it appears that this binding is too weak to be detected with the isolated I domain.

mAb to the Mac-1 I domain and mAb CBRM1/32 to the β-propeller domain each gave essentially complete inhibition of binding to iC3b and factor X. Although there was residual ligand binding after the I domain was deleted, the mAbs may give more complete inhibition by simultaneously restricting access to both the I domain and the top of the β-propeller domain. mAb CBRM1/32 recognizes the species-specific residue Arg^{534} in loop 2–3 at the top of β-sheet 6 (W6) in the predicted β-propeller (26). The I domain is inserted at the top of the β-propeller between W2 and W3 and is thus on the same "top" face of the β-propeller, an important ligand-binding interface for integrins that lack I domains (24, 57).

We found that the I domain is essential for the binding of Mac-1 to fibrinogen and heat-denatured BSA. Deletion of the I domain completely abrogated the binding of Mac-1 to these ligands. Consistent with these data, mAb to the I domain completely blocked the binding of wild-type Mac-1 to fibrinogen and denatured BSA, and mAb to the β-propeller domain only partially inhibited binding. These data suggest that fibrinogen and heat-denatured BSA bind only to the I domain or that the I domain is more essential than secondary contacts elsewhere in the integrin. This is consistent with earlier studies showing that recombinant I domain binds fibrinogen with high affinity (28).

Based on our results and previous work with isolated I domains, we propose that there are at least three functionally distinct ligand-binding mechanisms for Mac-1. Some ligands such as iC3b have ligand-binding regions both within and outside the I domain, and these sites can independently bind the ligand. For other ligands including factor X, sites both within and outside the I domain contribute to ligand binding, but the I domain by itself cannot bind the ligand. Finally, for ligands including fibrinogen and denatured BSA, the I domain is essential for ligand binding.

The β_2 integrin LFA-1 binds to the Ig superfamily members ICAM-1, ICAM-2, and ICAM-3 (19–21). Extensive mutagenesis studies have shown that several regions of LFA-1 are required for ligand binding, including the I domain and the β_2 subunit I-like domain conserved region (41, 43, 45, 58). In addition, experiments with the isolated I domain demonstrated that efficient ligand binding requires other domains of the LFA-1 molecule (59). We have used different expression systems and different cell types to analyze the binding of I-less LFA-1 to its ligands ICAM-1 and ICAM-3. Our data show that I-less LFA-1 does not bind to ICAM-1 or ICAM-3 under multiple conditions that support wild-type LFA-1 binding. Leitinger and Hogg (60) independently reached the same conclusion using an I domain deletion of LFA-1 in a paper published while this manuscript was in review. These results suggest that the I domain is absolutely essential for ligand binding in LFA-1. However, our data do not exclude the role of other domains of the LFA-1 molecule in making additional contacts or in modulating I domain-mediated ligand binding.

In summary, we have characterized the folding, heterodimer formation, and ligand binding of the β_2 integrins Mac-1 and LFA-1 in the absence of their I domains. Our data show that the I domain is not essential for the proper folding, heterodimer formation, and surface expression of these integrins. I-less Mac-1 does bind some ligands such as iC3b and factor X, but it does not bind other ligands such as fibrinogen and denatured BSA. These results indicate that there are several different ligand-binding sites in Mac-1. In contrast, LFA-1 does not bind its ligands in the absence of the I domain, indicating that the I domain is a crucial ligand-binding site in LFA-1. This study underscores the complexity of ligand binding by these I domain-containing β_2 integrins.

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