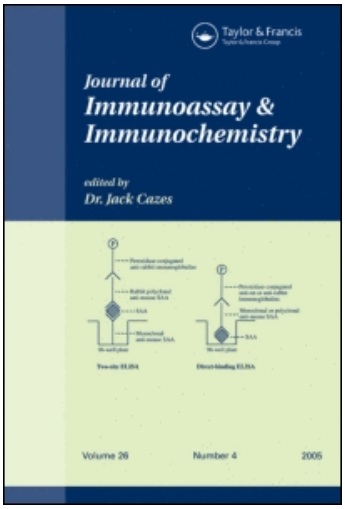

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Novel Tools for Functional Analysis of CD11c: Activation-Specific, Activation-Independent, and Activating Antibodies

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Abstract: Functions and binding properties of four CD11c-specific mAbs are described here. The mAb 496B stimulated, while 496K inhibited ligand binding of CD11c. The stimulatory mAb, 496B, as well as the inhibitory mAbs BU15 and 496 K appear to act allosterically, as they do not bind the CD11c I domain. The mAb 3.9 bound preferentially to activated forms of CD11c and the binding was divalent cation dependent. CD11c binding to 3.9 recapitulates many of the integrin-ligand interactions. Our data suggest that 3.9 is a competitive antagonist, BU15 and 496K are allosteric antagonists, and 496B is an allosteric agonist of CD11c. These mAbs provide a set of tools to study the functions of the dendritic cell marker, CD11c.

Keywords: CD11c, Ligand mimetic, I domain, Antibodies, Agonistic, Allosteric

INTRODUCTION

Integrins are a family of cell surface adhesion receptors that have been implicated in a wide variety of functions. These are Type I transmembane, heterodimeric molecules, consisting of one α chain and one β chain that are non-covalently associated. The CD18 integrins consist of four members with distinct α chains (αL, αM, αX, and αD), which are known as CD11a, CD11b, CD11c, and CD11d respectively, that share the same CD18 (β2) chain. Structurally, the extracellular region of the CD11/CD18 integrins has

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been defined in terms of multiple segments such as a headpiece, thigh, genu, calf1, and calf2.[1] The headpiece is composed of the β propeller domain and the I domain from the α chain and the I like domain from the β chain.[1,2] The CD18 integrins bind ligands via the α chain I domain in a metal ion dependent manner, whereas the β chain I like domain contributes to regulation of the binding activity.[3]

Structural studies have revealed a set of highly conserved residues in the I domains that provide five coordination sites with a single magnesium ion. Some of the residues involved in metal binding such as D-X-S-X-S are contiguous (CD11c residues 138 to 142) while others (T207, D240, and E244) are noncontiguous. Ligands of the CD18 integrins bind the I domain via providing the sixth coordination site to the magnesium ion.[4]

Activity of integrins, including the β2 integrins, is highly regulated.[5] Activated integrins bind ligands with higher affinity. The change in affinity is accompanied by distinct changes in integrin structure.[6–8] Broadly, three structural conformations of CD18 integrins have been defined: bent, extended with closed headpiece, and extended with open headpiece.[1,9] The bent from displays poor affinity toward the ligands. The extended form with closed headpiece binds ligand with intermediate affinity, while the extended form with open headpiece demonstrates highest affinity for ligands.[10] For CD11a, the conversion from bent to extended form occurs upon extension at the thigh/genu junction.[11]

Antibodies have been used as important tools to study integrin function as well as to delineate the structural elements that play important role in those functions. Several mAbs such as m24[12] and 327C[13] bind an activated state of CD18; on the other hand, mAbs such as CBR LFA1/2 activate the integrins upon binding.[14] The NKI-L16 mAb binds the CD11a/CD18 α chain specifically and activates it.[15] Ligand mimetic antibodies constitute another important class of mAbs. Similar to binding physiologic ligands, integrins bind to these antibodies in an activation- and divalent cation-dependent manner. For example, binding of CD11b to the mAbs 7E3[16] and CBRM1/5,[17] and binding of CD11a to the recently described phage antibody AL57[18,19] demonstrate ligand mimetic features. Some of the ligand mimetic antibodies contain ligand like sequences on CDRH3, the putative integrin-binding site.[19–21] Mapping of epitopes of these various classes of mAbs have provided important insights on the structural elements that regulate integrin activation and the switch from bent to extended conformation.

Although CD11c is highly expressed in important immune cells, such as the dendritic cells, compared to those of CD11a and CD11b the biology of CD11c is less well understood. Similarly, fewer CD11c mAbs are described, and the available mAbs are not well characterized. Here, we describe characterization of four anti-human CD11c mAbs, including a novel CD11c agonistic mAb, 496B, and three antagonistic mAbs, viz., 3.9,[22] BU15,[23] and 496 K. mAbs 496B, 496K and BU15 did not bind the

CD11c I domain, and hence may act allosterically. Of the three antagonistic mAbs, 496K and BU15 bound CD11c irrespective of the activation status of the integrin and their binding was independent of divalent cations. On the contrary, 3.9 binds to CD11c in an activation- and divalent cation-dependent fashion. Furthermore, mAb 3.9 binding to CD11c is localized to the I domain of the integrin, and the binding requires an intact MIDAS sequence. Divalent cation or the activating mutation I314/A stimulated 3.9 binding to the I domain. These observations, combined with 3.9-mediated strong blocking of CD11c binding to its multiple ligands, suggest that 3.9 preferentially recognizes active forms of CD11c.

EXPERIMENTAL

Cells, Antibodies, and Proteins

A cDNA for human CD11c was introduced in the JY cell line by electroporation and cells expressing CD11c were enriched by sorting with FITC-labeled BU15. These cells are termed JY[hCD11c]. Details of the development of the cell line are published elsewhere.[24] The cells were grown in RPMI supplemented with glutamine, sodium pyruvate, penicillin-streptomycin, and 10% fetal bovine serum. Anti-human CD11c mAbs, 3.9 and BU15 were from BioLegend (San Diego, CA) and The Binding Site (Birmingham, England), respectively. The human CD18 activating mAb 240Q was developed at ICOS Corporation and has been described.[13] All of the antibodies described here are of IgG1 isotype. Unless mentioned otherwise, purified antibodies were used in all assays.

For expression of the CD11c I domain, a fragment of the CD11c cDNA encompassing residues R128 to G319 was subcloned in the expression vector pET28a as an N-terminal $6 \times$ HIS tag protein and expressed in *E. coli*. CD11c I domain mutants, D138/A and I314/A were generated using a site-directed mutagenesis kit from Stratagene according to the manufacturer's protocol. Proteins were purified from *E. coli* lysates using affinity chromatography on NTA column and gel filtration chromatography through Superdex 200 according to standard protocol.

Cell Adhesion Assay

Immulon-4 96-well plates were coated with the indicated mAb at 10 μg/mL overnight in carbonate buffer, washed, and blocked with 1% human serum albumin (HSA) at room temperature for 1 h. The plates were washed in calcium-magnesiun-free PBS and used in adhesion assay. An overnight culture of JY[hCD11c] cells (density $\sim 0.5 \times 10^6$/mL) were harvested, washed, and resuspended in RPMI containing 0.1% HSA at a density of

1×10^6/mL. The cells were used in standard adhesion assay and adhesion was quantified as previously described.[25]

To study the effect of divalent cations on cell adhesion, the cells were washed with and resuspended in 10 mM HEPES buffer (pH 7.2) containing 140 mM NaCl and 2 mg/mL glucose. Adhesion assay was carried out in the presence of 5 mM EDTA, or 2 mM $CaCl_2$, or 10 mM $MgCl_2$, or 0.5 mM $MnCl_2$.

The rest of the assay protocol was the same as described above.

Flow Cytometry

Logarithmically growing JY[hCD11c] cells were diluted to 2×10^5/mL and allowed to grow overnight. The cells were harvested and resuspended at 3×10^6/mL in PBS. Portions of the cell suspension were adjusted to a final concentration of 1 mM EDTA or 2 mM $MgCl_2$. 100 μL aliquots of each cell suspension were incubated for 30 min. with each of the indicated mAbs (5 μg/mL) on ice in a 96 well plate. The cells were washed twice and incubated with FITC-labeled sheep anti-mouse IgG either in the presence of 1 mM EDTA or 2 mM $MgCl_2$. The cells were washed twice, fixed with 1% formaldehyde and fluorescence was read in a Becton Dickinson Facscan instrument.

ELISA

For I domain ELISA, Immulon-4 96-well plates were coated overnight at 4°C with purified recombinant CD11c I domain proteins at 5 μg/mL in PBS. The plates were washed twice in PBS containing 0.1% Tween 20 and blocked with 1% human serum albumin in 10 mM Tris (pH 8.0) containing 150 mM NaCl. The wells were incubated with the indicated mAbs at 1 μg/mL in the presence of 10 mM EDTA or 2 mM $MgCl_2$ at room temperature for 1 hr. The plates were washed three times and incubated with HRP-conjugated goat anti-mouse IgG at room temperature for 30 min. The plates were washed four times and color was developed with the HRP substrate, TMB.

Surface Plasmon Resonance (SPR)

SPR experiments were performed with a Biacore 2000 instrument (BIAcore, Uppsala, Sweden). mAb 3.9 was immobilized to the CM5 sensor chip (Biacore) via amine coupling (226 Response Units (RU) final surface density). Coupling of the mAb 3.9 was performed at 2 μg/mL in 10 mM NaAcetate/ pH 4.0. CD11c I domains were diluted into 10 mM HEPES (pH 7.4) containing 150 mM NaCl and 0.005% Tween 20 (HBS-P) (Biacore)

with either 1 mM $MgCl_2$ or 10 mM EDTA and perfused at a flow rate of 50 μL/min. All experiments were performed at 25°C. Data was double reference subtracted and fit globally with BiaEvaluation software v.4.1 using the 1:1 binding model.

RESULTS

Blocking and Activating mAbs of Human CD11c

We generated a set of anti-human CD11c mAbs using recombinant CD11c/CD18 as immunogen. For the identification and selection of the CD11c α chain-specific mAbs, hybridoma supernatants were screened against soluble CD11a/CD18 by ELISA. ELISA profiles of two CD11c-specific mAbs, 496B and 496K are shown in Figure 1. Both the mAbs reacted strongly with CD11c/CD18, but poorly to CD11a/CD18. Their binding to CD11a/CD18 was similar to that of the ELISA background as determined by the negative control. These data suggest that 496B and 496K do not bind to CD11a or the common CD18 chain. We further tested specificity of the mAbs against CD11a/CD18 and also against CD11b/CD18 by flow cytometry using the JY lymphoblastoid line transfected with either CD11b or CD11c. JY cells naturally express CD11a/CD18. The mAbs bound only the JY cells transfected with CD11c, but neither the untransfected cells nor the CD11b transfectants (data not shown), further establishing their specificity to the α chain of CD11c.

We tested functional properties of the mAbs in cell adhesion assays in which JY cells expressing human CD11c, JY[hCD11c] were allowed to adhere to an ICAM-1 coated surface in the presence of the PKC activator,

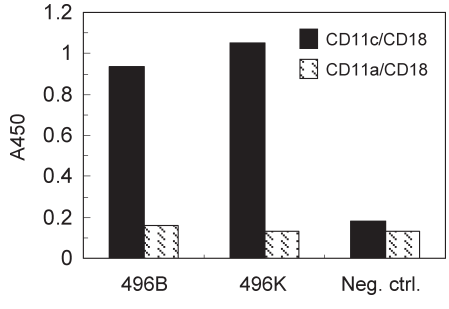

Figure 1. Selectivity of CD11c mAbs, 496B and 496K. Immulon-4 96-well plates were coated overnight with the indicated integrin heterodimers at 1 μg/mL and blocked. Hybridoma supernatants (50 μL) containing either the indicated test mAb or an irrelevant mAb was applied to the wells and binding was determined as described in Experimental. One representative example of several experiments is shown.

PMA, which activates multiple integrins, including CD11c. Since JY cells express a second ICAM-1-binding integrin, CD11a/CD18, the assays were conducted in the presence of a saturating concentration of the CD11a blocking mAb, TS1/22 to block its binding to ICAM-1. Figure 2a shows the effect of two of the newly generated mAbs, 496B and 496K on CD11c binding to ICAM-1. For comparison, we have also included cell adhesion data on the CD11c mAbs, 3.9 and BU15. As expected, both 3.9 and BU15 blocked the integrin binding to ICAM-1 (Figure 2a). Of the two new CD11c mAbs, 496K abolished JY[hCD11c] adhesion to ICAM-1. In contrast, 496B enhanced binding by about 3-fold, indicating that it functions as an agonist of CD11c.

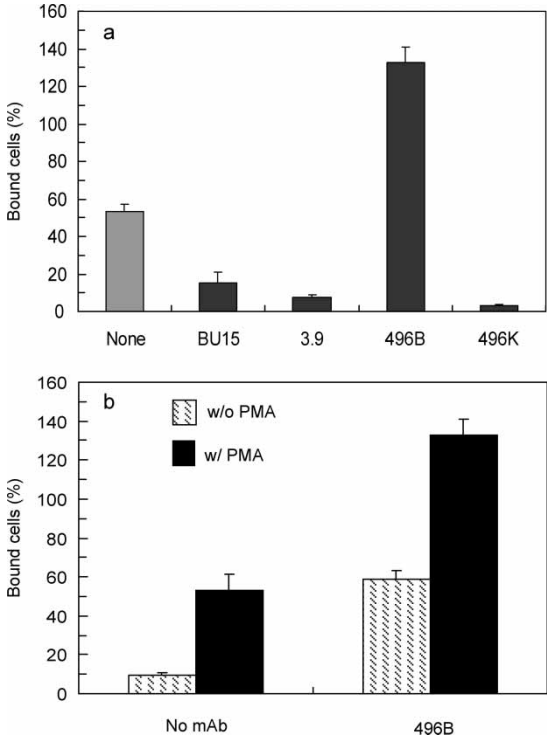

Figure 2. Blocking and activating anti-human CD11c mAbs. JY[hCD11c] cell adhesion to ICAM-1-coated plates was conducted as described in Materials and Methods. To suppress CD11a mediated adhesion to ICAM-1, the CD11a blocking mAb, TS1/22, was included in the assay. In the assay depicted in Fig. 2a, PMA (20 ng/mL) was included in all wells. The mAbs were used at 10 μg/mL. Effect of the mAb 496B in the presence or absence of PMA is compared in Fig. 2b. Cell adhesion was normalized using the values obtained from a set of wells that were coated with BU15, which was set at 100%. A representative example of 3–5 independent experiments is shown.

To determine whether the observed activation of CD11c by 496B is independent of the action of PMA, we compared the effect of 496B in the absence and in the presence of PMA. As shown in Figure 2b, compared to the adhesion in the presence of PMA, the absolute number of bound cells was lower in the absence of PMA. However, 496B enhanced JY[hCD11c] adhesion to ICAM-1 in the absence of PMA by about 6 fold. These data indicate that 496B can independently activate CD11c, and 496B-mediated activation of CD11c is additive to that of PMA.

mAb 3.9 Preferentially Binds to Activated CD11c

We were interested to determine whether any of the CD11c mAbs bind to the integrin in an activation dependent fashion and thereby serve as a reporter of CD11c activation. We modified the CD11c-mediated cell adhesion system, such that 96 well plates were coated with the antibodies, and adhesion of JY[hCD11c] cells to these wells were quantified. To activate CD11c, the cells were treated with PMA or the CD18-activating mAb 240Q [13], or a combination of both. As shown in Figure 3, cell adhesion to BU15-, 496B-, and 496K-coated wells was quantitatively similar, and the adhesion was insensitive to integrin activation. In contrast, the profile of JY[hCD11c] adhesion to the mAb 3.9 was distinct from those of the other mAbs.

In the absence of stimuli, cell adhesion to 3.9-coated wells was only $\sim$20% relative to those of other mAbs. Although mAb 240Q alone only

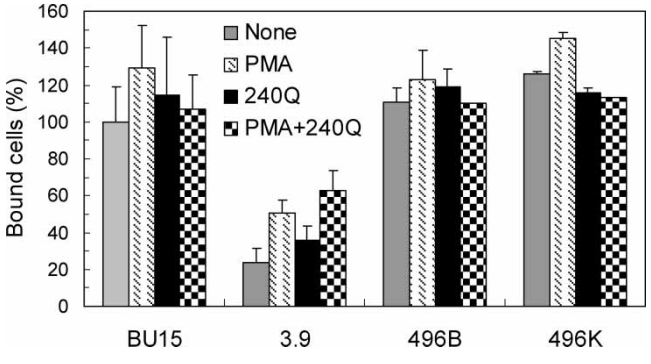

Figure 3. CD11c activation-dependent recognition of mAbs by JY[hCD11c]. The four anti-human-CD11c mAbs, BU15, 3.9, 496B, and 496K were used to coat an Immulon-4 96 well plate. JY[hCD11c] cells were stimulated with PMA (20 ng/mL), the β2 integrin activating mAb, 240Q, (10 μg/mL), or a combination of both. Cell adhesion to the mAb-coated wells was quantified as described in Experimental. Binding to all of the mAbs was normalized using the BU15 unstimulated adhesion values as 100%, and the particular mAb used for the four assay conditions is indicated along the X axis. One representative of 3 independent experiments is shown.

marginally stimulated cell adhesion, PMA or the combination of PMA and 240Q increased binding to 3.9-coated wells by 2- to 3-fold. These data demonstrate that CD11c binding to 3.9 is dependent on activation of the integrin. In the presence of both activators, cell adhesion to 3.9 was only ~60% compared to the other mAbs suggesting that only a subpopulation of CD11c is activated, and only the activated population is capable of binding to the mAb. Alternatively, it may be possible that 3.9 is a lower affinity mAb. However, subsequent affinity determination suggests this may not be the case (see below). In addition, compared to BU15, 3.9 was equivalent or more efficient in blocking JY[hCD11c] adhesion to ligand-coated wells (Figure 2a and data not shown). Thus, similar to physiologic ligands, 3.9 appears to bind to the subset of CD11c that are competent for ligand binding.

mAb 3.9 Binds to CD11c in a Divalent Cation Dependent Manner

The CD18 family of integrins contains multiple α and β subunit divalent cation binding sites, and their binding to ligands is dependent on the nature and availability of these ions. The α subunit I domain binds magnesium ion and mediates ligand binding in a metal ion dependent manner.[4] Binding of several mAbs to the integrins is also influenced by cations. Utilizing the JY[hCD11c] adhesion to mAb-coated wells, we tested whether binding of CD11c to the four antibodies requires divalent cations. As shown in Figure 4, adhesion of the cells to BU15-, 496B-, and 496K-coated wells was insensitive of the presence or absence of divalent cations. In contrast,

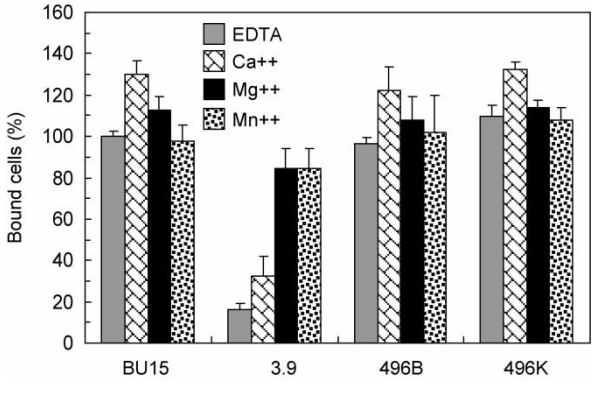

Figure 4. Divalent cation dependent JY[hCD11c] cell adhesion to mAbs-coated surface. The cells were washed and resuspended in 10 mM HEPES buffer (pH 7.2) containing 140 mM NaCl and 2 mg/mL glucose. Adhesion was carried out in the presence of EDTA or the divalent cations as indicated. Cell adhesion was normalized using the values obtained from the BU15-coated wells in the presence of EDTA, and the particular mAb used is indicated along the X axis.

adhesion of the JY[hCD11c] cells to the 3.9-coated surface produced a distinct profile. Compared to EDTA, calcium had no significant effect on cell binding to 3.9. However, stronger effects were observed in the presence of magnesium and manganese, both of which enhanced binding of JY[hCD11c] to 3.9 by more than 5-fold. A similar pattern of the divalent cation requirement of CD11c for binding to 3.9 was observed when the mAb binding to the JY[CD11c] or to the freshly isolated neutrophils and monocytes was studied using immunofluorescence and flow cytometry (data not shown). Since magnesium and manganese can activate the CD18 integrins,[16] these data suggest that 3.9 binds to activated forms of CD11c. Alternatively, it is also possible that 3.9 simply requires metal ions for binding irrespective of the activation status of the integrin. These data, together with the cell adhesion data, demonstrate that cell surface CD11c requires divalent cations to bind the mAb 3.9, a feature that is reminiscent of the integrin binding to physiologic ligands.

3.9 Binds to the I Domain of CD11c and the Binding is Divalent Cation- or Activation-Dependent

The α chain of the CD18 family of integrins contain an I domain that mediates ligand binding. Structural and mutagenesis studies demonstrate that the MIDAS sequence, including the residues D-X-S-X-S within the I domain, is critical for binding to metal ions and ligands.[4] Utilizing purified recombinant wild-type CD11c I domain, a D138/A mutant, and the activating I314/A mutant,[8] we determined binding properties of the four mAbs to CD11c I domain. Plates were coated with the purified I domain protein and antibody binding was determined by ELISA. As shown in Figure 5, none of the I domain proteins bind the three mAbs, 496B, 496K, and BU15. In contrast, a distinct binding profile of the mAb 3.9 to the I domains was observed. The wild-type CD11c I domain bound to 3.9 in the presence of magnesium. Inclusion of EDTA severely reduced binding to the wild-type I domain. The D138/A mutant version of the I domain did not bind to 3.9 under any of the conditions tested. 3.9 bound readily to the I314/A activated mutant I domain in the presence of magnesium. Removal of divalent cations reduced binding by about 40%. These data demonstrate that 3.9 binding to CD11c I domain is highly influenced by the presence of divalent cation and/or activation status of the I domain.

To further characterize binding properties of 3.9 to CD11c I domain, we studied 3.9 binding to the wild-type and I314/A I domain proteins by Surface Plasmon Resonance (SPR). To avoid bivalent interactions, the mAb was immobilized on the sensor chip and serial dilutions of the I domain proteins were injected. Results are shown in Figure 6 and Table 1. 3.9 bound the wild-type protein in the presence of magnesium with a K_D of 2.13×10^{-9} M. Depletion of magnesium with EDTA reduced binding affinity by nearly 200-fold ($K_D = 1.7 \times 10^{-7}$ M), due mainly to an increase

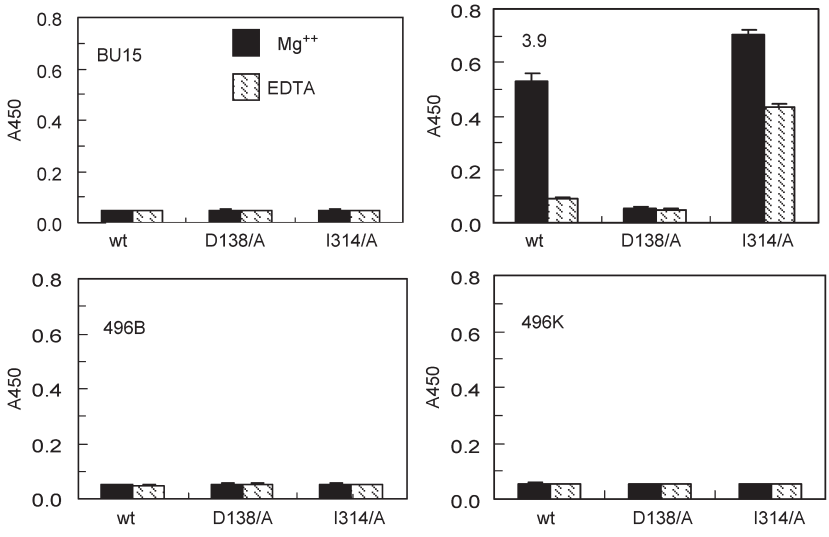

Figure 5. Recognition of CD11c I domains by mAbs. 96-well plates were coated overnight with purified CD11c I domain wild-type or the MIDAS mutant, D138/A, proteins, or the activating mutant I314/A (5 μg/mL). mAb binding to the I domain proteins was determined by ELISA as described in Experimental. Each panel shows binding data for the indicated mAb. One representative of at least three experiments is shown.

in the k_{off}. In contrast to binding the wild-type I domain, the mAb bound the mutant I domain in the presence of EDTA with a K_D that is similar to the wild-type protein binding in the presence of magnesium. However, mAb binding to the I314/A protein was stronger in the presence of magnesium ($K_D = 7.99 \times 10^{-10}$ M). These data corroborate the ELISA data and further demonstrate that the affinity of 3.9 toward the CD11c I domain can vary nearly 4,000-fold depending upon the binding conditions.

DISCUSSION

In the present study we have characterized the functions and binding properties of four human CD11c mAbs. Of the four mAbs, 496B activates CD11c-mediated cell adhesion to ICAM-1, and the 496B-mediated activation is additive to that of the inside-out integrin activator, PMA. Our data suggest that 496B-mediated activation of the integrin is independent of CD11c I domain, since it does not bind the isolated I domain. 496B bound to CD11c in the absence of divalent cations, and its epitope is constitutively expressed in neutrophils and monocytes (data not shown). Activating antibodies for other members of the CD18 integrins have been described. For example, the mAbs NKI-L16[15] and MEM-83[26] activate CD11a. Similarly, 6C1, a

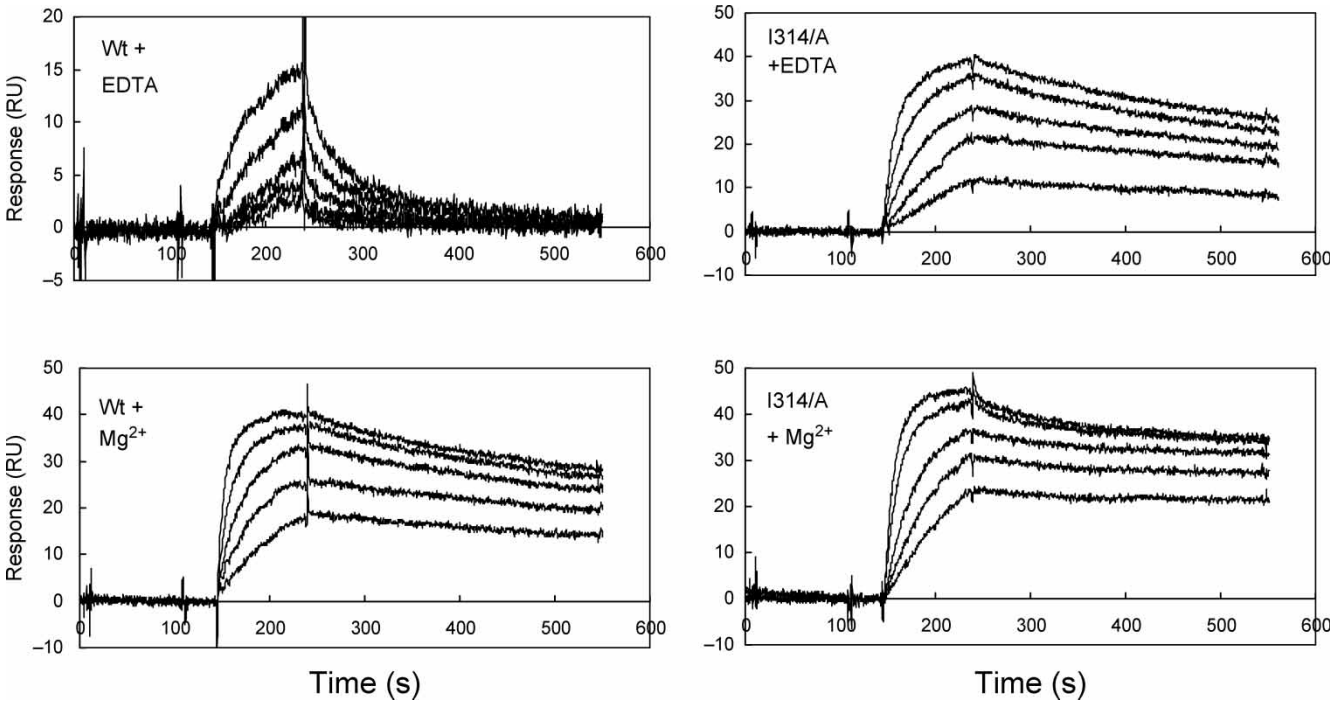

Figure 6. Surface plasmon resonance analysis of CD11c I domain binding to mAb 3.9. The wt or I314/A CD11c I domain was perfused onto 3.9-immobilized sensor chips in the presence of EDTA or $MgCl_2$ as indicated. The concentration of the I domains was 15.63, 31.25, 62.5, 125, and 250 nM.

Table 1. Kinetics of mAb 3.9 binding to CD11c I domain

I domain	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)
Wt + EDTA	7.36×10^4	1.25×10^{-2}	1.70×10^{-7}
Wt + $MgCl_2$	4.82×10^5	1.02×10^{-3}	2.13×10^{-9}
I314/A + EDTA	2.91×10^5	1.25×10^{-3}	4.29×10^{-9}
I314/A + $MgCl_2$	5.92×10^5	4.73×10^{-4}	7.99×10^{-10}

low affinity CD11b mAb, has been reported to activate the integrin.[27] Both MEM-83 and 6C1 recognize the I domains of the respective integrins.[27–28] In contrast, our data indicate that 496B does not appear to recognize CD11c I domain. This is similar to NKI-L16, which binds to the thigh domain of CD11a.[11] However, binding of NKI-L16 to CD11a requires divalent cations.[11] Although a small increase in 496B binding to CD11c was observed in the presence of EDTA by flow cytometry, 496B bound well to CD11c in the absence of the cations. We propose that 496B preferentially binds an inactive state and induces a conformational change resulting in the activation of a subpopulation of CD11c. Determination of the epitope of 496B should reveal important regulatory site(s) of CD11c.

Among the three blocking mAbs of CD11c studied here, BU15 and 496K bind in an activation- and divalent cation-independent manner. In experiments with neutrophils, 496K competed with BU15, while 3.9 did not compete with either (data not shown). Neither BU15 nor 496K bound the isolated I domain. This suggests that BU15 and 496K mediated inhibition of CD11c may be allosteric. The CD11a mAbs CBR LFA1/1 and YTA-1 have been reported to be allosteric inhibitors of CD11a binding to ICAM-1. CBR LFA1/1 binds the CD11a β propeller and YTA-1 binds an epitope consisting of the β propeller and the I-like domain of the β2 chain.[30] Important information on regulatory elements present in these regions of CD11a has been obtained through the determination of epitopes of these mAbs. Similarly, determination of BU15 and 496K epitopes on CD11c should provide information regarding CD11c regulatory sites.

We also describe several unique features of the CD11c mAb 3.9. Cell adhesion data demonstrate that binding of CD11c to 3.9 is enhanced upon activation of the integrin, and the mAb appears to recognize only a subset of the integrins present on the cell surface. Using domain exchange with CD11b and heterologous expression of the chimeric CD11b/CD11c molecules, 3.9 has been reported to bind the CD11c I domain.[31] The binding data with isolated I domain presented here is consistent with this binding site. Furthermore, we demonstrate that 3.9 binding to cell surface CD11c/CD18 and to the isolated wild-type CD11c I domain are dependent on the presence of divalent cations. In addition, presence of an intact MIDAS sequence is a requirement for 3.9 binding to the I domain. These features are similar to those of the CD11b antibodies 7E3[32] and CBRM1/5[17] as well as the recently described CD11a phage antibody, AL-57.[18,19] The murine CD11b mAb,

107 also displays ligand-mimetic features. However, in contrast to physiologic ligands, it binds preferentially to the low-affinity form of the integrin.[33] Interactions of these mAbs with their corresponding integrins parallel the respective integrin-ligand interactions in multiple ways, and hence these mAbs are portrayed as ligand-mimetic. Data presented here demonstrate some of these ligand-mimetic features in the binding profiles of CD11c to 3.9. However, the activating mutation of the CD11c I domain alleviated the divalent cation requirement of 3.9 binding to the integrin. This raises the possibility that for the wild-type I domain, 3.9 may recognize an open conformation generated by divalent cations such as magnesium.[4] Based on these properties, 3.9 may be considered a CD11c I domain activation-specific mAb.

In the SPR experiments, 3.9 displayed weakest binding to the wild-type I domain in the presence of EDTA ($K_D = 1.70 \times 10^{-7}$ M), intermediate binding to the wild-type I domain in the presence of magnesium or to the I314/A I domain in the presence of EDTA ($K_D = 2.13 \times 10^{-9}$ M and 4.29×10^{-9} M respectively). Strongest binding was observed to the I314/A I domain in the presence of magnesium ($K_D = 7.99 \times 10^{-10}$ M). Based on this data we hypothesize that the CD11c I domain can exist in multiple conformations. For CD11a and CD11b I domains, existence of at least three conformations with respect to ligand binding (closed, intermediate, and open) have been demonstrated experimentally.[10] The amino acid residues that are implicated in maintaining these conformations in CD11b I domain are perfectly conserved in the CD11c I domain (V297, F300, and L303) further strengthening our hypothesis. The intermediate affinity I domain of CD11a has been demonstrated to support firm adhesion and cell arrest in the context of extended α and β subunits.[34] More recently, electron microscopic studies have demonstrated conversions of bent to extended forms of CD11a and CD11c.[9] It will be interesting to determine the functional attributes of the different conformations of the CD11c I domain in the context of the extended integrin.

CONCLUSION

Here we present data describing three functional classes of human CD11c mAbs: 1) an allosteric agonistic mAb, 496B, 2) activation-independent and allosteric antagonistic mAbs, 496K and BU15, and 3) activation-specific and antagonistic mAb, 3.9. Determination of the epitopes of these mAbs is likely to reveal regulatory elements on CD11c. Furthermore, the features of 496B and 3.9 offer novel means to investigate CD11c functions.

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