BINDING BIOMECHANICS OF RECOMBINANT HUMAN BETA 2 INTEGRIN

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Summary LFA-1 and Mac-1 integrins play distinct roles in mediating the leukocyte slow rolling, firm adhesion and crawling by binding to intercellular adhesion molecule-1 (ICAM-1) ligand. To elucidate the underlying mechanisms, the ICAM-1 binding kinetics was compared between LFA-1 and Mac-1 molecules reconstructed with different affinity states, and corresponding micro-structural dynamics were investigated. Our data indicated that the affinity up-regulation from WT to HA is off-rate dependent for LFA-1 but on-rate dependent for Mac-1, which is consistent with conformational difference between LFA-1 and Mac-1. These results indicated that the binding kinetics of β_2 integrin is associated with structural features for their biological functions.

INTRODUCTION

LFA-1 ($\alpha_L\beta_2$, CD11a/CD18) and Mac-1 ($\alpha_M\beta_2$, CD11b/CD18) are two members of β_2 integrin subfamily and share common β_2 subunit noncovalently associated with respective α subunit. They are constitutively expressed on neutrophils and mediate various stages of leukocyte recruitment cascade by binding to the same ligand of ICAM-1. The slow rolling and firm adhesion of leukocytes is dependent on LFA-1 while the crawling is dependent on Mac-1[1]. We hypothesized that their distinct roles are likely attributed to the differences in the binding kinetics to ICAM-1 or in the diverse responses of outside-in and inside-out signaling. In this study, we compared the ICAM-1 binding features between soluble LFA-1 and Mac-1 with different affinity conformation. Also investigated were their corresponding conformational dynamics, which govern the binding kinetics. The outcomes would provide an insight in understanding the molecular mechanism for their cooperative functions and structure-function relationship.

METHODS

Fc-fused wild type low affinity (*LA*) human LFA-1 and Mac-1 molecules and their high affinity (*HA*) conformation mutants (LFA-1-K287C/K294C and Mac-1-Q163C/Q309C) were constrcuted[2]. Recombinant proteins were harvested *via* Fc domain using secondary antibody-coated 3μm-diameter beads and human ICAM-1 was physically adsorbed to 6 μm-diameter beads. Their binding affinity to ICAM-1 ligand was quantified respectively upon adhesion frequency assay using optical trap assay. The micro-structural dynamics of the complex were tested using molecular dynamics simulations (MDS) by comparing the conformational stability of LFA-1/Mac-1 α subunit I domain and the interaction between I domain-ICAM-1 D1/D3 domain.

RESULTS AND CONCLUSION

Recombinant β_2 integrin-Fc fusion molecules were identified using Western blotting analysis[3] (Fig. 1). Binding kinetics measurements indicated that the affinity difference between *HA* and *LA* is off-rate dependent for LFA-1 but on-rate dependent for Mac-1 (Fig. 2), which is presumably related to their distinct roles in initiating the leukocyte recruitment. These results were also consistent with MDS simulations in the two aspects from on-rate and off-rate viewpoints: 1) the binding "pocket" keeps accessible to the ligand for LFA-1 but switches from close to open for Mac-1 when the receptor transits from *LA* to *HA* state, suggesting that Mac-1 binding could be on-rate dependent; 2) forced lifetime of LFA-1-ICAM-1 bond is significantly enhanced when LFA-1 transits from *LA* to *HA* state, implying that the interaction might be off-rate related[4] (Fig.

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3). This work furthers the understandings in their function differences and structure-function relationship.

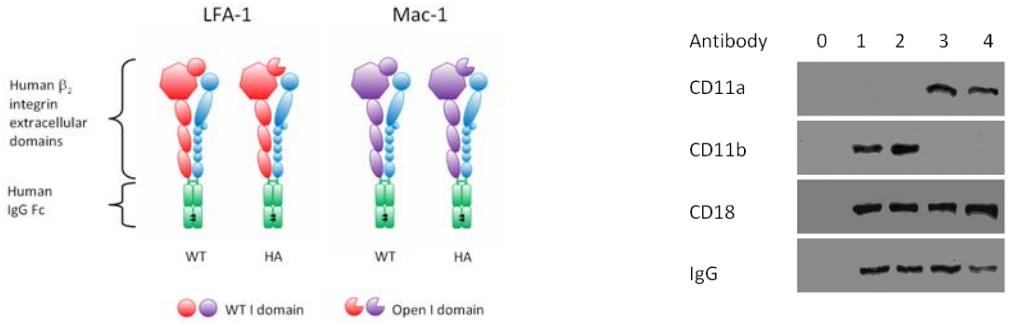

Figure 1. Schematic of soluble human β_2 integrin-Fc fusion proteins (left) and Western blotting analysis (right). Lanes 0, 1, 2, 3, and 4 were denoted as Mock, *LA* Mac-1, *HA* Mac-1, *LA* LFA-1, and *HA* LFA-1, respectively.

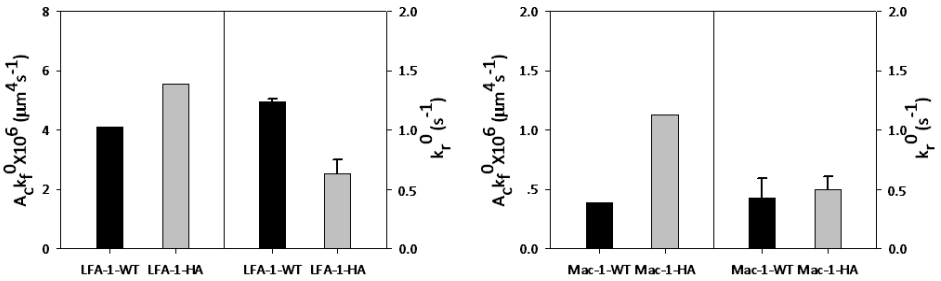

Figure 2. Kinetic parameters of LFA-1 and Mac-1 to ICAM-1. $A_c k_f^0$ and k_r^0 are the lumped on-rate and off-rate, respectively.

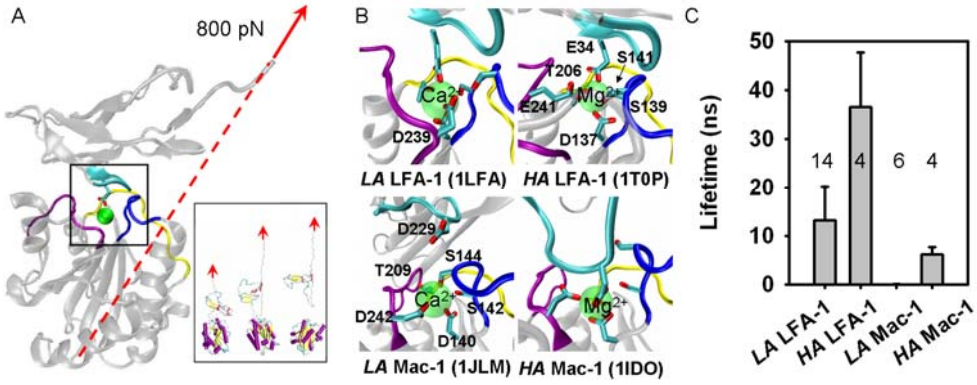

Figure 3. Key interactions between LFA-1/Mac-1 I domain and ICAM-1 D1/D3 domain (B) and bond lifetime (C) estimated *via* SMD simulation with 800-pN constant force pulling C-terminal of ICAM-1 D1 or D3 domain (A).

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