The Top of the Inserted-like Domain of the Integrin Lymphocyte Function-associated Antigen-1 β Subunit Contacts the α Subunit β-Propeller Domain near β-Sheet 3*

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We find that monoclonal antibody YTA-1 recognizes an epitope formed by a combination of the integrin αL and β2 subunits of LFA-1. Using human/mouse chimeras of the αL and β2 subunits, we determined that YTA-1 binds to the predicted inserted (I)-like domain of the β2 subunit and the predicted β-propeller domain of the αL subunit. Substitution into mouse LFA-1 of human residues Ser692 and Arg693 of the β2 subunit and Pro738, Thr739, Asp869, Ile875 and Asn877 of the αL subunit is sufficient to completely reconstitute YTA-1 reactivity. Antibodies that bind to epitopes that are nearby in models of the I-like and β-propeller domains compete with YTA-1 monoclonal antibody for binding. The predicted β-propeller domain of integrin α subunits contains seven β-sheets arranged like blades of a propeller around a pseudosymmetry axis. The antigenic residues cluster on the bottom of this domain in the 1–2 loop of blade 2, and on the side of the domain in β-strand 4 of blade 3. The I domain is inserted between these blades on the top of the β-propeller domain. The antigenic residues in the β subunit localize to the top of the I-like domain near the putative Mg2+ ion binding site. Thus, the I-like domain contacts the bottom or side of the β-propeller domain near β-sheets 2 and 3. YTA-1 preferentially reacts with activated LFA-1 and is a function-blocking antibody, suggesting that conformational movements occur near the interface it defines between the LFA-1 α and β subunits.

Lymphocyte function-associated antigen-1 (LFA-1) is a member of the leukocyte integrin family. LFA-1 (αLβ2; CD11a/CD18), Mac-1 (αMβ2; CD11b/CD18), p150,95 (αxβ2; CD11c/CD18), and αxβ2 (1, 2). The leukocyte integrins are heterodimers composed of a common β2 subunit noncovalently associated with different but structurally homologous α subunits (3). LFA-1 is expressed on the cell surface of all leukocytes. Upon activation, LFA-1 binds to its ligands, ICAM-1, -2, and -3 (4–6), and mediates important immunological functions including leukocyte adherence to endothelium, natural killing, and antigen-dependent T and B cell responses (7, 8).

Structure-function studies of LFA-1 are important to understand the molecular basis for cell adhesion through LFA-1. Three extracellular subregions of LFA-1 are critical in ligand binding. The first is a sequence of seven 60-amino acid repeats located in the N-terminal half of the αL subunit. These seven repeats are a common structural feature of all integrin α subunits. These repeats have been predicted to fold into a β-propeller domain with seven β-sheets (9). The β-propeller domain is toroidal in shape, with the β-sheets arranged around a pseudosymmetry axis like blades of a propeller. Each β-sheet may be termed a “W” after the topology of the four anti-parallel β-strands. Ligand binding has been localized to loops on the “upper” surface of the propeller, in β-sheets 2, 3, and 4 for the integrin α subunits α4, α5, and α6 (10–14). In contrast to α4, α5, and α6, the leukocyte integrins contain an additional domain of about 200 amino acids. It is inserted into a loop at the top of the β-propeller domain between β-sheets 2 and 3, and is designated the inserted (I) domain. I domain structures for αL and αM have been determined by crystallography (15, 16). The I domain folds into a doubly twisted α/β structure with a ligand binding site known as a metal ion-dependent adhesion site, or MIDAS, in a crevice on its upper face. The third region important for ligand binding by integrins is in the N-terminal half of the β2 subunit from residue 100 to 340, which is well conserved among different integrin β subunits. This conserved region has been predicted to fold like an I domain with a ligand-binding MIDAS motif (15, 17–19, 57). These three domains also interact with divalent cations, such as Mg2+, Mn2+, and Ca2+, which are required to regulate integrin-ligand interactions (4, 21–25).

Extensive studies including mutagenesis and mapping epitopes of function-blocking or activating antibodies have demonstrated that the I domain and the β-propeller domain of the αL subunit and the I-like domain of the β2 subunit cooperatively contribute to ligand binding for LFA-1 (26–30). Furthermore, although conformational change in I domains is now well documented (31–34), little is known about conformational change in the β-propeller or I-like domains of leukocyte integrins. Moreover, the structural basis for interactions between these domains remains unknown. Previously, there has been no direct evidence for structural association between the α subunit β-propeller domain and the β subunit I-like domain, despite their joint role in regulating ligand binding; mAb has been used to show that folding of epitopes in the I-like domain is dependent on association with the α subunit, and that folding of epitopes in the β-propeller domain is dependent on association with the β subunit (35, 36). This mutual dependence raised

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1 The abbreviations used are: LFA-1, lymphocyte function-associated antigen-1; mAb, monoclonal antibody; ICAM-1, intercellular adhesion molecule-1; I, inserted; MIDAS, metal ion-dependent adhesion site; FBS, fetal bovine serum.
the possibility of an intimate structural association between the β-propeller and I-like domains. The mAb used in the above studies were demonstrated to be completely dependent for binding on species-specific residues in either the LFA-1 α or β, but not both subunits (35, 36). For example, mAb used to study binding of the β-propeller domain of the human α2 subunit were reactive with α2β2 complexes whether the β2 subunit was of human, mouse, or chicken origin (36).

Mapping an antibody with an epitope combined from both integrin α and β subunits would elucidate structural information on intersubunit association. We describe here such a mAb, YTA-1. YTA-1 is specific for the human LFA-1 integrin but has properties that distinguish it from other antibodies to LFA-1 (37, 38). It reacts strongly with CD3+CD16+ large granular lymphocytes that function as natural killer cells, but not with other peripheral blood lymphocytes that express LFA-1. Furthermore, YTA-1 is mitogenic for natural killer cells and can activate natural killer cytotoxicity. The antibody was established to be specific for LFA-1 based on its ability to bind to transfecants expressing LFA-1 but not the related β2 integrins Mac-1 or p150,95. In distinction to other described antibodies to LFA-1, binding of YTA-1 to LFA-1 could be competed away by certain mAbs against both the α1 and β2 subunits (37). In this report, we demonstrate that YTA-1 recognizes an activation-dependent epitope on LFA-1 consisting of residues from both the β2 subunit and the α1 subunit. We identify these specific amino acid residues and their positions in models of the α1-β2-propeller domain and the β2-I-like domain. Direct association between these subunits is thus demonstrated and localized.

MATERIALS AND METHODS

Cell Lines and Monoclonal Antibodies—293T cells (a human renal epithelial transformed cell line) were grown in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (FBS), nonessential amino acids (Life Technologies, Inc.), 2 mM glutamine, and 50 μg/ml gentamicin. Jurkat (human T lymphoma cells) and SKW3 (human T lymphoma cells) were grown in RPMI 1640 medium with 10% FBS and 50 μg/ml gentamicin.

The mouse anti-human α1 mAb TS1/22, CBR LFA-1/10, CBR LFA-1/1, C25.2, TS2/9, S6F1, TS2/6, May.05/2, TS2/14, and 25-3-1; the anti-human β2 mAbs TS1/18, YFC118.3, YFC51, 1C11, GRF-1, CLB LFA-1/1, May.017, L130, CBR LFA-1/7, and CBR LFA-1/2; the anti-human LFA-1 YTA-1; and the rat anti-mouse α1, G25.2, TS2/4, S6F1, TS2/14, and 25-3-1; the anti-human β2 mAbs TS1/18, YFC118.3, YFC51, 1C11, GRF-1, CLB LFA-1/1, May.017, L130, CBR LFA-1/7, and CBR LFA-1/2; the anti-human LFA-1 YTA-1; and the rat anti-mouse α1 mAb M17/5.2 have been described previously (35–37, 39, 40).

Human Mouse Chimeric α1 and β2 Constructs—Human and mouse α1 and β2 cDNA were inserted in vector AprMS (29). As described previously (39, 40), chimeras were named according to the species origin of their segments. For example, h401m442h indicates residues 1–401 are from human, 402–442 are from mouse and 443 to the C terminus are from human. Chimeras and substitution mutants were generated by polymerase chain reaction (PCR) with 10% of fetal bovine serum (FBS), nonessential amino acids (Life Technologies, Inc.), 2 mM glutamine, and 50 μg/ml gentamicin. Jurkat (human T lymphoma cells) and SKW3 (human T lymphoma cells) were grown in RPMI 1640 medium with 10% FBS and 50 μg/ml gentamicin.

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Transfection—Plasmids for transfection were purified by Qiagen (Valencia, CA). 293T cells were transiently transfected with cDNA for human or mouse α1 and β2 subunits as indicated. B. Jurkat-β2 cells were stably transfected with the wild type human α1 subunit or mutated human α4 subunit in which the GFFKR sequence at the junction of the transmembrane and cytoplasmic domains was deleted (44). Transfectants were stained with TS1/18 mAb to α1, TS1/22 mAb to β2, or X63 myeloma IgG1 as control. Cells were then stained with fluorescein isothiocyanate anti-Ig and subjected to immunofluorescence flow cytometry.

Adhesion Assay—Binding of cells to human soluble ICAM-1 was examined as described (44). Briefly, purified human soluble ICAM-1 was absorbed to each well of flat-bottom 96-well plates by incubation overnight at 4°C. Non-specific binding sites were blocked with 1% bovine serum albumin at room temperature for 1 h. Cells were labeled with 2,7'–bis(carboxyethyl)-5(6)-carboxyfluorescein, acetoxyethyl ester and mixed in ICAM-1-coated wells with 50 μg/ml mAb YTA-1, TS1/22, or as control the X63 myeloma IgG1. After incubation at 37°C for 15 min, the unbound cells were removed on a Microplate Autowasher (Bio-Tek Instruments, Winooski, VT). The fluorescence content of total input cells (before washing) and the bound cells (after washing) in each well was quantified with a fluorescence concentration analyzer (IDEXX, Westbrook, ME).

α1 Subunit β-Propeller Model—Modeling was with Segment (45) of the An Intersubunit Association of LFA-1 Integrin

FIG. 1. mAb YTA-1 is specific for both subunits of human LFA-1. A, 293T cells were co-transfected with cDNA for human or mouse α1 and β2 subunits, as indicated. B, Jurkat-β2 cells were stably transfected with the wild type human α1 subunit or mutated human α4 subunit in which the GFFKR sequence at the junction of the transmembrane and cytoplasmic domains was deleted (44). Transfectants were stained with TS1/18 mAb to α1, TS1/22 mAb to β2, or X63 myeloma IgG1 as control. Cells were then stained with fluorescein isothiocyanate anti-Ig and subjected to immunofluorescence flow cytometry. The fluorescence content of total input cells (before washing) and the bound cells (after washing) in each well was quantified with a fluorescence concentration analyzer (IDEXX, Westbrook, ME).

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Adhesion Assay—Binding of cells to human soluble ICAM-1 was examined as described (44). Briefly, purified human soluble ICAM-1 was absorbed to each well of flat-bottom 96-well plates by incubation overnight at 4°C. Non-specific binding sites were blocked with 1% bovine serum albumin at room temperature for 1 h. Cells were labeled with 2,7’–bis-(carboxyethyl)-5(6)-carboxyfluorescein, acetoxymethyl ester and mixed in ICAM-1-coated wells with 50 μg/ml mAb YTA-1, TS1/22, or as control the X63 myeloma IgG1. After incubation at 37°C for 15 min, the unbound cells were removed on a Microplate Autowasher (Bio-Tek Instruments, Winooski, VT). The fluorescence content of total input cells (before washing) and the bound cells (after washing) in each well was quantified with a fluorescence concentration analyzer (IDEXX, Westbrook, ME).

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1alk and 1tbg. A final model was made with MODELLER using the entire LOOK model as the .ini file, and as templates: 1) three different 1alk files containing only the residues shown in Fig. 4 and Ca\(^{2+}\) ions, 2) the LOOK model of aL deleting the residues aligning with the 1alk loops and the residues shown in lowercase in Fig. 4 to enable the two cysteines in this region to form a disulfide using the PATCH DISULFIDE routine, and 3) circularly permuted 1tbg Protein Data Bank files starting with strand 4 of W1 as shown in Fig. 4 and also beginning with strand 4 of W2, W3, and W4 (see Ref. 9). One hundred models were made, and one was chosen that lacked knotted loops, contained Ca\(^{2+}\)-binding loops with conformations similar to that of 1alk, and had a score of -1.772 as determined with the QUACHK module of WHAT IF (49).

RESULTS

Monoclonal Antibody YTA-1 Recognizes Human LFA-1 on an Epitope Formed by a Combination of the aL and b2 Subunits—To test whether the YTA-1 mouse anti-human antibody was specific for both the human aL and b2 subunits, they were expressed in association with murine b2 and aL subunits, respectively. Binding of YTA-1 mAb to 293T cell transfectants was measured by immunofluorescence flow cytometry, and the total amount of LFA-1 surface expression was determined with mAb that react with human aL or b2 independently of the species origin of the associating subunit (35, 36); expression of LFA-1 on the cell surface requires association between the aL and b2 subunits (50, 51). Human LFA-1 and mouse/human hybrid LFA-1 were equivalently expressed, as examined by immunostaining with either TS1/18 mAb to b2 or TS1/22 mAb to aL (Fig. 1A). MAb YTA-1 was strongly reactive with human LFA-1, but not LFA-1 with human aL and mouse b2 or mouse aL and human b2 subunits (Fig. 1A). Thus, both the aL and b2 subunits have to be of human origin to form the YTA-1 epitope. The overall conformation of the mouse/human hybrid LFA-1 molecules was intact as shown by immunostaining with other anti-aL and b2 mAbs. Indeed, all other mAbs we have studied, including 11 directed to 7 different epitopes on the aL subunit, and 17 to 12 different epitopes on the b2 subunit, are fully reactive if only one of the two subunits is of human origin (35, 36).

We examined whether LFA-1 activation affected the expression of the YTA-1 epitope. Previous studies have shown that LFA-1 expressed in 293T cells is constitutively active in binding ICAM-1 (44) (and see Fig. 9 below). Therefore, we tested
An Intersubunit Association of LFA-1 Integrin

LFA-1 in Jurkat T lymphoma cells. A mutant Jurkat cell line (Jurkat-β2,7) deficient in endogenous αL subunit was stably transfected with wild type human LFA-1 αL subunit or an activated form of αL, in which the conserved GFFKR sequence motif in the cytoplasmic domain is deleted (44). The YTA-1 mAb bound much better to activated than wild-type LFA-1 whereas the TS1/22 mAb to LFA-1 bound similarly to activated and wild-type (Fig. 1B). The fluorescence intensity of YTA-1 staining of 293T cells cotransfected with chimeric L subunit or an upper left portion of the I-like domain (Fig. 6, upper left). Mapping was done with mouse-human chimeras named accord-

Mapping the YTA-1 Epitope on the Human αL Subunit—

Mapping was done with mouse-human chimeras named according to the species origin of their segments, e.g. h300m359h is an α subunit with human amino acid residues 1–300, mouse residues 301–359, and human residues 360 to C terminus. Previous work showed that “YTA-1 mAb lost reactivity with h300m442h but reacted with h300m359h; thus, at least a portion of its epitope localizes to residues 360–442” (29). To refine this mapping, three different segments of mouse sequence, 359–376, 377–400, and 401–442 were swapped into the human αL sequence. Chimeric αL subunits were co-transfected with human β2 into 293T cells followed by immunostaining with YTA-1 (Fig. 2). Swapping in mouse segments 377–400 or 401–442 had no effect on binding of YTA-1 mAb; however, swapping in 359–376 in chimera h359m376h reduced binding to less than half of that seen with human LFA-1. All chimeras were well expressed and folded, as determined by staining with other mAbs to the human β-propeller domain and mAb TS1/18 to β2 (data not shown).

Within region 359–376, seven amino acids differ between human and mouse αL. Mouse residues were therefore introduced into the human sequence one or two at a time (“knock-out” mutations) (Fig. 2). Three groups of residues had no effect. However, N367Q and I365V each reduced binding. Furthermore, the double mutant I365V/N367Q reduced binding by the same amount as h359m376h.

Although residues Ile365 and Asn367 appeared to be the only species-specific residues recognized by YTA-1 in the 359–442 segment, YTA-1 reacted more strongly with I365V/N367Q and h359m376h than with mouse αL (Fig. 2). To determine if residues in other segments were recognized by YTA-1, the I365V/N367Q mutation was introduced into a set of previously constructed αL chimeras in which small segments within region 1–359 of human αL were replaced with mouse sequences (29) (Fig. 3). Of all segments tested, only replacement of residues 74–92 with mouse sequence was additive with I365V/N367Q, and reduced binding to the same low level as seen with mouse αL. However, substitution h74m93h alone was unable to reduce YTA-1 binding (Fig. 3). Comparison of human and mouse αL sequences within region 74–93 reveals only three differences, at the conserved amino acid residues 78, 79, and 80. Therefore, substitution of only five residues of the human αL, Pro78, Thr79, Asp80, Ile365, and Asn367, is sufficient to abolish recognition of the YTA-1 epitope. According to the β-propeller model (9), these residues are located in adjacent β sheets: Pro78, Thr79, and Asp80 in β-sheet 2, and Ile365 and Asn367 in β-sheet 3 (Fig. 4).

To confirm the above “knock-out” results, “knock-in” mutants were made by introducing the corresponding human residues into mouse αL (Fig. 5). Knock-in mutation V365I/Q367N was sufficient to restore the same level of YTA-1 binding as with human αL and the individual V365I and Q367N mutants partially restored binding. The A78P/A79T/K80D knock-in partially reconstituted YTA-1 binding (Fig. 5), consistent with the inability of the I365V/N367Q knock-out to fully eliminate YTA-1 binding (Fig. 4). This confirms the above “knock-in” results, “knock-in” mutants were made by introducing the corresponding human residues in to mouse αL. (Fig. 5). Knock-in mutation V365I/Q367N was sufficient to restore the same level of YTA-1 binding as with human αL and the individual V365I and Q367N mutants partially restored binding. The A78P/A79T/K80D knock-in partially reconstituted YTA-1 binding (Fig. 5), consistent with the inability of the I365V/N367Q knock-out to fully eliminate YTA-1 binding (Fig. 4). Combination of the knock-in mutations at residues 78–80 with those at 365 and 367 showed that the knock-in mutations at 365 and 367 were sufficient for YTA-1 binding, and that knocking in residues 78–80 had little additional effect.

Mapping the YTA-1 Epitope on the Human β2 Subunit—The YTA-1 mAb binding site on the human β2 subunit was mapped using human/mouse β2 chimeras (Fig. 6, left). Immunostaining of 293T cells cotransfected with chimeric β2 and human αL, CD41 showed that region 302–344 is important for YTA-1 recognition. All chimeras in which region 302–344 was of mouse origin failed to stain with YTA-1, whereas all chimeras in which this region was of human origin stained as well as human β2 (Fig. 6, right). Region 302–344 is in the C-terminal portion of the I-like domain (Fig. 6, upper left). Controls confirmed that all chimeras stained equally well with mAb that

**Fig. 4. Sequence alignment for the LFA-1 α subunit β-propeller domain.** Each β-strand is designated by the β-sheet (W) in which it is present and strand position (S) in the sheet. Strand 1 is innermost and lines the 7-fold pseudosymmetry axis; strand 4 is outermost and forms the cylindrical side of the β-propeller. N- and C-terminal sequence segments come together to form W7. Antigenic residues recognized by YTA-1 mAb are underlined. The LFA-1 β-propeller domain was modeled with this alignment, as described under “Materials and Methods” using the transducin G protein β subunit as the β-propeller template and loops from the bacterial alkaline protease 1akl as templates for Ca2⁺–binding loops.
map to other epitopes on $\beta_2$ and with mAb TS1/22 to $\alpha_L$.

Only five residues differ between human and mouse $\beta_2$ in the segment 302–344: 302, 303, 325, 332, and 339. Specific human residues were “knocked out” by substituting them with mouse residues. The double mutation S302K/R303K completely abolished recognition by YTA-1 (Fig. 7A). By contrast, mutation of each of the other three species-specific residues in the 302–344 interval, E325D, H332Q, and N339Y, had no effect on recognition by YTA-1. The TS1/18 mAb was previously reported to block binding of YTA-1 mAb to LFA-1 (37). It has been mapped to residues Arg133 and His332, which are predicted to be present on adjacent $\alpha$-helices in the structure of the $\beta_2$ subunit I-like domain (57). Therefore, we also tested the R133Q/H332Q double mutation for recognition by YTA-1, and somewhat surprisingly, found that it abolished recognition by YTA-1. However, the individual substitution R133Q, like H332Q, had no effect on YTA-1 binding. The double mutation R133Q/N339Y also had no effect (Fig. 7A).

In reciprocal experiments, human → mouse “knock-in” mutations were introduced into the mouse $\beta_2$ subunit. The knock-in mutation K302S/K303R restored YTA-1 reactivity to the same level as seen with the fully human $\beta_2$ subunit (Fig. 7B). However, the “knock-in” mutation Q133R/Q332H had no effect at all. These results suggest that, in human $\beta_2$, residues Ser302/Arg303 represent a direct binding site for YTA-1, whereas the involvement of Gln133 and His332 is indirect.

Reconstitution of the YTA-1 Epitope on Mouse LFA-1—The above experiments indicated that the YTA-1 epitope may contain residues Pro78, Thr79, Asp80, Ile365, and Asn367 in the $\alpha_L$ subunit, and Ser302 and Arg303 in the $\beta_2$ subunit of human LFA-1. To test whether these residues are sufficient to form the YTA-1 epitope, mouse $\alpha_L$ and $\beta_2$ with these human → mouse “knock-in” mutations were co-transfected into 293T cells. YTA-1 recognition was quantified as in Fig. 2.

Competition of YTA-1 Binding by Other Antibodies to Human...
LFA-1—In our previous studies, we mapped a number of anti-LFA-1 antibodies to specific regions or residues in the \( \alpha_2 \) or \( \beta_2 \) subunits (35, 36, 57). To determine the relationship between these epitopes and the YTA-1 epitope, we tested these antibodies for their ability to compete with YTA-1 for binding to LFA-1. Transfected 293T cells expressing human LFA-1 or Jurkat cells expressing the active, GFFKR deletion mutant of LFA-1 were pre-incubated with an anti-LFA-1 mAb and subsequently immunostained with biotinylated YTA-1 (Table I). All antibodies were independently confirmed to bind to LFA-1 on these two cell types. Ten different mAb to \( \alpha_2 \) were tested, five of which were directed to epitopes in the \( \beta \)-propeller domain. Among these, only CBR LFA-1/1 blocked binding of YTA-1 to LFA-1 (Table I). This antibody was mapped to region 301–359 (36), nearby residues Ile\(^{365}\) and Asn\(^{367}\) of the YTA-1 epitope.

Among mAb to \( \beta_2 \), several mAb to the I-like domain blocked YTA-1 binding (Table I). TS1/18, GRF-1, YFC118.3 and YFC5.1 were directed to epitopes in the \( \beta_2 \) subunit on the YTA-1 epitope. Ten different mAb to the I-like domain blocked binding, May.017 and L130 (Table I). All antibodies were independently confirmed to bind to LFA-1 on these two cell types. mAb to two more C-terminal segments in the \( \beta \) subunit, CBR LFA-1/7 and CBR LFA-1/2, did not block binding.

**YTA-1 Is a Function-blocking Antibody**—We tested whether YTA-1 antibody inhibited LFA-1 binding to its ligand ICAM-1. Human LFA-1 overexpressed on 293T cells was constitutively active in binding to immobilized human ICAM-1 (Fig. 9A). However, binding to ICAM-1 was abolished if the cells were

![Fig. 7. Effect of specific amino acid substitutions in the \( \beta_2 \) subunit on the YTA-1 epitope.](image)

![Fig. 8. Reconstitution of the YTA-1 epitope in mouse LFA-1.](image)
pretreated with YTA-1 mAb or the TS1/22 mAb to the LFA-1 α subunit I domain (Fig. 9A). Furthermore, the inhibition by YTA-1 was concentration-dependent (Fig. 9B). Similar experiments were performed using the SKW3 T lymphoma cell line. LFA-1 is endogenously expressed on these cells and binds to ICAM-1 upon stimulation with the phorbol ester phorbol 12-myristate 13-acetate or the activating mAb CBR LFA-1/2 (Fig. 9C). In both cases, stimulated binding was inhibited with YTA-1 and TS1/22 mAb (Fig. 9C).

Human but not mouse LFA-1 binds to human ICAM-1 (52), and this species specificity has been mapped to the α2 subunit I domain (29). We tested whether any of the human → mouse substitutions that affected YTA-1 binding affected binding to ICAM-1. They did not (Fig. 9A), demonstrating lack of involvement of these residues in species-specific recognition of LFA-1. Furthermore, this demonstrated that these substitutions did not “de-activate” LFA-1. As expected, the substitutions abolished the ability of YTA-1 mAb to block binding of LFA-1 to ICAM-1 (Fig. 9A).

**DISCUSSION**

We provide direct evidence for association between the I-like domain of integrin β subunits and the β-propeller domain of integrin α subunits. We map the epitope of the anti-LFA-1 mAb YTA-1 to specific residues in these domains, and thus localize a region of close contact between the I-like and β-propeller do-
mains. Interestingly, we find that YTA-1 preferentially recognizes activated LFA-1 and blocks LFA-1 binding to its ligand ICAM-1, suggesting that this specific intersubunit association is related to the active conformation of LFA-1.

Models of the LFA-1 β-propeller and I-like domains are useful for understanding our findings in three dimensions. We made an αL subunit β-propeller model using the alignment with the transducin G protein β subunit β-propeller domain shown in Fig. 4, as described under “Materials and Methods” (Fig. 10). The approach was similar to that previously used to model αL and αM β-propellers (9, 32). Residues Pro78, Thr79, and Asp305 of the YTA-1 epitope are predicted to be in a turn or loop between β-strands 1 and 2 in β-sheet 2 (W2) of the αL subunit β-propeller (Fig. 4). Thus, they are on the lower surface of the β-propeller (Fig. 10). Residues Ile365 and Asn367 are predicted to be in β-strand 4 of W3 of the propeller (Fig. 4), located on the approximately cylindrical side of the β-propeller, about midway between the top and bottom (Fig. 10). Because β-strand 4 is the most challenging of the four β-strands in each sheet to align, the position within this β-strand should be considered tentative, whereas the alignment of β-strands 1 and 2 and hence the position of residues 78–80 in the model is straightforward. In the YTA-1 epitope, residues 365 and 367 are more important than 78–80. Introduction of the conservative substitutions V365I and Q367N into the mouse L subunit is related to the active conformation of LFA-1 and blocks LFA-1 binding to its ligand ICAM-1, suggesting that this specific intersubunit association is necessary for understanding our findings in three dimensions. We predicted to be in surface of the I-like domain model (Fig. 11, A and B). In the center of this surface is the “front” face of the I-like domain, which bears helices α1 and α6, which are adjacent in the structure (Fig. 11) (57). The current study identified residues Ser302 and Arg303 as the YTA-1 epitope on the β2 subunit. Residues Ser302 and Arg303 were necessary for the YTA-1 epitope, as shown by substitution for mouse residues, and sufficient for the YTA-1 epitope, as shown by substitution into mouse β2. Residues Ser302 and Arg303 are predicted to be in a turn between β-strand 5 and α-helix 5, at the top of the I-like domain (gold side chains, Fig. 11). In Fig. 11, other antigenic residues defined in β2 are shown as rose-pink side chains, and the positions of antigenic residues defined in β1 are shown as rose-pink lollipops. It is interesting that the antigenic residues extend only over one half of the surface of the I-like domain model (Fig. 11, A and B). In the center of this surface is the “front” face of the I-like domain, which bears helices α1 and α6, which are adjacent in the structure (Fig. 11) (57). The current study identified residues Ser302 and Arg303 as the YTA-1 epitope on the β2 subunit. Residues Ser302 and Arg303 were necessary for the YTA-1 epitope, as shown by substitution for mouse residues, and sufficient for the YTA-1 epitope, as shown by substitution into mouse β2. Residues Ser302 and Arg303 are predicted to be in a turn between β-strand 5 and α-helix 5, at the top of the I-like domain (gold side chains, Fig. 11). In Fig. 11, other antigenic residues defined in β2 are shown as rose-pink side chains, and the positions of antigenic residues defined in β1 are shown as rose-pink lollipops. It is interesting that the antigenic residues extend only over one half of the surface of the I-like domain model (gray lollipops, Fig. 11). Residues Ser302 and Arg303, in the β5-α5 loop, are on the top face of the I-like domain (Fig. 11A), and form what may be thought of as the upper-left corner of the antigenic surface (Fig. 11B). Glu175 in β2 is on the upper right corner, in a disulfide-bonded turn (Fig. 11B). Viewed from the top, Ser302/Arg303 and Glu175 are on opposite ends of the upper face, and in between them is the putative Mg2+ ion of the MIDAS of the I-like domain.

Our model and the data on the YTA-1 epitope suggest that the I-like domain associates closely at its “top left” corner in the β5-α5 loop with the β-propeller domain. It is intriguing that Ser302 and Arg303 are at the boundary between the front face, which bears antigenic and N-linked glycosylation sites, and the back face, which is devoid of antigenic residues and N-linked sites, except for one N-linked site near Arg303 on the α5 helix (Fig. 11C). Thus, the back face may be buried in an interface with the β-propeller domain, with Ser302 and Arg303 on the solvent exposed face near its boundary with the buried face, and hence near to the α subunit β-propeller, and to residues on its surface including Ile365 and Asn367.

The ability of many of the mAb directed to the β subunit I-like domain to competitively inhibit binding of YTA-1 is con-
FIG. 11. Stereodiagram of a theoretical model of the $\beta_2$ subunit I-like domain. A, top view; B, view from the antigenic “front” face bearing the $\alpha_6$, $\alpha_1$, and $\alpha_2$ helices; C, view from the “back” face bearing the $\alpha_3$, $\alpha_4$, and $\alpha_5$ helices. Residues Ser$^{302}$ and Arg$^{303}$ in the YTA-1 epitope are shown as gold side chains. Other $\beta_2$ antigenic residues are shown as rose-pink side chains (see Footnote 2), and positions that are antigenic in $\beta_1$ integrins (20, 56) are shown as pink lollipops with a large C$\beta$ atom and a C$\alpha$-C$\beta$ bond. Sites that are predicted to be N-glycosylated in at least 2
establish a point of contact between these domains, but not their orientation relative to one another. Interestingly, a second point of contact has very recently been revealed between the $\alpha_{1\beta_2}$ $\beta$-propeller domain and the $\beta_3$ I-like domain in elegant work by Takada and co-workers (53) that mapped ligand-mimetic antibodies. The residues recognized by these mAb in $\beta_3$ are present in the same disulfide-bonded loop that contains Glu$^{175}$ in $\beta_2$; thus, this contact region is on the top face of the I-like domain on the edge opposite from Ser$^{302}$ and Arg$^{303}$ (Fig. 11, A and B). This same loop has been demonstrated to bear specificity for ligand and has been termed the specificity-determining loop (53, 54). The contact residues in $\alpha_{1\beta_2}$ are in the 4–1 loops at the top edge of the $\beta$-propeller, between $\beta$-sheets 2 and 3, and between $\beta$-sheets 3 and 4. Thus, both YTA-1 to LFA-1 and the ligand mimetic antibodies to $\alpha_{1\beta_2}\beta_3$ contact the $\beta$-propeller on the side with $\beta$-sheet 3, but the ligand-mimetic-defined contact is at the top of the side, whereas the YTA-1-defined contact is on the middle to bottom of the side. With two points of contact, the relative orientation of the $\beta$-propeller and I-like domains can be predicted (Fig. 12). The top of the I-like domain is in contact with the side of the $\beta$-propeller domain. Furthermore, the edge of the I-like domain with $\beta$-strand 3 and $\alpha$-helix 2 is toward the top of the $\beta$-propeller, whereas the edge with $\beta$-strand 6 and $\alpha$-helix 6 is toward the bottom of the $\beta$-propeller. It is tempting to speculate that the back face of the I-like domain, which lacks antigenic residues and N-linked sites, is in contact with the $\beta$-propeller. If so, then the orientation defined by the epitopes would mean that with the top of I-like domain contacting the $\beta$-propeller near $\beta$-sheet 3, the bottom would extend toward $\beta$-sheet 5, rather than toward $\beta$-sheet 1 (Fig. 12). Note that the putative MIDAS motif is positioned in the middle of the interface between the $\beta$-propeller and I-like domain, as appropriate for a function in ligand binding, or in regulating the conformation of loops involved in ligand binding. The I-like domain MIDAS and the specificity-determining loop are well situated to interact with ligand-binding loops on the upper surface of the $\beta$-propeller in $\beta$-sheets 2, 3, and 4 of the $\alpha_{1\beta_2}$, $\alpha_4$, and $\alpha_5$ integrins (see Introduction for references).

The I domain is inserted into a loop at the top of the $\beta$-propeller domain, between $\beta$-sheets 2 and 3. Therefore, the bottom of the I domain is in close proximity to the top of the I-like domain. The C-terminal $\alpha$-helix of the I domain moves 10 Å down the side of the domain in a movement that is linked to a shift from a putative inactive to active ligand binding configuration at the top of the domain (31). Therefore, the interface between the $\alpha$ subunit $\beta$-propeller and $\beta$ subunit I-like domains is well positioned both to indirectly regulate ligand binding by I-domain-containing integrins, and directly participate in ligand binding by integrins that lack I domains. We have shown here that the YTA-1 mAb selectively binds to activated LFA-1. Thus, the interface it recognizes between the $\beta$-propeller domain and I-like domain appears to alter structurally during activation of LFA-1, and may be an important linkage in the machinery for inside-out signal transduction by integrins.

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REFERENCES

1. Springer, T. A. (1990) Nature 346, 425–433
2. Van der Vieren, M., Le Trong, H., Wood, C. L., Moore, P. F., St. John, T.,
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Staunton, D. E., and Gallatin, W. M. (1995) Immunity 3, 683–690
3. Kishimoto, T. K., O'Connor, K., Lee, A., Roberts, T. M., and Springer, T. A. (1995) J. Biol. Chem. 270, 16283–16284
4. Marlin, S. D., and Springer, T. A. (1987) Cell 51, 813–819
5. Staunton, D. E., Dustin, M. L., and Springer, T. A. (1989) Nature 339, 61–64
6. de Fougerolles, A. R., Stacker, S. A., Schwartz, R., and Springer, T. A. (1991) J. Exp. Med. 174, 283–287
7. Springer, T. A., Dustin, M. L., Kishimoto, T. K., and Marlin, S. D. (1987) Annu. Rev. Immunol. 5, 223–252
8. Larson, R. S., and Springer, T. A. (1990) Immunol. Rev. 114, 181–217
9. Springer, T. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 65–72
10. Kamata, T., Puzon, W., and Takada, Y. (1995) Biochem. J. 305, 945–951
11. Schiffer, S. G., Hemler, M. E., Lobb, R. R., Tizard, R., and Osborn, L. (1995) J. Biol. Chem. 270, 14227–14237
12. Irie, A., Kamata, T., Puzon-McLaughlin, W., and Takada, Y. (1995) EMBO J. 14, 5550–5556
13. Mould, A. P., Askari, J. A., Aota, S., Yamada, K. M., Irie, A., Takada, Y., Mardon, H. J., and Humphries, M. J. (1997) J. Biol. Chem. 272, 17283–17292
14. Kamata, T., Irie, A., Tokuhira, M., and Takada, Y. (1996) J. Biol. Chem. 271, 18610–18615
15. Lee, J.-O., Rieu, P., Arnaout, M. A., and Liddington, R. (1995) J. Cell Biol. 129, 2293–2299
16. Qu, A., and Leahy, D. J. (1995) Science 271, 223–2252
17. Tozer, E. C., Liddington, R. C., Sutcliffe, M. J., Smeeton, A. H., and Loftus, J. C. (1996) J. Biol. Chem. 271, 21978–21984
18. Tuckwell, D. S., and Humphries, M. J. (1997) J. Biol. Chem. 272, 20438–20443
19. Shih, D. T., Doettiger, D., and Buck, C. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10727–10731
20. Dransfield, I., and Hogg, N. (1989) EMBO J. 8, 12395–12398
21. Bajt, M. L., Goodman, T., and McGuire, S. L. (1995) J. Biol. Chem. 270, 19008–19016
22. Bajt, M. L., Goodman, T., and McGuire, S. L. (1995) J. Biol. Chem. 270, 19008–19016
23. Bajt, M. L., Goodman, T., and McGuire, S. L. (1995) J. Biol. Chem. 270, 19008–19016
24. Gailit, J., and Ruoslahti, E. (1988) J. Cell Biol. 106, 1013–1022
25. van Kooyk, Y., Weder, P., Heije, K., and Figdor, C. (1994) J. Cell Biol. 124, 1061–1071
26. Randi, A. M., and Hogg, N. (1994) J. Biol. Chem. 269, 12395–12398
27. Edwards, C. P., Champe, M., Gonzalez, T., Weissinger, M. E., Spencer, S. A., Presta, L. G., Berman, P. W., and Bodary, S. C. (1995) J. Biol. Chem. 270, 12363–12364
28. Bajt, M. L., Goodman, T., and McGuire, S. L. (1995) J. Biol. Chem. 270, 12363–12364