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Characterization of single amino acid substitutions in the β2 integrin subunit of patients with leukocyte adhesion deficiency (LAD)-1

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Abstract

Leukocyte Adhesion Deficiency 1 (LAD-1) is caused by defects in the β2 integrin subunit. We studied 18 missense mutations, 14 of which fail to support the surface expression of the β2 integrins. Integrins with the β2-G150D mutation fail to bind ligands, possibly due to the failure of the α1 segment of the βI domain to assume an α-helical structure. Integrins with the β2-G716A mutation are not maintained in their resting states, and the patient has the severe phenotype of LAD-1. The β2-S453N and β2-P648L mutants support the expression of integrins and adhesion functions. They should be re-classified as polymorphic variants.

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Keywords

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β2 (CD18) Integrin
Expression
Cell Adhesion

Abbreviations

LAD-1, Leukocyte adhesion deficiency-1; LFA-1, Leukocyte function associated antigen-1 (αLβ2); Mac-1, Macrophage antigen-1 (αMβ2); ICAM, Intercellular adhesion molecule; BSA, Bovine serum albumin; WT, Wild-type, NMR, Nuclear magnetic resonance

Highlights

- Complete the functional characterization on LAD-1 missense mutations.
- Systematic analysis of integrin adhesion using two-sample Hotelling's T-square test.
- G150D highlights the importance of α1 helix conformation in the βI domain with respect to ligand binding.
- Summarize *in vitro* characteristics related to all LAD-1 missense mutations identified to date in Table 1.

1. Introduction

Leukocyte Adhesion Deficiency-1 (LAD-1) is caused by defects in the *ITGB2* gene which codes for the β2 integrin subunit, resulting in the depressed expression of the β2 leukocyte integrin family members, the αLβ2, αMβ2, αXβ2 and αDβ2 integrins, or the expression of their non-functional forms. The αLβ2 integrin is also referred to as the leukocyte function associated antigen-1 (LFA-1), and CD11a/CD18 in the leukocyte antigen nomenclature; and similarly, αMβ2 the macrophage antigen-1 (Mac-1) and CD11b/CD18; αXβ2 the p150,95 antigen and CD11c/CD18; and αDβ2 the CD11d/CD18 antigen. The leukocytes of patients with LAD-1 are defective in various adhesion functions, including the migration from blood to tissue sites of infection and inflammation. Consequently, the patients suffer from recurrent bacterial and fungal infections. Depending on the severity of the disease, may die at an early age [1-3].

In 2012, van de Vijver et al updated the mutant alleles leading to LAD-1 [4]. They include 23 deletions, 2 insertions, 4 deletion/insertions, 10 nonsense mutations, 12 splice site mutations, and 35 missense mutations . Whereas the mutations in the first five categories invariably lead to the non-expression of the β2 integrins, the missense mutations could result in many molecular phenotypes. Most of them do not support integrin heterodimer expression, others support normal expression levels of integrin heterodimers which are non adhesive, yet others support subnormal level of integrin heterodimers with residual adhesion functions, and a few mutations support the expression of integrins in their non-resting states. Seventeen of the 35 missense mutations were reported previously for their capacity to support of integrin heterodimer expression, and their adhesion functions. More detailed information can

be found in the summary Table 1 [2, 5-29]. In this article, we complete this line of investigation on the remaining 18 missense mutations.

The location of the 18 missense mutations is shown in the schematic map in Figure 1. The alignment of these variants to the local sequences of the 8 human β integrin subunits is shown in Figure 2. The numbering of the amino acids of the polypeptide is such that “1” is for the initiation methionine.

2. Materials and methods

2.1. Antibodies and reagents

Hybridoma of mAbs were obtained from different sources: MHM24 (anti-αL blocking mAb) and MHM23 (β2 heterodimer specific mAb) [30] and H52 (β2-specific mAb) [31] were provided by Professor AJ McMichael (John Radcliff Hospital, Oxford, UK). KIM185 (anti-β2 activating mAb) [32] was obtained from Dr. MK Robinson (UCB, CellTech, UK). LPM19C (anti-αM blocking mAb) [33] and 1B4 (function blocking mAB, β2 heterodimer specific) [34] were purchased from ATCC. All mAb were purified from hybridoma supernatant using Hi-Trap protein G or A columns (Amersham Pharmacia Biotech). Preparation of recombinant human ICAM-1-Fc and ICAM-3-Fc was described previously [35]. Bovine serum albumin (fraction V, BSA) was purchased from Sigma-Aldrich.

2.2 cDNA expression plasmids

The pcDNA3 expression plasmids containing αL, αM, αX and β2 cDNA were described previously [22]. LAD-1 mutations were generated using site-directed

mutagenesis kit (Stratagene) following the manufacturer's protocols. All constructs were verified by sequencing (1st Base, Singapore).

2.3. Cell culture and transfection

Human embryonic kidney 293T (HEK293T) cells were purchased from ATCC, cells were cultured in DMEM (Hyclone) supplemented with 10% (v/v) heat-inactivated FBS (Hyclone), 100 IU/ml penicillin and 100 µg/ml streptomycin (Hyclone) at 37°C in 5% CO_2 humidified incubator. HEK293T cells were transfected with expression plasmids using Polyethylenimine (PEI) according to a previously described method [36].

2.4. Flow cytometry

Surface expression of the β2 integrins was determined by flow cytometry on a FACS caliber (BD Bioscience) [10] with the heterodimer specific mAb MHM23 [5,6]. detection was described previously [35]. Data were analyzed using Flowjo (Tree Star).

2.5. Cell adhesion assay

For ICAM-1 and ICAM-3 adhesion, 100 µl of 5 µg/ml goat anti-human IgG (Fc specific) (Sigma-Aldrich) in bicarbonate buffer (50 mM, pH 9.2) was introduced into each well in 96-well microtiter plates (Polysorb, Nunc Immno-Plate) and left overnight at 4°C. Non-specific sites were blocked with 130 µl of 0.5% (w/v) BSA in PBS for each well. 50 µl of ICAM-1-Fc or ICAM-3-Fc in PBS with 0.1% BSA (w/v) at 1 µg/ml was added into each well and left at RT for 2 hours. For BSA adhesion assay, 100 µl of 100 µg/ml BSA in bicarbonate buffer was coated on each well and

the plate was left overnight at 4°C. Non-specific sites were blocked with 130 µl of 0.2% (w/v) polyvinylpyrrolidone (PVP) in PBS for each well. In all cases the wells were washed twice with wash buffer (RPMI1640 with 10 mM HEPES and 5% v/v FBS) before adding cells. Transfected cells were harvested, washed and incubated with 1 µg/ml BCECF (Invitrogen) in wash buffer at 37°C for 20 min. Labeled cells were transferred to each wells with or without activation reagents (Mg^{2+}/EGTA: 5 mM $MgCl_2$ and 1.5 mM EGTA; Mn^{2+}: 0.5 mM $MnCl_2$; mAb KIM185: 10 µg/ml) and incubated at 37°C for 30 min. To establish specificity, appropriate antibodies were added to block adhesion. Unbound cells were removed by washing twice with wash buffer. Fluorescence signal, which correlates with the number of cells adhering to the ligand-coated well, was measured using a FL600 fluorescence plate reader (Bio-Tek instruments, Winooski, VT).

2.6. Peptide synthesis

Peptides were synthesized using 9-fluorenyl-methyloxycarbonyl (FMOC) solid-phase chemistry (Liberty Microwave Peptide Synthesizer, CEM North Carolina, USA) and Tentagel amide resin (Intavis AG, Germany). The peptides were cleaved from the resin with trifluoroacetic acid (TFA). The lyophilized peptides were dissolved in a water-acetonitrile mixed solvent, injected into a C18 HPLC column, and eluted with a linear gradient of water/acetonitrile, from 0% to 100% acetonitrile. Peptide purity was confirmed by electrospray ionization (ESI) mass spectrometry. The purified peptides were lyophilized overnight after the addition of 10 mM HCl, to remove possible TFA adducts.

2.7. NMR data collection and structure calculation

NMR experiments were performed at 298K using an Avance-II 700 NMR spectrometer with cryogenic probe. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as the internal reference for ^{1}H nuclei. NMR data were processed using TopSpin 3.1 (www.bruker-biospin.com) and analyzed using CARA (www.nmr.ch). Sequence-specific resonance assignments of the peptides were achieved from two-dimensional total correlation spectroscopy (TOCSY) and nuclear Overhauser effect spectroscopy (NOESY) spectra acquired in PBS buffer containing 0.5 mM glucose at pH 7.4. The peptide concentrations were 1 mM, and mixing times were 80 and 200 ms for TOCSY and NOESY, respectively. For structure calculation, NOE distance restraints were obtained from NOESY spectra. The short-range and medium range NOE connectivities were used to establish the sequence-specific ^{1}H NMR assignment and to identify elements of regular secondary structure. All the restraints used in the calculations are listed in Supplementary Tables 1 (β2 peptides) and 2 (β3 peptides). Structure calculations were performed using CYANA 3.0 [37, 38] and visualized using PyMOL (Delano Scientific). A total of 10 structures were calculated and the structure statistics are summarized in the Supplementary Tables.

2.8. Statistics

Adhesion profile of each mutant was compared to that of its corresponding wild-type. Each profile consisted of at least three conditions, which are highly correlated with one another. In order to study all conditions simultaneously, a multivariate two-sample comparison, Hotelling's T^2 test was conducted. This test is a multivariate analog of the ordinary t-test in the univariate statistics and extends the idea of the t-test to analyze statistical significance holistically for the combined set of conditions. Multivariate statistical methods have become increasingly popular and desirable in

the analysis of data from complex biological assays [39, 40]. It has the unique advantages of taking variant factors simultaneously and is capable of describing differences between graph patterns, which are the major concerns in this characteristic work aiming to understand the function profiling under comprehensive conditions for each of the mutants.

The binding values of conditions in Fig. 4A, 4C, 4D (namely sham, Mg^{2+}/EGTA or Mn^{2+}, KIM185,) were treated as a 3-dimensional vector. In Fig. 4B, one additional condition (Mg^{2+}/EGTA plus KIM185) returned a vector of 4 dimensions. A comparison was made between a group of vectors (triplet or quadruplet) of conditions for each mutant and that for the corresponding wild-type. Note that there was no evidence that assumption of homogeneous variance-covariance matrices was violated for all tests. Data were analyzed using SAS (statistical analysis system) software. It should be noted that only the data obtained without the blocking antibodies, which were used to establish binding specificity, were analyzed.

3. Results and Discussion

Each of the 18 mutations was introduced into a wild-type β2 integrin cDNA expression plasmid. These plasmids were co-transfected into HEK293T cells with the expression plasmids of the αL, αM, or αX subunit. Surface expression of the αLβ2, αMβ2 and αXβ2 integrins was determined by flow cytometry using the heterodimer specific mAb MHM23. The results on αLβ2 expression are shown in Figure 3. Four of the missense mutations G150D, S453N, P648L, and G716A supported the heterodimer expression of αLβ2. The αMβ2 and αXβ2 expression

profiles with the 18 β2 mutants are similar to those of αLβ2. They are shown in the Supplementary Figure A1. The 14 mutations which did not support heterodimer surface expression were capable in forming CD18 protein as tested by anti- β2 conformational mAb H52 and KIM185 after cell permeabilzation (data not shown).

The four mutants that support heterodimer expression were subjected to adhesion analyses. Normally, the wild-type αLβ2 would require either the presence of Mg^{2+}/EGTA or the activating mAb KIM185 for adhesion to ICAM-1, but would require the presence of both reagents to adhere to ICAM-3 [26, 41].

Tansfectants expressing αLβ2 of the S453N, and P648L mutations showed wild-type adhesion properties to ICAM-1 and ICAM-3. Cells expressing αLβ2 with G150D showed no adhesion to either ligand on all conditions (Figure 4A, B). Cells expressing αLβ2 with G716A showed similar adhesive property to ICAM-1 as the wild-type (no significant difference at the level of 0.05). However, the difference in adhesion appears more clearly in the binding to ICAM-3, in which αLβ2- G716A required only Mg^{2+}/EGTA or KIM185 to adhere to ICAM-3 (Figure 4B), suggesting that the overall adhesion property of αLβ2 with G716A is different from (more adhesive than) that of the wild-type.

Similarly, cells expressing αMβ2 and αXβ2 with the S453N, and P648L mutations were wild-type like in their adhesion to denatured BSA; whereas those with G150D showed no adhesion to the ligand. The distinction between the adhesion profiles of αMβ2 and αXβ2 with G716A, in comparison to their respective wild-types, is significant (Figure 4C, D).

3.1. S453N and P648L

The two proposed mutations, S453N and P648L, were reported in two individuals but their LAD-1 status was not very definitive. The two mutations were identified by sequencing genomic DNA exons and splicing sites of introns, no other mutation could be identified. With the results presented here, it is likely that they represent rare polymorphisms found in the *ITGB2* gene. In fact, recent statistics shows that S453N has a frequency of ~0.2% in European cohorts (RS#13865490).

3.2. G150D

The G150D mutation was identified in a patient born to unrelated parents. He has decreased surface expression of the β2 integrins on his neutrophils. The patient is a compound heterozygote, the other mutation is P178L. It was previously reported that the P178L mutation in the β2 integrin subunit does not support integrin heterodimer expression of the αLβ2, αMβ2 and αXβ2 integrins [19]. Thus the expression of the integrins on his leukocytes is due to the G150D allele. Our experimental results are in agreement with this finding. Two other mutations, S138P [12] and D231H [20], were reported previously to be capable to support heterodimer expression but not adhesion. S138 is the coordinating residue of the divalent cation binding motif MIDAS in the βI domain [42], and D231 that of the synergistic metal ion binding site SyMBS [43], and may therefore account for the lack of adhesion functions of these mutants. G150 is also located in the βI domain. In the closed and bent structure of the αXβ2 ectodomain, the α1 and α1’ helices of the βI domain are discontinuous, and G150 is located at the N-terminal of the α1’ helix [43]. Upon activation, the α1

helix of αXβ2 become extended and the G150 residue is inside the extended helix [44] (see Figure 5A and B).

We synthesized the β2 peptides spanning the α1 and α1' helices with the wild-type glycine or the mutant aspartic acid. NMR analysis showed that whereas the wild-type peptide with the glycine is a continuous helix, the one with the aspartic acid is not (Figure 5C and D). We may therefore tentatively argue that the aspartic acid would prevent the β2 integrins to assume its active state, resulting in integrins with no adhesion functions. However, such conclusion can only be established if the entire ectodomain structures of the wild-type and mutant integrin can be compared.

3.3. G716A

G716A is the first LAD-1 mutation found in the β2 transmembrane region. In a study on the effect of various residues in the transmembrane region by phenylalanine scanning, it was found that the mutation of this glycine to a phenylalanine would confer an intermediate activation state to the αLβ2 integrin. By model building, G716 of β2 faces the L1104 of αL in the αLβ2 heterodimer, and this orientation is also consistent with later NMR titration experiments of αL TM with β2 TM in micelles [45].

The replacement of G716 with a phenylalanine would have two bulky residues facing each other, resulting in the leg separation of the integrin, and its promotion to the next activation state [46]. In this study, we showed that by changing the glycine to an alanine is sufficient to cause activation. It seems that the proper packaging of

the transmembrane region at this site is necessary to maintain LFA-1 in the resting state.

Perhaps the more curious observation is why such mutations would confer LAD-1 to the patients, in particular with the very low expression of the β2 integrins on the patients' leukocytes. There were two cases reported previously. Similar to G716A, the C590R and R593C mutations were able to support the expression αLβ2 integrin, in an expression system using COS-7 cells, at the intermediate activation state [22]. However, unlike G716A, these two mutations do not support the expression of the αMβ2 and αXβ2 integrins in this artificial expression system. Recently, it was reported in a patient with the classic leukocyte adhesion deficiency symptoms including delayed umbilical cord separation and recurrent bacterial infection, but does not have the mutations in the genes associated with LAD-1 and LAD-II. His normal platelet function also excludes him for having LAD-III. Interestingly, his leukocytes were found to be hyperadhesive and slow in migration [47]. This report is therefore in line with our observation that LAD-1 patients expressing hyperadhesive integrins could display the symptoms of leukocyte adhesion deficiency.

4. Conclusion

We have characterized 16 novel missense mutations in the β2 integrin subunit. We have also shown that the mutation status of the S453N and P648L has to be re-evaluated and they are likely to be rare polymorphisms of the *ITGB2* gene. The mutants with the G150D and G716A have provided novel insights in the regulation of the structure and functions of the β2 integrins. A table that listed the *in vitro*

characteristics of all 35 LAD-1 missense mutations (including 16 in this work) was summarized (Table 1).

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Appendix A. Supplementary data

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Figure Legends

Fig. 1. The location of the LAD-1 mutations to the various domains of the β2 integrin subunit.

Fig. 2. Local sequence alignment of the 8 human integrin subunits flanking the mutated residues. The mutated residues are shown in bold at the top of the aligned sequences.

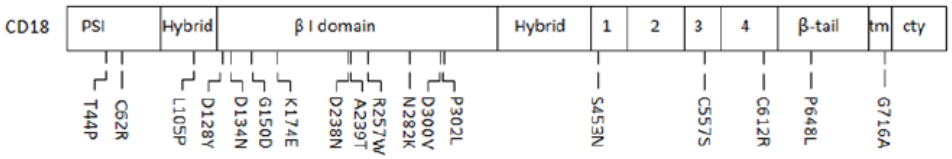
Fig. 3. Flow cytometry analysis of the surface expression of αLβ2 (LFA-1) transfectants with the various mutations. The mAb used was MHM23 which is specific for the β2 integrin heterodimers [30]. The background histograms were obtained with LPM19c (αM-specific).

Fig. 4. Adhesion of αLβ2 transfectants to ICAM-1 (A), and ICAM-3 (B). The forward bars were adhesion in the presence of the function-blocking mAb MHM24. Adhesion of αMβ2 (C) and αXβ2 (D) to denatured BSA. The forward bars were adhesion in the presence of the function-blocking mAb LPM19c (C) and 1B4 (D) respectively. In all cases, conditions of “no activating reagent” are showed by white bars, conditions with Mg^{2+}/EGTA

(for αLβ2 adhesion) or Mn^{2+} (for αMβ2 and αXβ2 adhesion) showed by grey bars, conditions with KIM185 showed in black, and conditions with Mg^{2+}/EGTA together with KIM185 showed in hatched bars. The significant level was set at 0.05: ns (not significant); * (p-value < 0.05).

Fig. 5. The resting (A) and active (B) structure of the βI domain of the αXβ2 integrin. The resting state was that of PDB ID: 3K6S (from [43]) and the active state was that of PDB ID: 4NEH (from [44]). The location of G150 was shown to be at the top of α1' helix in the resting state but in the extended α1 helix in the active state. Coordinating residues of SyMBS were labeled in yellow, that of MIDAS were labeled in magenta and that of ADMIDAS were labeled in white. The NMR structures of the β2 peptides spanning the α1 and α1' helices "SMLDDLRNVKKL(G/D)GDLLRALNEI" are shown with the wild-type glycine in (C) and the mutant aspartic acid in (D).

Fig. 1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β I domain
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cty
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G150D
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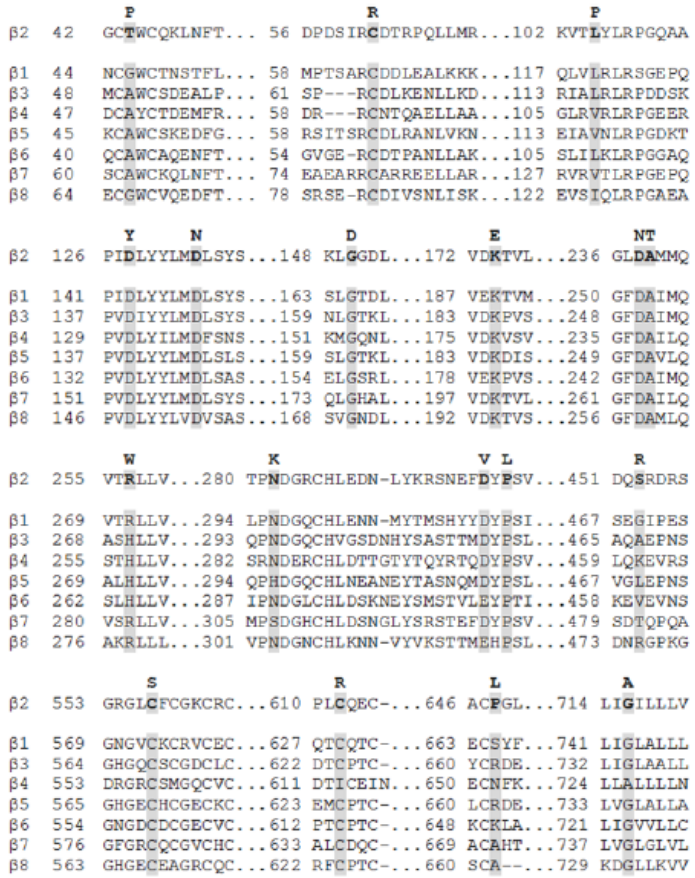
C557S
C612R
P648L
G716A

Fig. 2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β3 48 MCAWCSDEALP... 61 SP---RCDLKENLLKD...113 RIALRLRPDDSK
β4 47 DCAYCTDEMFR... 58 DR---RCNTQAELLAA...105 GLRVRLRPGEER
β5 45 KCAWCSKEDFG... 58 RSITSRCDLRANLVKN...113 EIAVNLRPGDKT
β6 40 QCAWCAQENFT... 54 GVGE-RCDTPANLLAK...105 SLILKLRPGGAQ
β7 60 SCAWCKQLNFT... 74 EAEARRCARREELLAR...127 RVRVTLRPGEPQ
β8 64 ECGWCVQEDFT... 78 SRSE-RCDIVSNLISK...122 EVSIQLRPGAEA
Y N D E NT
β2 126 PIDLYYLMDLSYS...148 KLGGDL...172 VDKTVL...236 GLDAMMQ
β1 141 PIDLYYLMDLSYS...163 SLGTDL...187 VEKTVM...250 GFDAIMQ
β3 137 PVDIYYLMDLSYS...159 NLGTKL...183 VDKPVS...248 GFDAIMQ
β4 129 PVDLYILMDFSNS...151 KMGQNL...175 VDKVSV...235 GFDAILQ
β5 137 PVDLYYLMDLSLS...159 SLGTKL...183 VDKDIS...249 GFDAVLQ
β6 132 PVDLYYLMDLSAS...154 ELGSRL...178 VEKPVS...242 GFDAIMQ
β7 151 PVDLYYLMDLSYS...173 QLGHAL...197 VDKTVL...261 GFDAILQ
β8 146 PVDLYYLVDVSAS...168 SVGNDL...192 VDKTVS...256 GFDAMLQ
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β1 269 VTRLLV...294 LPNDGQCHLENN-MYTMSHYYDYPSI...467 SEGIPES
β3 268 ASHLLV...293 QPNDGQCHVGSDNHYSASTTMDYPSL...465 AQAEPNS
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β6 262 SLHLLV...287 IPNDGLCHLDSKNEYSMSTVLEYPTI...458 KEVEVNS
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S R L A
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β1 569 GNGVCKCRVCEC...627 QTCQTC-...663 ECSYF...741 LIGLALLL
β3 564 GHGQCSCGDCLC...622 DTCPTC-...660 YCRDE...732 LIGLAALL
β4 553 DRGRCSMGQCVC...611 DTICEIN...650 ECNFK...724 LLALLLLN
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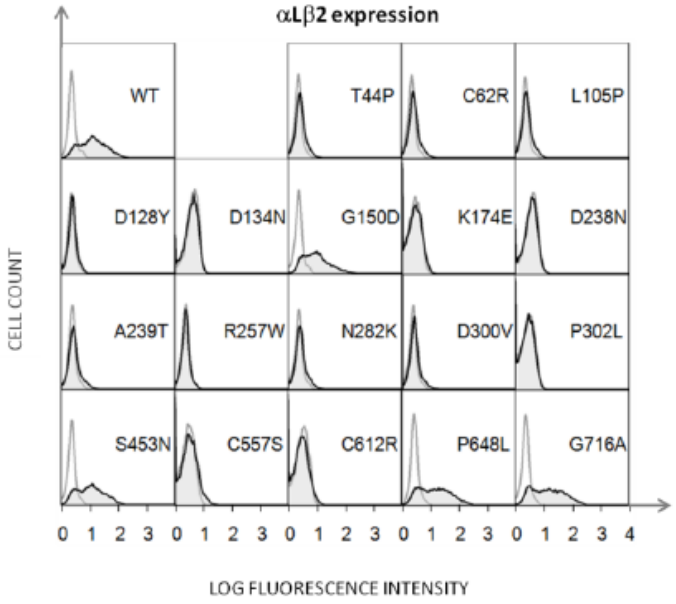
β6 554 GNGDCDCGECVC...612 PTCPTC-...648 KCKLA...721 LIGVVLLC
β7 576 GFGRCQCGVCHC...633 ALCDQC-...669 ACAHT...737 LVGLGLVL
β8 563 GHGECEAGRCQC...622 RFCPTC-...660 SCA--...729 KDGLLKVV

Fig. 3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C62R
L105P
D128Y
D134N
G150D
K174E
D238N
A239T
R257W
N282K
D300V
P302L
S453N
C557S
C612R
P648L
G716A
CELL COUNT
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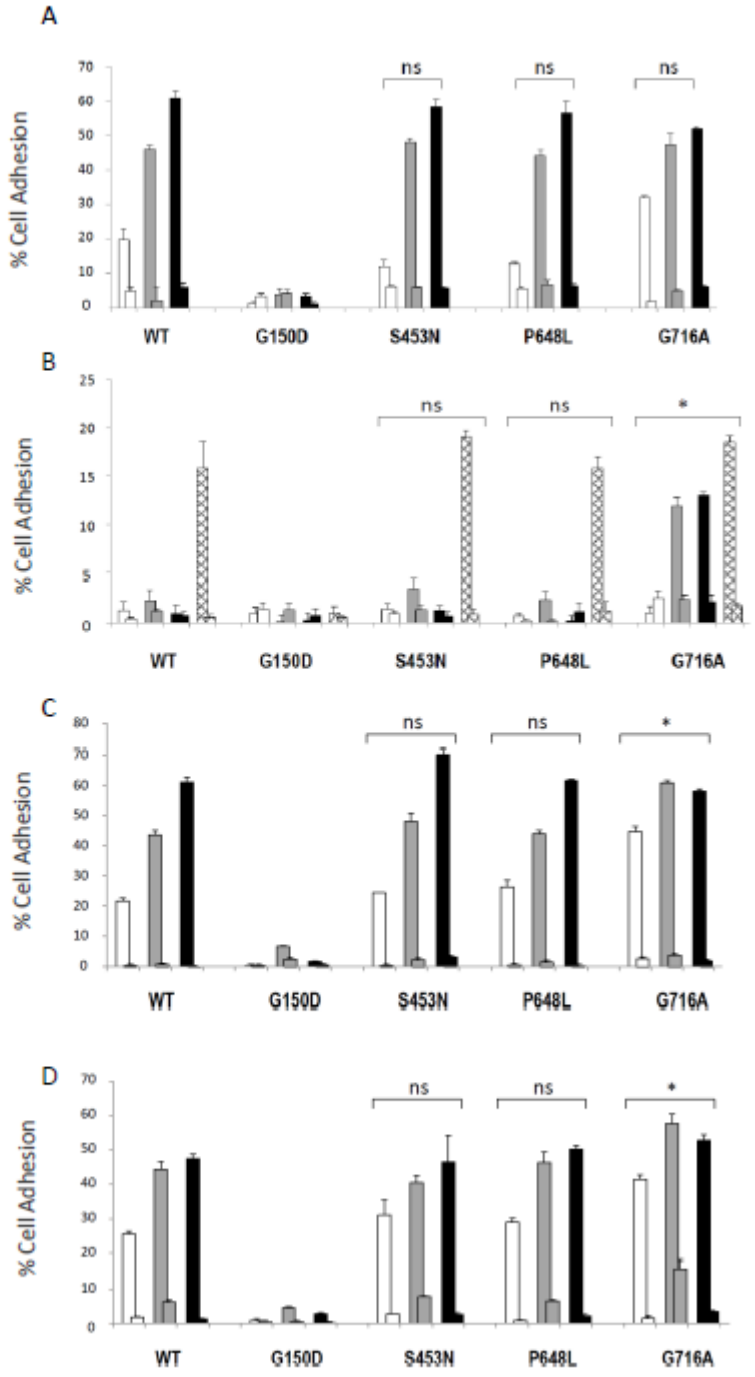
LOG FLUORESCENCE INTENSITY

Fig. 4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ns
ns
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WT
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B
% Cell Adhesion
ns
ns
*
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G150D
S453N
P648L
G716A
C
% Cell Adhesion
ns
ns
*
WT
G150D
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WT
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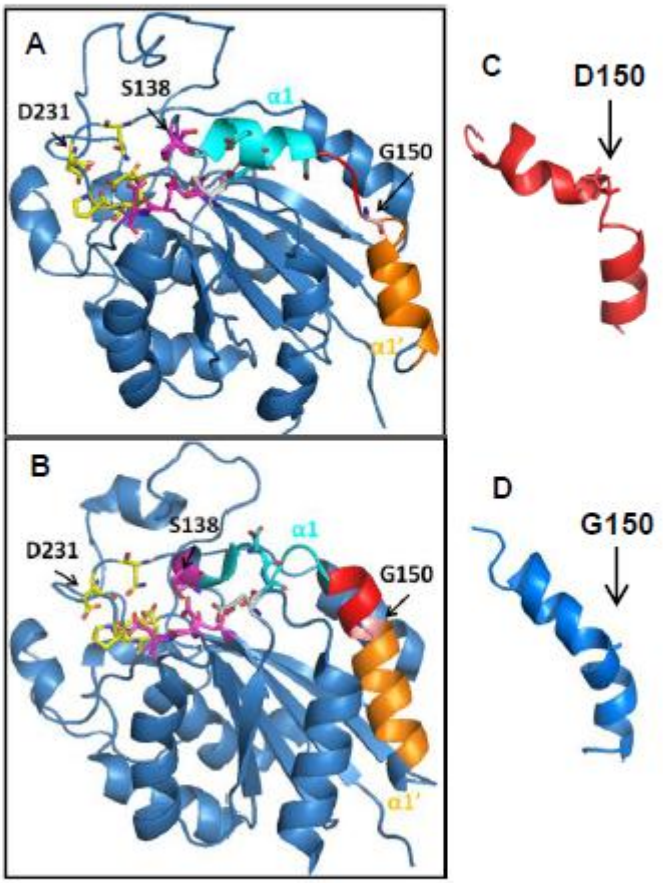
G716A

Fig. 5



Table

Mutation	Domain	Cons[a]	*Expression*	*Adhesion*	Ref.
M1K	PSI	8	ND[d]		[5]
C36S	PSI	8	Diminished expression of αLβ2; αMβ2 and αXβ2	Constitutive αLβ2 adhesion to ICAM-1	[4] [6]
T44P[b]	PSI	1	No		[4]
C62R[b]	PSI	8	No		[4]
L105P[b]	Hybrid	4	No		[4] [7]
D128N	βI	8	ND[d]		[8]
D128Y[b]	βI	8	No		[4] [9]
Y131S	βI	8	No		[10]
D134N[b]	βI	8	No		[4] [11]
S138P	βI	8	Normal	No adhesion function	[12]
L149P	βI	6	No		[13] [14]
G150D[b]	βI	8	Normal	No adhesion function	[15]
G169R	βI	8	No		[13] [16]
K174E[b]	βI	8	No		[4]
P178L	βI	8	No		[17] [18] [19]
D231H	βI	8	Normal αLβ2 expression, diminshed αMβ2 and αXβ2 expression	No adhesion function	[20]
D238N[b]	βI	8	No		[4]
A239T[b]	βI	8	No		[9]
W252R	βI	8	No		[21]
R257W[b]	βI	4	No		[4]
A270V	βI	6	No αLβ2 expression, diminished αMβ2 and αXβ2 expression	No adhesion function	[22]
G273R	βI	6	No		[12]
N282K[b]	βI	6	No		[4]
G284S	βI	7	No		[10] [14]
D300V[b]	βI	6	No		[4] [23]
P302L[b]	βI	8	No		[4]
A341P	βI	3	Detectable expression for αLβ2, no expression for αMβ2 and αXβ2	Detectable adhesion on ICAM-1 with αLβ2	[22]
N351S	βI	8	Normal αLβ2 expression and diminished αMβ2 and αXβ2 expression[e]	Active αLβ2 adhesion to ICAM-1 and ICAM-3. Diminished adhesion properties of αMβ2 and αXβ2[e]	[6] [24] [25] [26]
S453N[b,c]	IEFG-1	1	Normal	Normal	[4]
C557S[b]	IEFG-3	8	No		[4]
C590R	IEFG-4	8	Diminished αLβ2 and αXβ2 expression, no αMβ2 expression	Constitutive αLβ2 adhesion to ICAM-1, minimal adhesion for αMβ2 and αXβ2	[22]
R593C	IEFG-4	7	Diminished αLβ2 and αXβ2 expression, no αMβ2 expression	Constitutive αLβ2 adhesion to ICAM-1, minimal adhesion for αMβ2 and αXβ2	[2] [22] [27]
C612R[b]	IEFG-4	8	No		[4] [28] [29]
P648L[b,c]	β tail	1	Normal	Normal	[4]
G716A[b]	TM	7	Normal	Constitutive αLβ2 adhesion to ICAM-1, elevated adhesion for αMβ2 and αXβ2 to denatured BSA	[4] [9]

a: Conservation among the 8 human integrin β subunits

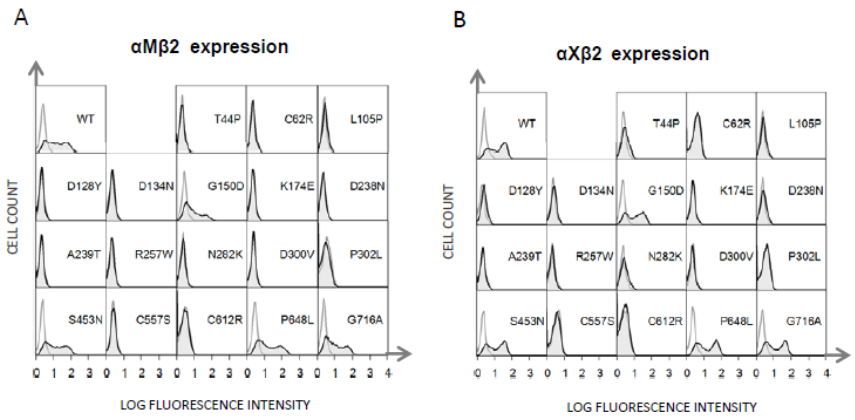
b: This study

c: Most likely rare polymorphisms

d: ND, not done

e: S.K.A. Law, unpublished result

Supplementary Figure A1



Supplementary Figure A1. Flow cytometry analysis of the surface expression of (A) CD11b/CD18 (Mac-1); and (B) CD11c/CD18 (p150,95) transfectants with the various mutations. The mAb used was MHM23 which is specific for the CD18 integrin heterodimers [30]. The background histograms were obtained with MHM24 (CD11a-specific).