The leukocyte integrin p150,95 (CD11c/CD18) as a Receptor for iC3b

Activation by a Heterologous β Subunit and Localization of a Ligand Recognition Site to the I Domain

Caroline A. G. Bilsland, Michael S. Diamond, and Timothy A. Springer

The Center for Blood Research and Department of Pathology, Harvard Medical School, Boston, MA 02115

p150,95 is a member of the leukocyte integrin family of adhesion proteins. Compared with LFA-1 and Mac-1, p150,95 is less well functionally characterized. Although p150,95 has complement receptor activity for iC3b and has been designated complement receptor type 4, transfected cells expressing p150,95 do not bind iC3b-sensitized cells. We report that cells cotransfected with a human p150,95 α subunit and a chicken, but not human, β subunit bind IgM-iC3b-coated erythrocytes, suggesting that interactions between the α and β subunits can regulate p150,95 adhesiveness. Furthermore, purified human p150,95 binds to cell-bound iC3b-coated erythrocytes. Because binding to iC3b by cellular and purified p150,95 is specifically abolished by mAbs that localize to the I domain of p150,95, we suggest that the I domain of the p150,95 α subunit is an important ligand recognition site for iC3b.

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The adhesive interactions mediated by the leukocyte integrins play an important role in the immune system (1, 2). This subset of the integrin family, LFA-1 (αLβ2; CD11a/CD18), Mac-1 (αMβ2; CD11b/CD18; complement receptor type 3 (CR3)) and p150,95 (αXβ2; CD11c/CD18; CR4), consists of αβ heterodimers composed of a common β subunit (β2, CD18) noncovalently linked with a unique α subunit. Each of these receptors can bind multiple ligands. LFA-1 binds to three known ligands, ICAM-1 (3), ICAM-2 (4), and ICAM-3 (5). Mac-1 is involved in a number of myeloid cell-adhesive functions and binds several ligands, including iC3b (6, 7), fibrinogen (8, 9), factor X (10), and ICAM-1 (11, 12). The ligands of p150,95 are less well characterized, but appear to overlap in part those of Mac-1. p150,95 is expressed on macrophages, monocytes, granulocytes, activated B cells, and some T cell populations (13-17). p150,95 binds iC3b (18, 19), iC3b-opsonized particles (20, 21), and fibrinogen (15, 22) and is involved in the adhesion of monocytes and neutrophils to endothelium and other cells and substrates (14, 23–26).

The study of p150,95 has been complicated by the apparent overlapping ligand-binding properties of related adhesion proteins that are expressed on the same cell. On myeloid cells, Mac-1 is invariably coexpressed with p150,95. Several experiments have suggested that p150,95 shares with Mac-1 a divalent cation-dependent specificity for iC3b. The first evidence for the iC3b-binding capability of p150,95 came from the biochemical isolation of both p150,95 and Mac-1 by iC3b-Sepharose affinity chromatography of solubilized spleen cells and neutrophils (18).

Further study confirmed that p150,95 isolated from U937 cells could bind to iC3b-Sepharose, but was unable to demonstrate that p150,95 on the surface of U937 cells could mediate binding of IgM-iC3b-coated erythrocytes (E-IgM-iC3b) (19). Subsequently, conditions of low ionic strength were found to enable iC3b rosetting that was dependent on p150,95. When neutrophils or cultured macrophages were treated with mAb to p150,95 alone, no inhibition was seen, but when CR3 activity was blocked with three different mAbs to Mac-1, additional inhibition was obtained with a mAb to p150,95 that was attributable...
to the CR4 (20). In another study, mAbs to p150,95 were able to give inhibition of up to 50% of iC3b rosetting in the absence of treatment with mAb to Mac-1 (21). This inhibition was enhanced by treatment with cytochalasin D in U937 cells, and in 5/7 alveolar macrophage but not in 2/7 preparations of other alveolar macrophages. In studies on iC3b-opsonized Leishmania major binding to differentiated macrophages, 85% of the attachment depended on Mac-1, and only a small level of binding to purified p150,95 was demonstrated (27).

By cotransfecting the α and β subunits of p150,95, it should be possible to study its function in the absence of other complement receptors. Transfection of LFA-1 and Mac-1 in COS and CHO cells has yielded functional integrins able to bind the same ligands as these integrins on their native cells, including ICAM-1 and iC3b (28, 29). However, it has not been possible to demonstrate binding of E-IgM-iC3b to COS or CHO cells transfected with p150,95 (29).

In the present study, we have made the surprising observation that cotransfection of a heterologous chicken β2 subunit with human αX in COS cells allows binding of E-IgM-iC3b, in comparison to the wild-type human p150,95, which does not form rosettes with E-IgM-iC3b. Thus, we speculate that interactions between the αX and β2 subunits may regulate ligand binding. We find that purified p150,95 adsorbed to a substrate is also active in binding E-IgM-iC3b.

The leukocyte integrins are among the largest and thus most structurally complex molecules known on the surface of white blood cells. The αX and β2 subunits of p150,95 have extracellular domains of 1,081 and 677 amino acids, respectively, and typical transmembrane and cytoplasmic domains (30, 31). The αX subunit, like αL and αM, has an inserted or I domain of about 190 amino acids not found in many other integrins, and three EF-hand-like putative divalent cation-binding sites in the extracellular domain. Although the p150,95 αX subunit was the first of the leukocyte integrin α subunits to be cloned, little is known of the functional significance of its different structural features. Chimeric α subunits constructed from segments of the αX and αM subunits have been expressed and studied for binding to iC3b-opsonized erythrocytes (29). Although αMβ2 and not αXβ2 expressed in COS or CHO cells bound E-IgM-iC3b, a binding site in αM could not be mapped with chimeras, because several reciprocal chimeras each expressed iC3b-rosetting activity. Because the conformation of several of these chimeras was disrupted, it was speculated that noncomplementary interactions between αM and αX segments may have helped to express a binding site for iC3b. A binding site for several ligands for Mac-1, including iC3b, fibronectin, and ICAM-1, was mapped to the I domain by using the chimeras to map the epitopes of function-blocking mAbs (29). A Mg²⁺-binding site that is required for iC3b binding has also been mapped to the Mac-1 I domain (32). In this paper, we have used the αM-αX chimeras and the rosetting activity of purified p150,95 and transfectants expressing the human αX and chicken β2 subunits to map an iC3b binding site in p150,95 by localizing epitopes recognized by function-blocking mAbs.

**Materials and Methods**

**Cell lines and mAbs**

The SV40-transformed African green monkey kidney fibroblastoid cell line, COS-7 (33), was used for transfection.

The following mAbs were used in this study: CBRM1/23 (anti-CD11b, IgG2a) (29); CBM1/29 (anti-CD11b, IgG1) (29); CBP150/4G1 (anti-CD11c, IgG2a) (26); CBP150/2C1 and CBP150/2E1 (anti-CD11c) (26); SHCL3 (anti-CD11c, IgG2b) (16); 3.9 (anti-CD11c) (17); and BL-4H4 (anti-CD11c) (24); and M1/87 (35). mAb 3.9 and BL-4H4 were obtained through the 4th International Leukocyte Workshop.

**iC3b-coated erythrocyte preparation**

Erythrocytes were sensitized with IgM (E-IgM) and C5-deficient human complement (E-IgM-iC3b) as previously described (29, 36). Previous studies have shown that on erythrocytes prepared with IgM and whole serum as complement source almost all of the C3b is converted to iC3b and very little, if any, is converted further to C3d (37). Sheep erythrocytes (Colorado Serum Company, Denver, CO) were washed once in HBSS/15 mM HEPES, pH 7.3/1 mM MgCl₂, resuspended in 10 ml of the same buffer (6 × 10⁹ cells) and incubated with a 1:128 dilution of heat-inactivated anti-Forssman IgM mAb (M1/87) supernatant (35) for 60 min at room temperature with end-over-end rotation. E-IgM was washed twice and resuspended in 1.8 ml HBSS/15 mM HEPES pH 7.3/1 mM MgCl₂/1 mM CaCl₂. Half of the E-IgM was then treated with 100 µl C5-deficient human serum (Sigma Chemical Co., St. Louis, MO) and incubated for 60 min at 37°C with end-over-end rotation. The resulting E-IgM-iC3b were then washed twice more and resuspended in 3 ml HBSS/15 mM HEPES, pH 7.3/1 mM MgCl₂/1 mM CaCl₂. We have previously shown that this reagent forms rosettes with receptors specific for iC3b but not for C3b (6, 29). Neutrophil rosetting with E-IgM-iC3b is 99% inhibited with CBRM1/29 mAb to Mac-1 (29).

**Protein purification and adhesion assay**

p150,95 was purified from hairy cell leukemia spleen detergent lysates by immunoaffinity chromatography (26, 27). Mac-1 and LFA-1 were purified from leukocyte lysates (12, 27). Adhesion to purified proteins involving potentially low binding affinities was analyzed using a tip plate assay (12). Briefly, purified proteins in elution buffer containing 1% glycerol were diluted 1:15 or 1:20 in 10 mM Tris, pH 8.0/150 mM NaCl/2 mM MgCl₂ (TSM) to a final concentration of 0.2 µg/ml and adsorbed (25-µl spots) onto 6-mm petri dishes for 90 min at room temperature. Nonspecific binding sites were blocked with 1% heat-treated BSA/PBS. The petri dishes were treated with blocking or nonblocking mAbs for 30 min at room temperature before 1 × 10⁵ E-IgM-iC3b or E-IgM were added. After a 1-h incubation at 37°C, unbound erythrocytes were removed by gentle swirling of the binding buffer and removal using a bulb pipette. This washing procedure was performed three to five times. Erythrocytes bound to the purified proteins were scored by light microscopy. The number of erythrocytes bound were those counted in 20 squares along the edges of a 10×10 square ocular grid.

**COS cell transfection and adhesion assay**

COS cells were transfected with ApoM constructs expressing the leukocyte integrin β2 and α subunits using the DEAE-dextran method (38). COS transfecteds were suspended using trypsin-EDTA and seeded one day before assay into six-well tissue culture plates (5 × 10⁴ cells/well) (39). Just before assay, nonadherent transfecteds were removed by three washes with HBSS/15 mM HEPES, pH 7.3/1 mM MgCl₂/1 mM CaCl₂/1% BSA, whereupon 1 × 10⁵ E-IgM or E-IgM were added.
THE I DOMAIN AND ADHESIVENESS OF p150,95 FOR iC3b

**Results**

**Binding of hybrid chicken/human p150,95 to E-IgM-iC3b**

Although several reports have shown that p150,95 binds to iC3b (18–21), human p150,95, when expressed in COS or CHO cells, does not bind iC3b (29). In the course of studies on the chicken β2 integrin subunit, we made the surprising observation that although human p150,95 expressed in COS cells did not bind E-IgM-iC3b, the interspecies hybrid, containing the chicken integrin β2 subunit complexed with the human αX subunit, did bind iC3b-opsonized erythrocytes (Fig. 1). COS cells expressing human Mac-1, a well-characterized iC3b receptor, were used as a positive control and rosetted strongly with E-IgM-iC3b (Fig. 1). Hybrid HaX/ChP2 Mac-1 bound iC3b-E to equivalent levels (Fig. 1). Whereas 100% of COS cells expressing wild-type or hybrid Mac-1 formed rosettes, approximately 30% of COS cells expressing hybrid p150,95 did so; however, the rosettes with hybrid p150,95 were as florid as those with Mac-1, in some cases completely covering the COS cell surface. COS cells expressing LFA-1 with either a human or chicken β2 subunit demonstrated absolutely no E-IgM-iC3b rosetting (Fig. 1), as did mock-transfected COS cells (data not shown). Both the interspecies hybrid and human LFA-1 were well expressed (41). None of the transfected cells rosetted with E-IgM (data not shown).

The interspecies HaX/Chβ2 complex was expressed as efficiently as the human HaX/Hβ2 complex in terms of percent positive cells and fluorescence intensity (Fig. 2). Human αX alone was not expressed efficiently in COS cells. Surprisingly, αM transfected alone was consistently expressed efficiently in COS cells (Fig. 2) and enabled iC3b rosetting (Fig. 1), although only 10% as efficiently as αMβ2. Immunoprecipitation from [35S]labeled COS cells confirmed surface expression in cells transfected with αM alone and showed coprecipitation of a band that may represent an endogenous COS cell integrin β subunit (data not shown).

**Binding of E-IgM-iC3b to purified human p150,95**

We wished to extend our functional studies on chicken and human hybrid p150,95 to human p150,95. Previously, p150,95 in detergent lysates was purified along with CR1, CR2, and Mac-1 (CR3) on iC3b-Sepharose (18, 19); however, it has not been demonstrated that purified p150,95 can mediate rosetting with iC3b-opsonized erythrocytes. Purified leukocyte integrin proteins were coated onto plastic, and their ability to bind iC3b-opsonized erythrocytes determined by using a tip plate assay. Significant binding of E-IgM-iC3b was observed to purified human p150,95 (Fig. 3A) and to the positive control protein human Mac-1 (Fig. 3B). No binding of E-IgM-iC3b was observed to uncoated areas of the plate (Fig. 3A and B, upper right corner) or to the related leukocyte integrin protein LFA-1 (Fig. 3C) or to uncoated plastic (Fig. 3D). No binding to any of the substrates was observed with E-IgM (data not shown).

**Epitope mapping of anti-p150,95 Abs**

CHO cell transfectants expressing wild-type p150,95, Mac-1, or p150,95/Mac-1 chimeras (29) were used to map the epitopes recognized by mAbs to p150,95 (Fig. 4). mAbs 3.9 and BL-4H4 were localized to the I domain.
FIGURE 2. Flow cytometric analysis of COS cells expressing human and human-chicken hybrid p150,95 and Mac-1 molecules. COS cells were transfected with vectors expressing the indicated subunits or with vector alone (mock), stained with mAb to αX (CBRp150/2E1) or αM (CBRM1/23) and FITC anti-mouse Ig, and subjected to flow cytometry.

FIGURE 3. Binding of E-iC3b to purified integrin proteins coated on plastic. Photomicrographs of E-iC3b binding to purified p150,95 (A), Mac-1 (B), LFA-1 (C), or no integrin protein (D). Purified proteins were adsorbed (25-μl spots) onto 6-cm petri dishes for 90 min at room temperature. Nonspecific binding sites were blocked with 1% heat-treated BSA-PBS. E-IgM-iC3b (1 × 10⁷) were added and incubated at 37°C for 60 min, after which unbound erythrocytes were removed by gentle swirling of the buffer and removal using a transfer pipette. The outer edge of the circular protein spot is shown in panels (A) and (B), including the area outside the spot with no bound E-iC3b, to emphasize the specificity of binding.

of αX by their ability to bind to a discrete subset of p150,95/Mac-1 chimeras (Fig. 4). Two mAbs (CBRp150/2C1 and CBRp150/4G1) have been mapped to the C-terminal region of αX (26), and one (SHCL3) has been mapped to a discontinuous epitope requiring the presence of both the N-terminal and divalent cation-binding regions (29). The Mac-1 mAb CBRM1/29 has been localized to the I domain of αM (29).

**Functional inhibition with mAbs: localization of epitopes associated with E-iC3b rosetting**

The two systems we developed for studying the function of p150,95 in rosetting with iC3b-opsonized particles, transfectants expressing human αX with chicken β2 and purified human p150,95 adsorbed to a substrate, allowed us to test the effect of mAbs on function and correlate this with epitope localization. With COS cells expressing hybrid HaX/Chβ2 p150,95 only two of five mAbs tested, 3.9 and BL-4H4, were found to completely abolish E-iC3b rosette formation (Fig. 5). The epitope recognized by both these mAbs mapped to the I domain of αX (Fig. 4). This observation suggests that the I domain is, or constitutes an important part of, the iC3b-binding site of p150,95. The other three mAbs, CBRp150/2C1, CBRp150/4G1, and SHCL3, reduced rosetting only approximately twofold (Fig. 5). These mAbs mapped to distinct regions of the αX subunit (Fig. 4).

In the assay of adherence of E-IgM-iC3b to purified human p150,95, binding was also completely abolished by the I domain Abs 3.9 and BL-4H4 (Fig. 6). mAbs to other regions of αX again only reduced binding by approximately twofold. All of the p150,95 mAbs were specific, as none inhibited binding of E-IgM-iC3b to Mac-1 (Fig. 6). Conversely, E-IgM-iC3b binding to Mac-1 was inhibited only by an anti-αM Ab (Fig. 6). Again, the specificity of the assay was demonstrated by lack of binding of E-IgM-iC3b to purified LFA-1 (Fig. 6) and by lack of binding of E-IgM to any of the substrates (data not shown).

**Discussion**

We describe here the use of human αX/chicken β2 p150,95 and purified human p150,95 to measure the function of p150,95 in rosetting with iC3b-opsonized particles in systems in which no other complement receptors are present. Our studies suggest that the I domain of the p150,95 α subunit is a major ligand recognition site for iC3b.

Early studies showed that detergent-solubilized p150,95 could bind to and be purified with iC3b-Sepharose affinity.
columns (18, 19). Our own study has extended these studies by showing that purified p150,95 can function as a receptor for iC3b-opsonized particles. Initial attempts to demonstrate iC3b-binding by p150,95 on the monocytic cell line U937 were unsuccessful (19). Subsequently, a cell-surface iC3b receptor activity was shown for p150,95 with neutrophils, monocyte-derived macrophages (20), and alveolar macrophages (21). However, these studies on cells required extensive blockade with three mAbs to the CR3 (Mac-1) and with mAb or polyclonal antisera to CR1 (CD35) before a smaller contribution made by p150,95 could be observed (20, 21). On other cells expressing high levels of p150,95, such as PMA-stimulated U937 cells, which express up to $3 \times 10^5$ sites/cell, no iC3b receptor activity attributable to p150,95 was detectable unless cells were treated with cytochalasin D (21).

Our studies extend these results by showing that on transfected cells that lack other complement receptors, p150,95 functions as a receptor for iC3b-opsonized erythrocytes. Together with our studies with purified p150,95, this demonstrates that cooperation with other complement receptors is not required and that p150,95 is sufficient for expression of CR4 activity.

Our studies have also clarified an apparent conflict in the literature between the studies cited above and one from our own laboratory that showed that when human p150,95 was expressed in transfected CHO or COS cells, no iC3b binding was demonstrated (29). By contrast, when transfected in COS cells, LFA-1 (42) and Mac-1 (12) are constitutively active. However, this latter finding does not appear to hold true for p150,95. Accumulating evidence suggests that regulation of leukocyte integrins may involve removal of a restraint that basally keeps integrins in an inactive state. Interestingly, purified LFA-1, Mac-1, and p150,95 are all constitutively active when immobilized on plastic (Refs. 12, 26, 43, and this report). When the β subunit cytoplasmic domain is partially truncated in COS cells, ligand binding by LFA-1 is lost, but when fully

**FIGURE 4.** Mapping of mAb epitopes with chimeric p150,95/Mac-1 α subunits. The restriction sites (e = EcoRV, b = BglII and a = AflII) used to create the chimeric molecules are indicated by arrows and in the chimeric molecule name. CHO cells, stably cotransfected with chimeric α subunits and the human β2 subunit (29), were stained with p150,95 mAb and subjected to immunofluorescence flow cytometry. mAb binding to the wild-type and chimeric p150,95 and Mac-1 molecules was scored as follows: (+++) indicates that 100% of the cells stained strongly with the mAb, (+++) indicates that 100% of cells were positive but with a lower fluorescence intensity, and (+) indicates that only a subpopulation of the cells stained positively with the mAb.
After a 60-min incubation at 37°C, unbound erythrocytes at room temperature before \(1 \times 10^7\) E-lgM-iC3b were added. Wells were incubated with or without mAbs for 30 min at room temperature before 1 \(1 \times 10^7\) E-lgM-iC3b were added. After a 60-min incubation at 37°C, unbound erythrocytes were removed by gentle swirling of the buffer and removal using a transfer pipette. E-lgM-iC3b bound to purified leukocyte integrin proteins were scored by light microscopy. The percent of rosetting cells was divided by the percent of COS cells positive with p150,95 or Mac-1 subunit mAb in immunofluorescence flow cytometry. BL-4H4, 3.9, CBRp150/2C1, CBRp150/4G1, and SHCL3 are anti-\(\alphaX\) mAbs. CBRM1/29 is an anti-\(\alphaM\) mAb that has been mapped to the 1 domain. There were 0 rosettes in the presence of mAbs 3.9 and BL-4H4.

![Figure 5](image-url)

**Figure 5.** COS cell transfectants binding E-iC3b in the presence of Abs. COS cell transfectants were suspended and reseeded in six-well tissue culture plates one day before assay. Wells were incubated with or without mAbs for 30 min at room temperature before \(1 \times 10^7\) E-lgM-iC3b were added. After a 60-min incubation at 37°C, unbound erythrocytes were removed by gentle swirling of the buffer and removal using a transfer pipette. Rosettes (>10 erythrocytes/COS cell, >100 cells examined) were scored by light microscopy. The percent of rosetting cells was divided by the percent of COS cells positive with p150,95 or Mac-1 subunit mAb in immunofluorescence flow cytometry. BL-4H4, 3.9, CBRp150/2C1, CBRp150/4G1, and SHCL3 are anti-\(\alphaX\) mAbs. CBRM1/29 is an anti-\(\alphaM\) mAb that has been mapped to the 1 domain. There were 0 rosettes in the presence of mAbs 3.9 and BL-4H4.

truncated, it is largely regained (44). Because it seems unlikely that the chicken \(\beta2\) integrin subunit would fortuitously be better adapted to binding human iC3b than the human \(\beta2\) integrin subunit and because the HoM/Ch\(\beta2\) and HoM/H\(\beta2\) complexes bind iC3b equally well, the likely interpretation is that an intersubunit restraint on \(\alphaX\) conformation is loosened with the chicken \(\beta2\) subunit so that \(\alphaX\) can more readily adopt the conformation that binds iC3b. It may be significant in this regard that the association between \(\alphaX\) and \(\beta2\) is more difficult to disrupt with denaturing conditions than the association between \(\alphaL\) and \(\beta2\) or \(\alphaM\) and \(\beta2\) (45). In further support of this idea, activation of iC3b binding also seems to be triggered in certain \(\alphaX/\alphaM\) subunit chimeras, perhaps by non-complementary intra-\(\alpha\) subunit interactions that alter conformation (29). The chicken \(\beta2\) subunit extracellular domain is less conserved with the human than the cytoplasmic domain (41). Because of this, and because all key cytoplasmic residues and regions important in regulation of LFA-1 adhesiveness (46) are conserved, our speculations on why the interspecies hybrid p150,95 molecule is activated have focused on the extracellular domain. However, it is also possible that the few differences in the chicken \(\beta\) subunit cytoplasmic domain may alter CR4 activity.

Although binding an overlapping repertoire of ligands, p150,95 and Mac-1 show differences in cell surface expression and perhaps regulation. During monocyte differentiation into tissue macrophages, there is a switch in expression of the predominant iC3b receptor from Mac-1 to p150,95 (13, 17). The basis of this change is not clearly understood. It has been proposed that p150,95 and Mac-1 have different cytoskeletal attachments, resulting in differences in mobility in the membrane (21). In a study on alveolar macrophages and PMA-differentiated U937 cells, it was observed that the more numerous p150,95 molecules were less able to form rosettes with E-iC3b than were the less numerous but apparently more mobile Mac-1 molecules (21). Treatment with cytochalasin D increased the ability to modulate CR3 and CR4, decreased the CR3-dependent rosettes, and increased CR4-dependent rosettes (21).

Leukocyte integrin \(\alpha\) subunits have two interesting motifs in the extracellular domain, a ~190 amino acid insert or "I" domain and three divalent cation-binding repeats (30, 42, 47–50). I domains have been found in only a subset of the integrin \(\alpha\) subunits, namely the three leukocyte or \(\beta2\) integrins and the \(\beta1\) integrins VLA-1 and -2 (51, 52). I domains are structurally homologous to domains in Factor B, von Willebrand factor, and other proteins (42, 49, 53). We found that in two different assays of iC3b-rosetting function, only mAbs that mapped to the I domain of \(\alphaX\) completely blocked binding of iC3b. This...
suggests that the I domain is an important par of the ligand recognition site. mAbs to other regions gave only partial inhibition, suggesting that the epitopes recognized in these regions may be near but do not overlap with the iC3b-binding site. Studies on Mac-1 have shown that the I domain is an important recognition site for four ligands: iC3b, ICAM-1, fibrinogen, and the ligand for neutrophil homotypic aggregation (29). Patterns of Ab inhibition suggest that the ligand binding sites within the I domain of Mac-1 are overlapping but not identical (29). A mAb that recognizes a functionally active subpopulation of Mac-1 molecules on activated leukocytes maps to the I domain (54). Furthermore, a Mg$^{2+}$-binding site in the I domain of Mac-1 is required for ligand binding (32). Analysis of Abs that activate the leukocyte integrin LFA-1 identified an epitope that maps to the I domain of the α subunit, suggesting that this domain may regulate binding to ICAM-1 (55), but a direct role in ligand binding has not yet been demonstrated. The p150,95 molecule is the second example of an integrin in which the I domain has been shown to have a prominent role in ligand binding. It will be interesting to determine whether this will be generalizable to LFA-1, which does not bind iC3b, and to the β1 integrins that contain I domains, VLA-1 and VLA-2.

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