Interaction of the Fungal Pathogen Candida albicans with Integrin CD11b/CD18: Recognition by the I Domain Is Modulated by the Lectin-Like Domain and the CD18 Subunit

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Interaction of the Fungal Pathogen *Candida albicans* with Integrin CD11b/CD18: Recognition by the I Domain Is Modulated by the Lectin-Like Domain and the CD18 Subunit

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Interactions of microorganisms with integrins are central to the host defense mechanisms. The leukocyte integrin CD11b/CD18 is the principal adhesion receptor on leukocytes for *Candida albicans*, a major opportunistic pathogen. In this study we have investigated the roles of three regions within the receptor, the inserted (I) and lectin-like domains within the CD11b subunit, and the CD18 subunit, in CD11b/CD18-*C. albicans* interactions. We report four major findings. 1) A mutation in CD18 exerts a dominant negative effect on the function of the CD11b/CD18 complex. This interpretation is based on the observation that in the absence of CD18, the CD11b subunit alone binds *C. albicans* well, but a single point mutation at Ser138 of CD18 abolishes CD11b/CD18 binding of the fungus. 2) The lectin-like domain is not sufficient for CD11b/CD18-*C. albicans* interactions. Rather, the lectin-like domain appears to influence CD11b/CD18 binding activity by modulating the function of the I domain. 3) The I domain is the primary binding site for *C. albicans* in the receptor and is sufficient to support an efficient interaction. 4) We have identified specific amino acid sequences within the I domain that engage the microorganism. Compared with other ligands of CD11b/CD18, *C. albicans* has some unique as well as common contact sites within the I domain of the receptor. Such unique contact sites may underlie the ability of *C. albicans* to modulate CD11b/CD18 function and raise the possibility for selective interference of the microorganism-host leukocyte interactions.

Pathogenic organisms use a variety of cell surface receptors to gain entry into host cells and to bypass the natural defense mechanisms, such as the oxidative burst (1, 2). One of the most prominent receptors used in this fashion is the leukocyte adhesion receptor CD11b/CD18. This integrin is the primary leukocyte receptor for a number of pathogens, including *Histoplasma capsulatum* (3), * Blastomyces dermatitidis* (4), *Mycobacterium tuberculosis* (5), *Leishmania* sp. (6), *Bordetella pertussis* (2), and *Candida albicans*. CD11b/CD18 is the principal adhesion receptor for *C. albicans* on neutrophils, macrophages, and lymphocytes (7, 8), and this interaction leads to suppression of the immune response to the microorganism (7). As the major cause of hospital-acquired fungal infections (9), the recognition of *C. albicans* by CD11b/CD18 has major pathogenetic consequences. Nevertheless, only limited information is available on the molecular mechanisms involved in recognition of this fungus by the receptor. The primary goal of this study was to identify the critical segments within CD11b/CD18 that compose the binding pocket for *C. albicans*.

Integrins are a large family of αβ heterodimeric adhesion receptors and can be divided into subfamilies according to their common β subunits. CD11b/CD18 (αMβ2, Mac-1, CR3) is a member of the CD18 subfamily of integrins, which also contains CD11a/CD18 (αLβ2, LFA-1), CD11c/CD18 (αxβ2), and a recently discovered fourth member, α6/CD18(α6β2) (10). CD11b/CD18 is found on all major classes of leukocytes, including virtually all neutrophils and macrophages as well as subsets of NK cells, T cells, and B cells (3, 10). CD11b/CD18 has been shown to play key and often the principal role in leukocyte adhesion, extravasation, migration, phagocytosis, and degranulation-cytotoxicity (3). Two domains have been implicated in the binding functions of CD11b/CD18 (3). The integrin recognizes protein ligands via a segment of about 200 amino acids in the CD11b subunit, termed the I domain (10). CD11b/CD18 recognizes mannose and β-glucan carbohydrate structures by a lectin-like ligand binding domain also in the CD11b (α) subunit (11). I domain ligands include ICAM-1, C3bi, fibrinogen (Fg), and the helminth ligand neutrophil inhibitory factor (NIF) (12–15). The lectin domain ligands include zymosan, *Saccharomyces cerevisiae*, and nonspecific activators of cell-mediated immunity such as lentinan (11). For *Leishmania* sp., *B. dermatitidis*, and *B. pertussis* the protein ligands that bind to the I domain and the carbohydrate ligands that bind to the lectin domain have been identified (1, 2). Previous studies have suggested that recognition of *C. albicans* (8), as well as other fungi (4), by CD11b/CD18 depends upon both the I domain and the lectin domains within its CD11b subunit.

The structural basis for the ligand binding to these two domains of CD11b/CD18 has begun to emerge. The crystal structure of the CD11b I domain was solved and revealed a novel divalent cation coordination site, the MIDAS motif, which is essential for the ligand binding functions of CD11b/CD18 (16). Certain ligands of CD11b/CD18 bind to the expressed I domain in a cation-dependent manner (17–20), and many CD11b/CD18 blocking Abs map to the I domain (21). The lectin-like adhesion site of CD11b/CD18
was also mapped using carbohydrate ligands (11). This domain resides in an area proximal to the cell membrane and exhibits a broad specificity for β-glucan (glucose) and mannan (mannose) polysaccharides. Subsequent studies showed that binding of such carbohydrates to the lectin domain of CD11b resulted in priming of the receptor for phagocytosis and cytotoxicity, which involved a tyrosine kinase, a Mg-dependent conformational change in the I domain, and exposure of the mAb CBRM1/5 activation epitope (22). Consistent with this observation, certain mAbs can activate CD11b/CD18 adhesion by binding to the lectin domain (23). These studies support data showing that the I and the lectin domains are both involved in CD11b/CD18-mediated adhesion to intact microorganisms, including C. albicans (5, 8).

Previously, we have used the homologue-scanning mutagenesis strategy (24) to analyze the binding site for the I domain ligands of CD11b/CD18 (25). This strategy takes advantage of the known crystal structures of the CD11a and CD11b I domains and entails swapping homologous structural motifs, either α-helical segments, β-sheets, or their connecting loops from CD11a into CD11b to identify requisite sites for ligand binding function while conserving the structure of CD11b/CD18. Mutated CD11b/CD18 receptors containing CD11b I domain segments of 6–10 amino acids “swapped” with their corresponding segments of CD11a I domain have been stably expressed in 293 cells. We have demonstrated previously that these mutant CD11b/CD18 receptors exhibit the conformation of the wild-type receptor by a variety of criteria (25). Using these mutant cell lines, we have shown that the I domain binding pockets for NIF, C3bi, and Fg are overlapping but not identical (26). Recently, these mutants were used to map the CD11b/CD18 binding pocket for NIF (25). The experiments in the present study describe the utilization of these mutants to map the segments of the CD11b/CD18 I domain that mediate adhesion to C. albicans and identify C. albicans as a unique CD11b/CD18 ligand. Additional mutant receptors have been used to examine the roles of the lectin-like domain and the CD18 subunit in the recognition of this fungal ligand.

Materials and Methods

Fungal culture

C. albicans (S8716, American Type Culture Collection, Manassas, VA) was provided by Dr. Herbert L. Mathews, Loyola University of Chicago (Maywood, IL), and used throughout this investigation. Cultures were stored at 25°C on Sabouraud’s dextrose agar (Difco, Detroit, MI). Cells used for experimentation were cultured overnight at 37°C on Sabouraud’s dextrose agar, collected as isolated colonies, washed once in complete HBSS, and cultured in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μM nonessential amino acids, and 2 mM l-glutamine (all from BioWhittaker, Walkersville, MD) as well as 50 μM 2-ME. The cells were distributed at 2.5 × 10⁶ cells/ml into Costar 24-well plates (Becton Dickinson, Lincoln Park, NJ) with 1500 U/ml IL-2 (Sigma, St. Louis, MO), and they were harvested after 7 days at 37°C in 5% CO₂. These cells were >99% lysed as judged by Wright staining.

Calcein labeling of 293 cells and lymphocytes

Human 293 kidney cells and IL-2-activated lymphocytes were labeled with the fluorochrome calcein-AM (Molecular Probes, Eugene, OR) for use in adhesion assays. The calcein-AM solution was prepared fresh for each experiment using 50-μg aliquots provided by the manufacturer by initial solubilization in 10 μl of DMSO followed by 950 μl of HBSS without Ca²⁺ or Mg²⁺. Cells were suspended at a concentration of 10⁶/ml in DMEM F-12 with 0.5% BSA (Sigma) and 5 μg/ml calcein-AM (100 μl) for 30 min at 37°C in 5% CO₂. Labeling was determined to have no effect on cell viability as assessed by trypan blue staining.

Adhesion of lymphocytes to C. albicans

This assay was performed as described previously (28) with some modifications. Briefly, C. albicans hyphae were prepared by growth of 10⁷ yeast in 50 μl of RPMI 1640/well (without serum) for 3 h at 37°C in flat-bottom 96-well plastic plates (Corning, Cornings, NY). After 3 h, 90–100% confluent of the cultures were removed from the sterile Pasteur pipette in HBSS, and cultured in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μM non-essential amino acids, and 2 mM l-glutamine (all from BioWhittaker, Walkersville, MD) as well as 50 μM 2-ME. The cells were distributed at 2.5 × 10⁶ cells/ml into Costar 24-well plates (Becton Dickinson, Lincoln Park, NJ) with 1500 U/ml IL-2 (Sigma, St. Louis, MO), and they were harvested after 7 days at 37°C in 5% CO₂. These cells were >99% lysed as judged by Wright staining.

Cell lines and culture

The human kidney 293 fibroblastoid cell line was maintained as described previously (27) in DMEM-F12 plus 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine (all from BioWhittaker, Walkersville, MD). Adherent cells were removed for passage and experiments using enzyme-free cell dissociation buffer (Life Technologies, Grand Island, NY). Construction of 293 cells stably expressing wild-type (WT) or mutant forms of CD11b/CD18 has been previously described (26). Before use in adhesion assays, expression levels of CD11b/CD18 were verified to be similar for all mutant and WT cells using FACS analysis. CD11b/CD18 mutants with low expression levels were enriched for clones with expression levels similar to WT using limiting dilution followed by FACS analysis to select for higher expressing clones. For cases in which this step was required, data were pooled from at least two separate clones. CD11b/CD18 expression also was verified at the time of the experiments to insure similar levels of CD11b/CD18 expression between WT and mutant cells. The mean fluorescent intensities for the WT and mutants used in this study are approximately 300 when stained with an anti-CD11b mAb (OKM1), compared with 5 for mock-transfected cells. All FACS analysis was performed with FACStar (Becton Dickinson, San Jose, CA) in the core flow cytometry facility at The Cleveland Clinic supported by the Keck Foundation. The expression levels of the cell lines used in this study differed by <50%. There was no correlation between the adhesive activity and the CD11b/CD18 expression levels within this range. For example, two subclones of WT-CD11b/CD18 with mean fluorescence intensities of 358 and 697 had 2.5 × 10⁶ and 1.5 × 10⁷ adherent cells, respectively, when a total of 5.0 × 10⁶ cells were added to the assay. The average was 1.99 ± 0.39 × 10⁴ adherent cells over 20 independent experiments.

IL-2-activated human lymphocytes

Human PBMC were obtained by venipuncture from normal donors with informed consent and were isolated using Ficoll-HistoPaque (Pharmacia, Piscataway, NJ) by centrifugation at 2000 × g for 25 min. Lymphocytes at the interface were removed from the sterile Pasteur pipette in HBSS, and cultured in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μM nonessential amino acids, and 2 mM l-glutamine (all from BioWhittaker, Walkersville, MD) as well as 50 μM 2-ME. The cells were distributed at 2.5 × 10⁶ cells/ml into Costar 24-well plates (Becton Dickinson, Lincoln Park, NJ) with 1500 U/ml IL-2 (Sigma, St. Louis, MO), and they were harvested after 7 days at 37°C in 5% CO₂. These cells were >99% lysed as judged by Wright staining.

The NIF protein was provided by Corvas International (La Jolla, CA). The mAbs used were as follows: OKM1 (anti-CD11b, IgG2b), 44a (anti-CD11b, IgG1), 15M201 (anti-CD11b, IgG1), IB4 (anti-CD18, IgG2a), and W6/32 (anti-MHC class I, IgG1; all from American Type Culture Collection). These Abs were purified from conditioned tissue culture medium using a column of recombinant protein G as described by the manufacturer (Zymed, San Francisco, CA). All anti-human mAbs were of mouse origin, and the secondary Ab used for immuno-fluorescence analysis was FITC goat anti-mouse IgG (Zymed). Protein concentrations were determined spectrophotometrically.

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Adhesion of different cell types to C. albicans

3% CO₂ before washing. Adhesion of different cell types to the C. albicans form of to the beled with calcein, were added over a range of cell concentrations by growing the yeast for 3 h at 37°C in 96-well plates. The assessed the ability of the WT-CD11b/CD18 receptor expressed on high levels on cell surfaces as functional heterodimers. We initially (26), have demonstrated that CD11b/CD18 can be expressed at comparable to IL-2-activated human lymphocytes.

Numerous studies (10, 12, 29), including those from our laboratory, have demonstrated that the IL-2-activated human lymphocytes adhesion to C. albicans. Previous studies have shown that adhesion of such activated lymphocyte preparations to C. albicans is CD11b/CD18 mediated (30). Fig. 1 shows that the extent of adhesion of both cell types to the hyphae was similar. In contrast, 293 transfectants expressing CD11a/CD18 at a similar level as the WT-CD11b/CD18 transfectants adhered minimally to the hyphae; the extent of adhesion of the CD11a/CD18 transfectants was comparable to that of the mock-transfected cells.

To further verify the role of CD11b/CD18 in cell adhesion to C. albicans, the effects of known inhibitors of CD11b/CD18 function were examined on adhesion of the WT-CD11b/CD18 transfectants and the IL-2-activated lymphocytes to the hyphae in parallel (Fig. 2). These inhibitors included several mAbs (OKM1, 44a, and IB-4) to different regions of CD11b/CD18. The anti-MHC class I mAb W6/32, which does bind to the 293 transfectants, was used as a negative control. mAb 44a to the I domain of CD11b strongly inhibited the adhesion of WT-CD11b/CD18 transfectants and IL-2-activated lymphocytes to the hyphae; adhesion of both cell types was inhibited by >80%. Treatment with the control mAb (W6/32) had no effect on the adhesion of either cell type to the hyphae. Consistent with a role for the I domain of CD11b in hyphal recognition, NIF, a CD11b/CD18 I domain-specific ligand (25), eliminated adhesion of both the WT-CD11b/CD18 transfectants and IL-2-activated lymphocytes to the hyphae. In addition, an anti-CD18-blocking mAb, IB4, also strongly inhibited (~80% inhibition) WT-CD11b/CD18 and IL-2-activated lymphocyte adhesion to the hyphae. OKM1, a mAb that blocks the function of the lectin domain of CD11b (11), inhibited adhesion of WT-CD11b/CD18 cells and IL-2-activated lymphocytes by 50%, and a further increase in the OKM1 concentration did not substantially increase the extent of inhibition. Taken together, these data demonstrate the role of CD11b/CD18 in mediating cell adhesion to C. albicans hyphae and are consistent with a contribution of multiple domains in recognition of the pathogen by the receptor (8).
Roles of the integrin subunits in CD11b/CD18-mediated adhesion to C. albicans

To dissect the roles of individual CD11b and CD18 chains in adhesion, we conducted binding experiments with the transfected cells expressing only the CD11b subunit or the heterodimeric WT-CD11b/CD18 (Fig. 3). Cell surface expression of CD11b in the absence of CD18 on 293 cells and lack of association with other endogenous β subunits have been verified previously using several independent approaches, including FACS analysis with a CD11b-specific mAb (44a) and a CD18-specific mAb (IB4) and surface labeling and immunoprecipitation (25). The cells expressing only CD11b bound hyphal wells, although at a reduced level compared with WT-CD11b/CD18 (54–76% of WT). To ensure that the decreased adhesion was not a result of small differences in receptor expression, several subclones of the CD11b-only cells were developed and tested. These subclones expressed from 50–100% of WT levels of CD11b by FACS analyses, and no correlation was found between expression level and the extent of adhesion among these cell lines (not shown). The specificity of CD11b-C. albicans interaction was verified using the specific blocking mAbs noted above. Both OKM1 and 44a inhibited adhesion of CD11b cells to hyphae, while the CD18-specific mAb IB4, which inhibited binding of the cells expressing the heterodimer (see above), had no effect on adhesion of CD11b-only cells. Thus, the CD11b subunit alone is sufficient to mediate hyphal binding and suggests that the CD18 subunit does not provide a major contact site for C. albicans.

We next examined the effects of a CD18 mutation (S138A) on CD11b/CD18 adhesion. Previous studies (26, 29) have shown that this mutation or ones at the immediately adjacent oxygenated residues, D134 and S136, block the capacity of CD11b/CD18 to bind certain ligands, including fibrinogen and C3bi. As shown in Fig. 3, mutation of Ser138 to Ala abolished CD11b/CD18 adhesion to C. albicans. Cell surface expression of the WT and mutant CD11b/CD18 was similar as judged by FACS (26). Therefore, although CD18 is not required for adhesion to C. albicans, i.e., CD11b in the absence of CD18 supports cell adhesion, mutation of Ser138 abrogates the adhesive activity of the CD11b/CD18 complex.

Roles of the lectin-like domain in CD11b/CD18-mediated adhesion to C. albicans

To investigate the role of the CD11b lectin-like domain in adhesion to C. albicans, cells were developed expressing a heterodimer CD11b(Q190-S197)/CD18. Adhesion of 5 × 10⁴ calcein-AM-labeled WT CD11b/CD18, CD11b-, CD11b/CD18(S138A)-expressing or mock-transfected human 293 cells to C. albicans were assessed. Adhesion of CD11b-expressing cells was performed in the presence of different mAbs (15 µg/ml) as described in Fig. 2. Data are expressed as the mean percentage of WT CD11b/CD18 adhesion from triplicate wells of two experiments.

in which the I domain of CD11a was substituted with that of CD11b (termed L/M). CD11a/CD18 has been shown to lack the lectin binding activity found in CD11b/CD18 (11). In the absence of the CD11b lectin-like domain, the chimeric CD11a/CD18-expressing cells (L/M), which contains only the I domain of CD11b, bound C. albicans well, ranging from 60–82% of the WT-CD11b/CD18 adhesion (Fig. 4). Verifying the specificity, the wild-type CD11a/CD18-expressing cells failed to adhere to the fungi. These data demonstrate that the CD11b/CD18 lectin-like domain is not required for CD11b/CD18-mediated adhesion to hyphae.

The absence of the lectin-like domain in CD11a/CD18 was confirmed. Known carbohydrate ligands of this site (11), N-acetyl-D-glucosamine and methyl-β-D-glucopyranoside, were assessed for their ability to inhibit adhesion of the WT-CD11b/CD18 and the chimeric receptor (L/M)-expressing cells to the hyphae (Fig. 5). Each carbohydrate was used at a concentration of 150 mM, previously shown to maximally inhibit the function of the lectin-like domain (11). β-D-glucose was used as a negative control. As

FIGURE 3. Adhesion of human 293 cells expressing mutant CD11b/CD18 receptors to C. albicans. Adhesion of 5 × 10⁴ calcein-AM-labeled WT CD11b/CD18-, CD11b-, CD11b/CD18(S138A)-expressing or mock-transfected human 293 cells to C. albicans were assessed. Adhesion of CD11b-expressing cells was performed in the presence of different mAbs (15 µg/ml) as described in Fig. 2. Data are expressed as the mean percentage of WT CD11b/CD18 adhesion from triplicate wells of two experiments.

FIGURE 4. Comparative adhesion to C. albicans of 293 cells expressing WT CD11b/CD18, WT CD11a/CD18, or CD11a/CD18 containing the I domain of CD11b. Adhesion to C. albicans of 3 × 10⁴ calcein-AM-labeled cells was assessed in triplicate wells as described in Fig. 1. Cells compared in these experiments were WT CD11b/CD18, WT CD11a/CD18, chimeric CD11a/CD18 containing the I domain of CD11b (L/M), and mock-transfected (Mock) cells. Data are presented as the mean percentage of WT CD11b/CD18 adhesion ± SD of three experiments.

FIGURE 5. Carbohydrates competitively inhibit adhesion of WT CD11b/CD18, but not L/M cells or the constitutively active mutant CD11b(Q190-S197)/CD18. Adhesion to C. albicans of 5 × 10⁴ calcein-AM-labeled WT CD11b/CD18, L/M chimeras, and the constitutively active CD11b(Q190-S197)/CD18 mutant was assessed in the presence of 150 mM of each carbohydrate. The carbohydrates used were N-acetyl-D-glucosamine (NAGD), methyl-β-D-glucopyranoside (MGP), and β-D-glucose. Data are expressed as the mean percent adhesion from triplicate wells of two experiments. For each cell type, adhesion in the absence of carbohydrates was assigned a value of 100%.

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shown in Fig. 5, N-acetyl-d-glucosamine and methyl-β-glucopyranoside inhibited adhesion of the WT-CD11b/CD18 cells by 60 and 70%, respectively, demonstrating a contribution of the lectin-like domain in CD11b/CD18 to adhesion. In contrast, these carbohydrates had no effect on L/M adhesion (Fig. 5). In separate experiments, we evaluated the effects of these sugars on CD11b/CD18-NIF interaction. At 150 mM, none of the three carbohydrates had any effect on NIF binding to the transfected cells as judged by FACS analysis using biotinylated NIF and avidin-FITC conjugate (25) (data not shown).

To test the hypothesis that modulation of CD11b/CD18 function by the lectin-like domain depends upon its influence on the conformational state of the I domain, we examined the effects of the inhibitory carbohydrates on a constitutively active CD11b/CD18 mutant, CD11b(Q190-S197)/CD18. This mutant contains a substitution within its I domain and is constitutively active with respect to the binding of activation-dependent CD11b/CD18 ligands, such as Fg (31). Consistent with its active state, this mutant exhibited enhanced binding to C. albicans; the adhesion of the CD11b(Q190-S197)/CD18 mutant cells was equal to or greater than that of the WT-CD11b/CD18 at higher cell concentrations (not shown). As shown in Fig. 5, the inhibitory carbohydrates, N-acetyl-d-glucosamine and methyl-β-glucopyranoside, had no effect on adhesion of this constitutively active CD11b/CD18 receptor, in contrast to their potent inhibition of the WT-CD11b/CD18 receptor.

Mapping the C. albicans binding region within the CD11b I domain

Since mAbs to the CD11b I domain and NIF blocked CD11b/CD18 adhesion to C. albicans (Fig. 2), and since the chimeric CD11a/CD18 receptor containing the CD11b I domain adhered well to the fungus (Fig. 4), we sought to localize the specific segments within the CD11b/CD18 I domain that mediate this adhesion. The adhesion to C. albicans of 10 homologue-scanning mutants within the I domain was assessed. These previously described mutants contain swaps of homologous small structural elements, α-helices, β-sheets, and connecting loops, of the CD11a I domain into the CD11b I domain (25). The adhesion of these mutants to C. albicans hyphal was assessed over the range of cell concentrations shown in Fig. 1, but only data from a representative concentration of 5 × 10^5 cells are shown (Fig. 6). The 10 mutants assessed were CD11b(P147-R152), CD11b(M153-T159), CD11b(Q190-S197), CD11b(E178-T185), CD11b(R208-K217), CD11b(K245FG), CD11b(D248-Y252), CD11b(E253-R261), CD11b(D273-K279), and CD11b(R281-I287). Adhesion of these mutants to the hyphal fell into two general categories; those that are similar to WT-CD11b/CD18, including CD11b(M153-T159), CD11b (Q190-S197), CD11b (E178-T185), CD11b(K245FG), and CD11b(D273-K279) (Fig. 6a), and those that demonstrate virtually no adhesion to C. albicans, including CD11b(P147-R152), CD11b(R208-K217), CD11b(D248-Y252), CD11b(E253-R261), and CD11b(R281-I287) (Fig. 6b).

Discussion

The Center for Disease Control has noted that the incidence of life-threatening opportunistic fungal infections have increased an alarming 500-fold since 1980, and C. albicans is responsible for 70–80% of all opportunistic fungal infections (9). A principal reason for the high mortality in C. albicans and other fungal infections is the ability of fungal surface structures (mannoproteins) to interact with leukocytes and modulate the immune response to these organisms, enabling evasion of the immune response in patients who are often already ill from other causes such as prema-

![FIGURE 6](http://www.jimmunol.org/)
influence CD11b/CD18-mediated adhesion to the fungal hyphae. Accordingly, we only compared cells that expressed equivalent levels of WT and mutated forms of CD11b/CD18 as assessed by FACS analyses. These analyses were performed with each cell type before and after adhesion experiments, and low expressing mutants were subcloned to select for high expression equivalent to WT. Thus, the expression level of the transfectants used in our study differed by <50%. In addition, WT-CD11b/CD18 and mock-transfected cells as well as background binding for each cell type to wells containing no fungus were determined as internal controls for each experiment. The physiologic relevance of the CD11b/CD18-expressing 293 cells used in our study was verified by the demonstration that these cells behaved qualitatively and quantitatively similarly to IL-2-activated lymphocytes, which are known to use CD11b/CD18 to bind C. albicans (30). Furthermore, CD11a/CD18-expressing 293 cells did not bind to hyphae specifically, and this integrin does not play a role in C. albicans binding to leukocytes (8, 30). The failure of CD11a/CD18 to recognize the hyphae allowed implementation of the homologue-scanning strategy in which segments of CD11a were exchanged for the corresponding segments of CD11b to map the I domain contact segments for C. albicans.

The role of the CD11b subunit in CD11b/CD18 ligand binding is well documented (17, 20, 21, 32) and is confirmed in this study. Transfected cells expressing only CD11b bound specifically to the hyphae. An mAb to the CD11b subunit that blocked binding of the heterodimeric receptor to the fungus remained inhibitory, whereas a mAb to the CD18 subunit lost inhibitory function. The CD11b cells did not bind to C. albicans as efficiently as the WT cells (65% WT; Fig. 3), but the modest reduction in binding precludes the existence of a substantial number of direct contacts between the hyphae and CD18. However, the function of the CD18 subunit in C. albicans binding to the heterodimeric receptor or, for that matter, of other ligands to this or other integrins is significant but remains difficult to define. The D134XSXS sequence of CD18 has been previously demonstrated to be critical for the recognition of several CD11b/CD18 ligands. Mutation of D134, S136, or S138 eliminated adhesion of CD11b/CD18-expressing cells to dOva, C3bi, ICAM-1, and fibrinogen (26, 29), and C. albicans can be added to this list. The homologous region within β3 also is critical to the ligand binding function of this integrin subfamily (33), and a naturally occurring point mutation at one of these residues has been reported to fully inactivate these receptors (34). Recently, it has been proposed that the β subunits of integrins, including CD18, contain an I domain-like structure, and the DXSXS sequence is part of a MIDAS motif providing cation coordination sites (35–38) and also may contain direct ligand contact sites as well (39–41). However, as demonstrated in this study, cells expressing CD11b alone adhered well to C. albicans, and removal of the entire CD18 subunit had limited effects on the recognition of this ligand, suggesting that, at least for this ligand, the CD18 subunit is not required for ligand engagement. Thus, the consequence of mutation of Ser138 is the creation of a dominant negative effect, which masks the intrinsic activity of the CD11b subunit. The mechanism of this effect is unclear. One possibility is that the absence of this residue changes the cation coordination complex to one that is incompatible with ligand binding. Clearly, the relationship between ligand and cation binding is vital to integrin function (42).

Previous studies with C. albicans (8) and with other ligands (22, 43) have implicated the lectin-like domain in CD11b/CD18 functions. In this study, the lectin domain blocking mAb OKM1 and two carbohydrate ligands of the lectin-like domain significantly reduced the adhesion of both WT transfectants and IL-2-activated lymphocytes. Although a large proportion of the surface of C. albicans is composed of β-glucan polymers, this domain is not sufficient for binding of C. albicans to the receptor. This conclusion is supported by the observations that 1) certain receptors with mutations only in the I domain and without alterations in the lectin domain failed to adhere to C. albicans; and 2) the chimeric CD11a/CD18 receptor that contained only the I domain of CD11b and lacked the lectin-like domain (the carbohydrates that inhibited WT adhesion had no effect on the function of the chimeric receptor) was able to bind the fungus to an extent similar (80%) to that of the WT-CD11b/CD18 cells. The mechanism by which the lectin-like domain modulates CD11b/CD18 function appears to reside in its modulation of I domain conformation. The mutant CD11b/CD18 receptor, CD11b(Q190-S197)/CD18, contains an activating mutation within the I domain (31), and the inhibitory carbohydrates did not block C. albicans binding by this activated receptor. These data and interpretation are consistent with the capacity of the lectin domain-specific mAb VIM12 to exert an activating effect on I domain-mediated adhesion of CD11b/CD18 as observed in association with homotypic aggregation (23). Recently, several studies have suggested the existence of a promiscuous lectin binding site with somewhat distinct sugar specificity within CD11a/CD18 (44). In the same study, N-acetyl-D-glucosamine was shown to inhibit the association between CD11a/CD18 and the FcγR333 receptor (44). Although this polysaccharide had no effect on L/M chimera adhesion to hyphae (Fig. 5), our data cannot rule out the possibility that a lectin site with different carbohydrate specificity may exist in CD11a/CD18 and could participate directly in L/M chimera interactions. Hyphae binding to a low affinity I domain may require the assistance of the lectin domain (in the cases of WT and L/M chimera), whereas a high affinity I domain can overcome the requirement for an additional contact site in the lectin domain (in the case of the CD11b(Q190-S197) mutant). Nevertheless, since cells expressing WT CD11a/CD18 and mutant CD11b/CD18 with intact lectin domain did not bind hyphae, the direct contribution from the lectin domain to hyphae binding, if any, cannot be substantial. The lectin domain also could be involved in directly priming CD11b/CD18 for antifungal cytotoxicity, a possibility that was not addressed in this study (22, 30).

That the I domain provides the major contact interface for C. albicans is supported by the following experiments. First, CD11b I domain-specific mAbs and the high affinity CD11b/CD18 ligand (NIF) that has been shown to interact exclusively with the I domain (25) blocked CD11b/CD18 adhesion to C. albicans. Second, the chimeric CD11a/CD18 receptor containing the CD11b I domain adhered well to the fungi. Accordingly, we sought to localize the functional segments within the I domain using our existing homologue-scanning mutants. Of the 10 representative mutants we tested, five (CD11b (M153-T159), CD11b (Q190-S197), CD11b (E178-T185), CD11b (K245FG), and CD11b (D273-K279)) had similar C. albicans adhesion to WT-CD11b/CD18 (Fig. 6a). Of note are mutants CD11b (K245FG) and CD11b (D273-K279). CD11b (K245FG) has approximately 60% the adhesive activity of WT-CD11b/CD18. This mutant binds significantly less well to C3bi (~20% of WT; our manuscript in preparation), thus distinguishing C. albicans from C3bi as a CD11b/CD18 ligand. The CD11b (D273-K279) mutant has been shown previously to exhibit virtually no adhesion to Fg (26), although it bound well to hyphae. This result distinguishes C. albicans from Fg as a CD11b/CD18 ligand. Five other mutants, including CD11b (P147-R152), CD11b (R208-K217), CD11b (D248-Y252), CD11b (E253-R261), and CD11b (R281-I287), demonstrate virtually no adhesion to C. albicans (Fig. 6b). The first four of these segments have been shown to constitute the NIF binding site within the I domain (Fig. 7) (25). Thus, C. albicans shares an overlapping ligand binding site with
NIF, providing the molecular basis for the potent inhibitory activity of NIF toward C. albicans binding. However, the CD11b (R281-I287) and CD18 (S138A) mutations have no effect on NIF recognition (25) while eliminating adhesion to C. albicans, thus setting these hyphae apart from NIF as a microbial ligand for CD11b/CD18. Taken together, our data show that the NIF binding site (P147-R152, R208-K217, D248-R261) represents the core structure of a broad surface that is involved in establishing the oligospecificity of the CD11b/CD18 integrin toward its multiple and unrelated ligands. The C. albicans recognition site encompasses the entire NIF binding site plus an additional segment (R281-I287; Fig. 7).

In summary, this study has provided important information regarding the roles of the CD18 subunit and the lectin-like domain of CD11b in the function of the CD11b/CD18 receptor. It also represents the first mapping of a pathogenic human microorganism to an integrin receptor and demonstrates that relative to previously mapped protein ligands for CD11b/CD18, C. albicans is a unique CD11b/CD18 ligand. Such a unique interaction may very well underlie the demonstrated ability of C. albicans to modulate CD11b/CD18 function. Greater understanding of this unique interaction could provide avenues for novel adhesion-based antimicrobial or antiinflammatory therapies.

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References


