The Binding Sites for Competitive Antagonistic, Allosteric Antagonistic, and Agonistic Antibodies to the I Domain of Integrin LFA-1

Chafen Lu, Motomu Shimaoka, Azucena Salas and Timothy A. Springer

*J Immunol* 2004; 173:3972-3978; doi: 10.4049/jimmunol.173.6.3972
http://www.jimmunol.org/content/173/6/3972

References
This article cites 43 articles, 18 of which you can access for free at:
http://www.jimmunol.org/content/173/6/3972.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
The Binding Sites for Competitive Antagonistic, Allosteric Antagonistic, and Agonistic Antibodies to the I Domain of Integrin LFA-1

Chafen Lu, Motomu Shimaoka, Azucena Salas, and Timothy A. Springer

We explore the binding sites for mAbs to the α I domain of the integrin αLβ2 that can competitively inhibit, allosterically inhibit, or activate binding to the ligand ICAM-1. Ten mAbs, some of them clinically important, were mapped to species-specific residues. The results are interpreted with independent structures of the αL I domain determined in seven different crystal lattices and in solution, and which are present in three conformational states that differ in affinity for ligand. Six mAbs bind to adjacent regions of the β1α1 and α3α4 loops, which show only small (mean, 0.8 Å; maximum, 1.8 Å) displacements among the eight I domain structures. Proximity to the ligand binding site and to noncontacting portions of the ICAM-1 molecule explains competitive inhibition by these mAbs. Three mAbs bind to a segment of seven residues in the β5α6 loop and α6 helix, in similar proximity to the ligand binding site, but on the side opposite from the β1α1/α3α4 epitopes, and far from noncontacting portions of ICAM-1. These residues show large displacements among the eight structures in response to lattice contacts (mean, 3.6 Å; maximum, 9.4 Å), and movement of a buried Phe in the β5α6 loop is partially correlated with affinity change at the ligand binding site. Together with a lack of proximity to noncontacting portions of ICAM-1, these observations explain variation among this group of mAbs, which can either act as competitive or allosteric antagonists. One agonistic mAb binds distant from the ligand binding site of the I domain, to residues that show little movement (mean, 0.5 Å; maximum, 1.0 Å). Agonism by this mAb is thus likely to result from altering the orientation of the I domain with respect to other domains within an intact integrin αLβ2 heterodimer. The Journal of Immunology, 2004, 173: 3972-3978.

Antibodies are widely used to study ligand binding by proteins. For example, in the fields of immunology and cell adhesion, mAbs are frequently selected and used in experimental studies based on their ability to inhibit or stimulate receptor-ligand interactions. However, the relationship between the Ab epitope and the receptor-ligand interface is often unknown. In this study, we examine this relationship in the particularly biologically interesting case of Abs to an integrin inserted (I) domain, which exists in multiple conformational states that regulate affinity for ligands.

The integrin LFA-1 (αLβ2, CD11a/CD18) binds to ICAM-1, -2, and -3, and regulates adhesive functions and migration of lymphocytes and most other leukocytes (1, 2). Like many integrins, the adhesiveness of LFA-1 is dynamically regulated by signals from the cytoplasm (inside-out signaling) (3, 4). Many integrin α subunits, including αL, contain an I domain of ~200 aa. The I domain mediates ligand binding by I domain-containing integrins (2). The I domain adopts the von Willebrand factor A domain/dinucleotide-binding fold similar to that of small G proteins, and has a unique divalent cation coordination site designated the metal ion-dependent adhesion site (MIDAS).

Integrin I domains exist in so-called open and closed conformations, and a recently described intermediate conformation, that differ in affinity for ligand (4–6). Mutationally introduced disulfide bonds have been used to stabilize the αL I domain in a high affinity open conformation and an intermediate affinity intermediate conformation (6–8). The affinity for ICAM-1 of I domains mutationally stabilized in the open and intermediate conformations is 10,000-fold and 500-fold higher, respectively, than the closed conformation. The closed conformation is energetically favorable for the isolated wild-type I domain. The affinity and kinetics of the “locked open” isolated I domain for ICAM-1 are within experimental error of values obtained for active intact αLβ2 (8, 9), showing that the binding site for ICAM-1 is contained wholly within the I domain. Multiple structures are available for the αL I domain in each of these conformational states, including one structure of a complex between the αL I domain and ICAM-1 (6, 10–13).

Numerous mAbs to the αL and β2 subunits of LFA-1 have been well characterized functionally. Many inhibit ligand binding, and a few are stimulatory (14–17). Furthermore, studies with αLβ2 containing an I domain locked in the high affinity conformation have revealed distinct classes of inhibitory mAbs (18). All tested mAbs to the β2-I-like domain that inhibit binding to ICAM-1 of wild-type αLβ2 fail to inhibit binding of αLβ2 with the mutant high affinity, locked open I domain. This finding demonstrates that the β2-I-like domain has a regulatory, rather than direct, role in ligand binding, and further suggests that mAbs to the β2-I-like domain are allosteric rather than competitive antagonists. In the same study, mAbs to the αI domain fell into two classes. One class inhibits both wild-type and locked open αLβ2, whereas the other inhibits only...
wild-type αβ. These findings suggest that mAbs to the α I domain may function either as competitive or allosteric antagonists (18). A third class of mAb to the αL I domain enhances binding to ICAM-1 (17, 19).

Despite these observations, the basis of agonism and the two different classes of antagonism remain unclear. Stimulated by the availability of structures of the αL I domain in different conformational states and in complex with ICAM-1 (6), we have mapped I domain epitopes to individual species-specific amino acid residues to gain insights into how mAb to the I domain can either competitively inhibit, allosterically inhibit, or stimulate binding to ICAM-1. The mechanism of action of mAb to the αL I domain is also of great interest clinically, because such mAb can prevent graft-vs-host disease following bone marrow transplantation (20), and clinically benefit patients with moderate to severe plaque psoriasis (21).

Materials and Methods

Monoclonal Abs

The mouse anti-human αL (CD11a) mAbs TS1/11, TS1/12, TS1/22, TS2/4, TS2/6, TS2/14, mouse anti-β2 (CD18) mAb TS1/18, and the nonbinding myeloma IgG X63 were described previously (22). mAbs F8.8, BL5, May.035 (23), 25-3-1 (24), and CBR LFA-1/9 (25) were obtained through the 5th International Leukocyte Workshop (26). mAb MEM83 (17, 19) was generously provided by Dr. V. Horejsi (Institute of Molecular Genetics, Prague, Czech Republic).

Cell lines

Human embryonic kidney 293T cells were cultured in DMEM medium supplemented with 10% FBS, 2 mM glutamine, and 50 µg/ml gentamicin. Stable K562 cell lines that express wild-type LFA-1 or the isolated αL I domain were described previously (7).

cDNA construction and expression

Human and mouse αL chimeras in expression vector AprM8 were described previously (14). Overlap extension PCR (27, 28) was used to generate single or multiple human-to-mouse substitution mutations in the αL I-domain. Wild-type αL cDNA in AprM8 was used as template for the first PCR. The outer left primer for PCR extension was complementary to vector sequence containing the EcoRI site in the multiple cloning site 5’ to the αL cDNA, and the outer right primer was 3’ to the EcoRI site at nt 1818 near the middle of the coding region of the αL cDNA. The inner primers were designed for each individual mutation and contained overlapping sequences. The final PCR products with outer primers were digested with EcoRI and used to replace the corresponding fragment in the wild-type αL cDNA in AprM8. Clones with the correct orientation were selected, and the entire inserted segment was sequenced to confirm its correctness.

Wild-type or mutant αL cDNA was transiently cotransfected with wild-type β2 cDNA in vector AprM8 in 293T cells as described (29). Two days after transfection, cells were harvested for flow cytometric analysis.

Flow cytometry

Flow cytometry was as described (29). mAb TS1/11, TS1/12, and the nonbinding IgG X63 were used as hybridoma supernatants at 1/20 dilution, mAbs TS1/22, TS2/4, TS1/18, and MEM83 at 15 µg/ml purified IgG, and the International Leukocyte Workshops mAbs F8.8, BL5, May.035, 25-3-1, and CBR LFA-1/9 were used at 1/100 dilution.

Results

Epitope expression was examined by cotransfection of mutant or wild-type αL cDNA and wild-type β2 cDNA in 293T cells followed 2 days later by staining with mAb, FITC anti-mouse IgG, and quantitative immunofluorescence flow cytometry. For each mutation, at least two independent clones were tested by transfection, and expression was measured in at least three independent transfections. Representative αLβ2 293T transfectant flow cytometry histograms have previously been shown (29). The nine function-blocking mAbs studied here have previously been mapped using αL chimeras in which six different I domain segments of 30 to 60 residues were replaced with mouse sequence (14). These previous results with the chimeras were confirmed (Tables I and II), and the activating mAb MEM83 was also mapped to the same segments (Table III). Segments of mouse sequence that resulted in reduced reactivity were subdivided into subregions, and subregions showing reduced reactivity were further dissected into individual human-to-mouse amino acid substitution. All mutants studied here were expressed equally as well as wild-type αLβ2, and were expressed as αLβ3 complexes, as shown by mAb TS2/4 that

Table I. Competitive antagonistic Abs to the β1α1 and α3α4 loops

<table>
<thead>
<tr>
<th>Human-to-Mouse Substitution</th>
<th>F8.8</th>
<th>CBR LFA-1/9</th>
<th>BL5</th>
<th>May.035</th>
<th>TS1/11</th>
<th>TS1/12</th>
</tr>
</thead>
<tbody>
<tr>
<td>h18m153h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P144R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h153m183h</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>h184n215h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R189N/D191N</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>A194V/K197G/H198S</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>K197G</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>H198S</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>K200Q/H201P/L203F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K200Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H201P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L203F</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>G210R</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>h217m248h</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>h249m303h</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>h300m359h</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Epitope</td>
<td>P144</td>
<td>P144</td>
<td>P144</td>
<td>K197</td>
<td>K197</td>
<td>K197</td>
</tr>
<tr>
<td></td>
<td>K200</td>
<td>K200</td>
<td>K197</td>
<td>H201</td>
<td>H201</td>
<td>H201</td>
</tr>
<tr>
<td></td>
<td>H201</td>
<td>H201</td>
<td>H201</td>
<td>L203</td>
<td>L203</td>
<td>L203</td>
</tr>
</tbody>
</table>

* Mean-specific fluorescence intensity, i.e., with background X63 IgG1 fluorescence intensity subtracted, for each mutant Ab was determined as percentage of wild type, and averaged for all independent transfections (n ≥ 3). +++++++, comparable to wild type; ++++++, 70–90% of wild type; +++++, 50–70% of wild type; +++, 30–50% of wild type; +, < 30% of wild type; −, indistinguishable from the X63 control.
maps to the $\alpha_L$ $\beta$-propeller domain and requires $\beta_3$ association for reactivity (30).

Six of the inhibitory mAbs require human residues 185–215 for reactivity, and three of these also require residues 119–153 (Table I). Single amino acid substitutions had already been prepared in the 119–153 subregion, and as previously described (14), only the Pro$^{144}\rightarrow$Arg substitution in this subregion affected binding, and it completely abolished binding of all three mAb (Table I). Within the region of residues 184–215, four of the mAbs require human residues in subregion 194–198 (Table I). Individual substitutions show that residue Lys$^{197}$ is the only species-specific residue required in this subregion. All six of the mAbs require the subregion of residues 200–203 (Table I), which includes species-specific residues Lys$^{200}$, His$^{201}$, and Leu$^{203}$. Individual substitutions show that two Abs require Lys$^{200}$, all six require His$^{201}$, and a different subset of two Abs requires Leu$^{203}$. Thus, the six mAbs map to four distinct epitopes (Table I, bottom row), all contained within the structurally contiguous $\beta_1$-$\alpha L$ and $\alpha_3$-$\alpha_4$ loops of the $\alpha_L$ I domain (Fig. 1). These same loops each contain residues that contact ICAM-1 (Fig. 1), correlating with the observation that all six mAbs are competitive antagonists of ICAM-1 (18).

A different set of three antagonistic mAbs to the $\alpha_L$ I domain require the segment containing residues 250–303 (Table II). Only the subregion of 266–272 affected Ab binding. Single amino acid substitutions show that mAb TS1/22 recognizes residues 266 and 270, mAb TS2/14 recognizes residues 270 and 272, and mAb 25-3-1 recognizes residues 266, 267, 268, 270, and 272. Thus, these three mAbs recognize three distinct epitopes in the $\beta_3$-$\alpha L$ loop and the short $\alpha_4$ helix (Fig. 1). This loop and helix are not far from the ICAM-1 binding site, undergo reshaping between the closed and open conformations, and also undergo reshaping as a consequence of crystal lattice contacts. These observations are not inconsistent with the ability of TS1/22 but not TS2/14 and 25-3-1 to strongly inhibit ligand binding by locked open $\alpha_L^0\beta_3$ (see Discussion).

The agonistic mAb MEM83 requires two human regions, residues 153–183 and 217–248 (Table III). Only residue Asp$^{185}$ is required in the first region. In region 217–248, substitution Glu$^{218}\rightarrow$His lowered mAb MEM83 reactivity (Table III). Substitution of all residues in this region lowered reactivity more than Glu$^{218}\rightarrow$His, showing a combinatorial effect of one or more additional residues. The MEM83 epitope thus requires residues in the contiguous $\beta_3$-$\alpha_2$ loop and the $\alpha_4$ helix, which locate far from the MIDAS (see below).

### Discussion

The use of mAbs to inhibit or stimulate cell-cell interactions is widespread, particularly in immunology, and far-reaching conclusions are drawn from such studies. We have attempted to understand the mechanism of action of a set of Abs to LFA-1 with different inhibitory or stimulatory effects that are widely used in the research literature and are of clinical importance. The epitopes for 10 such mouse anti-human integrin $\alpha_L$ Abs have been mapped in this study using human-to-mouse species-specific amino acid substitutions. This information can now be evaluated with respect to the structure of a mutant intermediate affinity $\alpha_L$ I domain complexed with domains 1 and 2 of ICAM-1 (6) (Fig. 2). A recent structure of a mutant high affinity $\alpha_L$ I domain bound to domain 1 of ICAM-3 reveals an almost identical set of contact residues on the I domain, and an essentially identical orientation between the $\beta$ I domain and the ICAM. Moreover, the information can also be evaluated with respect to eight independent $\alpha_L$ I domain structures (Table IV). These structures are present in the three different conformational states termed closed, intermediate, and open, which regulate affinity of $\alpha_L$ for ICAMs. They are also present in seven different crystal packings or in solution (Table IV), allowing conformational variation of loop structures, as influenced by crystal packing interactions, to be evaluated. What the epitope mapping together with the structural studies can teach us about

---

**Table II.** Competitive and allosteric antagonistic Abs to the $\beta_3$-$\alpha_6$ loop and $\alpha_6$ helix

<table>
<thead>
<tr>
<th>Human-to-mouse Substitution</th>
<th>TS1/22</th>
<th>TS2/14</th>
<th>25-3-1</th>
<th>Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>h18m153h</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>h153m183h</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>h18m215h</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>h217m248h</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>h249m303h/K252H</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>I255H</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>Q266V/T267S/K268V/E269Q/S270K/E272K</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Q266V</td>
<td>-</td>
<td>++++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>T267S</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>K268V</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>E269Q</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>S270K</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>E272K</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>K276I</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>K280E/A282V/S283E</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>h300n599h</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Epitope</td>
<td>Q266</td>
<td>S270</td>
<td>Q266</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S270</td>
<td>E272</td>
<td>T267</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K268</td>
<td>S270</td>
<td>E272</td>
<td></td>
</tr>
</tbody>
</table>

Class competitive allosteric allosteric

---

**Table III.** An agonistic Ab to the $\beta_3$-$\alpha_2$ loop and the $\alpha_4$ helix

<table>
<thead>
<tr>
<th>Human-to-mouse substitution</th>
<th>MEM83 Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>h118m153h</td>
<td>++++</td>
</tr>
<tr>
<td>h153m183h</td>
<td>++++</td>
</tr>
<tr>
<td>K159R</td>
<td>++++</td>
</tr>
<tr>
<td>S176D/Y177C/K178R</td>
<td>++++</td>
</tr>
<tr>
<td>S176D</td>
<td>++++</td>
</tr>
<tr>
<td>Y177C</td>
<td>++++</td>
</tr>
<tr>
<td>K178R</td>
<td>++++</td>
</tr>
<tr>
<td>D182T/S184L</td>
<td>-</td>
</tr>
<tr>
<td>D182T</td>
<td>-</td>
</tr>
<tr>
<td>S184L</td>
<td>++++</td>
</tr>
<tr>
<td>h18m215h</td>
<td>++++</td>
</tr>
<tr>
<td>h217m248h</td>
<td>-</td>
</tr>
<tr>
<td>A216V/T217A/E218H</td>
<td>++++</td>
</tr>
<tr>
<td>E218H</td>
<td>++++</td>
</tr>
<tr>
<td>R221K</td>
<td>++++</td>
</tr>
<tr>
<td>L224S</td>
<td>++++</td>
</tr>
<tr>
<td>L235V</td>
<td>++++</td>
</tr>
<tr>
<td>T243S</td>
<td>++++</td>
</tr>
<tr>
<td>S245K</td>
<td>++++</td>
</tr>
<tr>
<td>h249m303h</td>
<td>++++</td>
</tr>
<tr>
<td>h300m599h</td>
<td>++++</td>
</tr>
<tr>
<td>Epitope</td>
<td>D182</td>
</tr>
<tr>
<td></td>
<td>E218</td>
</tr>
</tbody>
</table>

*See legend to Table I.

---

mechanism of inhibition or stimulation by the Abs is discussed for each of the three epitope groups in turn.

The epitopes in the \( \beta_1 \)-\( \alpha_1 \) and \( \alpha_3 \)-\( \alpha_4 \) loops recognized by mAbs F8.8, CBR LFA-1/9, BL5, May.035, TS1/11, and TS1/12, are located on the “top” and “side” faces of the \( \alpha_1 \) domain, close to the ICAM-1 binding site centered on the MIDAS (Fig. 2). Indeed, the \( \beta_1 \)-\( \alpha_1 \) and \( \alpha_3 \)-\( \alpha_4 \) loops contain residues that coordinate the MIDAS metal ion and that contact ICAM-1 (Fig. 1). Although species-specific residues are present in ICAM-1 contacts in the \( \beta_1 \)-\( \alpha_1 \) and \( \beta_4 \)-\( \alpha_5 \) loops (Fig. 1), they are not recognized by any mAbs characterized to date. The mAbs to the \( \beta_1 \)-\( \alpha_1 \) and \( \alpha_3 \)-\( \alpha_4 \) loops all block ligand binding to \( \alpha_1 \) domains recognized by one or more mAbs are highlighted a different color for each of the three epitope groups. I domain residues contacting ICAM-1 in the crystal structure (6) are highlighted in green. Metal-coordinating MIDAS residues are underlined. Every 10th residue is dotted.

Three factors appear to be important to explain the finding that all tested mAbs to the \( \beta_1 \)-\( \alpha_1 \)/\( \alpha_3 \)-\( \alpha_4 \) epitopes are competitive antagonists of ICAM-1 binding. First, there is little difference among the eight independent \( \alpha_1 \) structures in this region. The deviations in \( C \alpha \) atom positions of epitope residues average 0.6–0.9 Å, and do not exceed 1.8 Å among the eight structures (Table V). Furthermore, there is little movement in this region between the open and closed conformations. The lack of structural variation in the \( \beta_1 \)-\( \alpha_1 \) and \( \alpha_3 \)-\( \alpha_4 \) loops from one structure to another, despite differing lattice contacts, suggests that these loops are relatively rigid. Therefore, there is little scope for Ab binding to perturb loop structure, and change the distance of the loops from the ICAM-1 binding site or change the orientation of the Ab binding site relative to the orientation of bound ICAM-1. Second, the ICAM-1 binding site is located very close to the \( \beta_1 \)-\( \alpha_1 \)/\( \alpha_3 \)-\( \alpha_4 \) epitopes, as measured by either the distance between the nearest of the ICAM-1 contact and epitope residues, or the average distance of the nearest contact residue to all epitope residues (Table V). An average Ab-protein Ag recognition surface is \(~800 \) Å\(^2\) (34), corresponding to a circle with a radius of \(~15 \) Å. Therefore, the ICAM-1 contact surface and the Ab contact surfaces in the \( \beta_1 \)-\( \alpha_1 \)/\( \alpha_3 \)-\( \alpha_4 \) loops are predicted to overlap, or to be sufficiently close, so that a bound Ab Fab would occupy some of the same space as a bound ICAM-1. Third, ICAM-1 binds to the \( \alpha_1 \) domain with an orientation such that portions of domains 1 and 2 of ICAM-1 are very close to the \( \beta_1 \)-\( \alpha_1 \)/\( \alpha_3 \)-\( \alpha_4 \) epitope and a bound Fab would project toward the junction between domains 1 and 2 (Fig. 2). Because the Fab portion of an Ab is \(~100 \) Å in diameter, an Ab to the \( \beta_1 \)-\( \alpha_1 \)/\( \alpha_3 \)-\( \alpha_4 \) epitope would sterically block ICAM-1 binding even whether it did not necessarily occlude the contact site. This was confirmed by docking of a Fab to the epitope using molecular graphics.

We mapped mAbs TS1/22 and 25–3–1 to residues 266–272. It is puzzling that another study mapped the TS1/22 and 25–3–1 mAbs to a completely different site at residues Ile\(^{126}\) and Asn\(^{129}\), in the linker between the \( \beta \)-propeller domain and I domain (31). In our own studies, the lack of reactivity of these mAbs with region 126–129 was previously (14), and in the present study, validated with \( \alpha_1 \) chimeras h153m359, h249m303h, and Q266V/T267S/K268V/Q271S, and with absence or diminished reactivity with different single amino acid substitution mutants in this region. In the other study (31), mapping to residues Ile\(^{126}\) and

![FIGURE 1](http://www.jimmunol.org/Downloadedfrom)
Asn\textsuperscript{129} was supported by only one \(\alpha_L\) chimera, termed H/M53, containing mouse residues in this region, and was counterindicated by the lack of effect by individual amino acid substitutions at Ile\textsuperscript{126} and Asn\textsuperscript{129}. Furthermore, no \(\alpha_L\) chimeras containing mouse substitutions covering the region 266–272 to which we map TS1/22 and 25-3-1 were studied (31). The effect of H/M53 was specific for particular Abs, and was particularly intriguing because it introduced mouse residues recognized by rat anti-mouse mAbs, M17/4 (35) and I21/7 (36); M17/4 is widely used to block \(\alpha_L\)\(\beta_2\) function in mouse models of disease (37, 38). Our mapping data suggest that chimera H/M53 inadvertently included mouse sequence in the region 266–272.

The mAbs TS1/22, TS2/14, and 25-3-1 recognize species-specific residues in the \(\beta_5\)-\(\alpha_6\) loop and \(\alpha_6\) helix (hereinafter called the \(\beta_5\)-\(\alpha_6/\alpha_6\) epitopes), on the opposite side of the MIDAS from the \(\beta_1\)-\(\alpha_1\)/\(\alpha_3\)-\(\alpha_4\) epitopes (Fig. 2). Despite mapping to similar epitopes, the TS1/22, TS2/14, and 25-3-1 mAbs differ considerably functionally. TS2/14 and 25-3-1 completely block adhesion to ICAM-1 of cells expressing activated wild-type \(\alpha_L\)\(\beta_2\), but under identical conditions fail to block, or block only weakly, adhesion of cells expressing the locked open, high affinity \(\alpha_L\) K287C/K294C mutant in \(\alpha_L\)\(\beta_2\) heterodimers or the isolated I domain (18, 39). These findings suggest differences in the mode of inhibition, such as direct competition by TS1/22 mAb and allosteric or mixed competitive/noncompetitive inhibition by TS2/14 and 25-3-1 mAb. mAb 25-3-1 is important clinically; it has been used in multiple clinical studies to prevent graft-vs-host disease following bone marrow transplantation (20, 24).

Multiple factors appear to be important to explain the differences among mAbs to the \(\beta_5\)-\(\alpha_6/\alpha_6\) epitopes and the finding that some of them act other than as competitive antagonists. First, there are large differences in the position of the epitopes among the eight independent \(\alpha_L\) structures, on average 3.0–3.9 \(\AA\)/residue, and as much as 9.4 \(\AA\)/residue (Table V). These differences are mostly attributable to crystal lattice contacts. There is no general correlation between the overall closed, intermediate, and open conformation of the \(\alpha_L\) I domain and \(\beta_5\)-\(\alpha_6/\alpha_6\) helix conformation (6); however, Phe\textsuperscript{265} within the \(\beta_5\)-\(\alpha_6\) loop swings away from the \(\beta_4\)-\(\alpha_5\) loop to accommodate the closed-to-open transition at the MIDAS (6). Phe\textsuperscript{265} is in the \(\beta_5\)-\(\alpha_6\) loop, immediately adjacent to both ICAM-1-contacting residues Lys\textsuperscript{263} and His\textsuperscript{264}, and \(\beta_5\)-\(\alpha_6/\alpha_6\) epitope residues 266–272 (Fig. 1). The movement of Phe\textsuperscript{265} provides a mechanism whereby an Ab-enforced conformation of the \(\beta_5\)-\(\alpha_6\) loop could be allosterically coupled to the closed conformation of the \(\alpha_L\) I domain, resulting in an allosteric mechanism

![FIGURE 2. Stereodigram of the ICAM-1 contact surface and epitopes on the \(\alpha_L\) I domain. The \(\alpha_L\) I domain is shown as a molecular surface and ICAM-1 is shown as a black Ca worm trace. Residues on the \(\alpha_L\) I domain with atoms within 3.5 \(\AA\) of ICAM-1 are green and the MIDAS Mg\textsuperscript{2+} is red. Species-specific residues in the epitopes are color coded the same as in Fig. 1: magenta for F8.8, CBR LFA-1/9, BL5, May.035, TS1/11, and TS1/12; orange for TS1/22, TS2/14, and 25-3-1; and cyan for MEM83. Figure prepared from the \(\alpha_L\) I domain complex with domains 1 and 2 of ICAM-1 (6) using GRASP (44), Bobscript (45), and Raster 3D (46).](http://www.jimmunol.org/)

<table>
<thead>
<tr>
<th>Table IV. (\alpha_L) I domain structures</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Data Bank Code</td>
<td>Characteristics</td>
<td>Residue (Å)</td>
<td>Space Group</td>
<td>Unit Cell (a, b, c) (Å)</td>
</tr>
<tr>
<td>ILFA</td>
<td>Wild type</td>
<td>1.8</td>
<td>C2</td>
<td>131.1, 45.4, 66.1</td>
</tr>
<tr>
<td>IZON</td>
<td>Wild type</td>
<td>2.0</td>
<td>P2(_1), P2(_1), P2(_1)</td>
<td>63.1, 63.7, 40.2</td>
</tr>
<tr>
<td>ICQP</td>
<td>Wild type + antagonist</td>
<td>2.6</td>
<td>P2(_1), P2(_1)</td>
<td>72.7, 77.7, 91.8</td>
</tr>
<tr>
<td>IDQG</td>
<td>Wild type in solution</td>
<td>3.5</td>
<td>P1</td>
<td>46.6, 62.9, 81.5</td>
</tr>
<tr>
<td>IMJN</td>
<td>Intermediate affinity mutant</td>
<td>1.3</td>
<td>P2(_1), P2(_1)</td>
<td>40.4, 57.4, 66.9</td>
</tr>
<tr>
<td>IMQ8</td>
<td>Intermediate affinity mutant + ICAM-1</td>
<td>3.3</td>
<td>P1</td>
<td>46.6, 62.9, 81.5</td>
</tr>
<tr>
<td>IMQA</td>
<td>High affinity mutant</td>
<td>2.5</td>
<td>C222 (_1)</td>
<td>61.9, 121.3, 54.1</td>
</tr>
<tr>
<td>IMQ9</td>
<td>High affinity mutant, pseudoliganded</td>
<td>2.0</td>
<td>P4(_2), P4(_2)</td>
<td>35.6, 35.6, 269.6</td>
</tr>
</tbody>
</table>
for inhibiting binding of ICAM-1. Thus, there is a plausible mechanism for explaining allosteric inhibition by mAb to β5-α6 epitopes. Second, as stated above and shown in Table V, residues in the β5-α6 epitope can be quite near the ICAM-1 contact site; however, because of the flexibility in this region, Abs could bind to and stabilize markedly different conformations of the loop, with some Abs overlapping the contact site, and others not. Third, in contrast to the β1-α1/3-α4 epitopes, ICAM-1 binds with an orientation such that noncontacting regions of domains 1 and 2 of ICAM-1 are far from the β5-α6 epitope (Fig. 2). Therefore, there is much less scope for regions outside of the Ag binding site of a bound Fab moiety to clash with a bound ICAM-1, and thereby give competitive inhibition. Docking of a Fab using molecular graphics showed that only particular orientations clashed (data not shown).

The agonistic mAb MEM83 (17, 19) recognizes residues Asp182 on the β3-α2 loop, and Glu218 at the end of the α4 helix. These residues are located on a face of the αI domain opposite from and distant from the ligand binding site, and close to the bottom of the I domain that interfaces with the β-propeller domain (Fig. 2). There is no significant conformational change in the mAb MEM83 epitope, with mean and maximum displacements of 0.5 and 1.0 Å between the eight structures (Table V). Furthermore, the conformation of this region is not correlated with shape-shifting at the ligand binding site. Therefore, agonism by MEM38 appears to be accounted for by its effect on the relative orientation of domains within the αIβ3 heterodimer. Mutations at the bottom of the αI and αI I domains activate αIβ3 and αIβ2 (40–43), suggesting that the bottom side of the I domain that connects to the β-propeller domain regulates ligand binding by the I domain. It is likely that mAb MEM83 activates LFA-1 binding to ICAM-1 by changing the orientation of the I domain relative to other domains in the αIβ3 heterodimer, which could possibly lead indirectly to conformational change within the I domain at the ICAM-1 binding site.

In summary, our study illustrates diverse mechanisms whereby Abs can activate or inhibit ligand binding by conformationally regulated surface molecules. A rich dataset of eight independent αI domain structures has allowed us to interpret the epitope mapping data presented in this study. The results explain how one group of six mAbs that bind to a conformationally stable region of the αI I domain in the β1-α1 and α3-α4 loops, despite some differences in the precise constellation of residues recognized in this region, always strongly inhibit ligand binding. They explain the effects of another group of three mAbs, which bind almost as close to the ligand binding site but on the opposite side of the MIDAS, to the β5-α6 loop and α6 helix. This region is conformationally highly plastic as shown by the influence of crystal lattice contacts, and has some coupling to conformational change at the ligand binding site that regulates affinity for ICAM-1. This explains why these three mAb behave so differently, with one appearing to be a competitive inhibitor, and the other two appearing to be mixed or noncompetitive inhibitors. Finally, the agonistic mAb binds distant to the ligand binding site to a region that does not undergo significant conformational change, suggesting that it alters interdomain relationships within αIβ3 heterodimers. A full explanation for the agonistic effect of MEM83 mAb will require a structure of the full αIβ3 ectodomain, or at least its ligand-binding headpiece.

Acknowledgments
We thank T. Xiao for figure preparation, and W. Yang for additional experiments.

References