INTRODUCTION  Firm leukocyte adhesion and transendothelial migration during inflammatory responses are largely mediated by the interaction of the leukocyte function-associated antigen-1 (LFA-1) with its ligands, intercellular adhesion molecules (ICAM) -1 and -2 (1). It is important to understand these interactions under an external pulling force as they must resist external forces due to blood flow in vivo. In this study, atomic force microscopy (AFM) was used to measure the unbinding of LFA-1 from ICAM-1 and -2.

METHOD  The AFM force measurements were performed on an apparatus designed to be operated in the force spectroscopy mode (2). LFA-1-expressing Jurkat cells were attached to the tip of an AFM cantilever by concanavalin A (con A)-mediated linkages and interacted with a surface coated with ICAM-1 or ICAM-2 (3). Measurements of unitary LFA-1 and ICAM-1 or ICAM-2 unbinding forces were obtained under conditions that minimized contact between the Jurkat cell and the substrate. An adhesion frequency of <30% in the force measurements ensured that there is a >85% probability that the adhesion events are mediated by single bonds. Data were corrected for hydrodynamic drag (4) (5). The damping coefficient is the slope of the linear fit and is about 2 pN•s/µm (6).

RESULTS AND DISCUSSION  The single molecule measurements covered three orders of magnitude in force loading rate (50-60000 pN/s). These measurements allowed us to determine the energy landscapes corresponding to the dissociation of the LFA-1/ICAM-1 and LFA-1/ICAM-2 complexes as well as the energetic determinants of the complexes at equilibrium and under the influence of an external force. The force spectra (i.e., unbinding force versus loading rate) revealed a fast and a slow loading regime. For both interactions, binding of LFA-1 was elevated by the addition of Mg^{2+}, a cofactor that stabilizes
the LFA-1/ICAM-1 and -2 interactions only in the slow loading regime. This is an indication that the divalent cation-dependent inner activation barriers of both interactions regulate the ability of the complexes to resist a pulling force, while the equilibrium dissociation constants of both complexes are regulated by the energetics of the outer activation barrier. The LFA-1/ICAM-1 interaction was characterized by a steep inner activation barrier and a wide outer activation barrier. The LFA-1/ICAM-2 force spectrum exhibited a much less steep inner activation barrier and lower adhesion forces. These results can be attributed to differences in both function and structure. While ICAM-1 is expressed only during inflammation, ICAM-2 is constitutively expressed on resting endothelium and was recently found to play a role in angiogenesis (7) (8, 9) (10). Binding site differences in the ring of the hydrophobic amino acids of both ICAM-1 and ICAM-2, which surround the glutamic acid residues that bind to the I domain of LFA-1, suggest a weaker interaction and a lengthening of the inner barrier.

ACKNOWLEDGMENT We thank C. Freites for technical support. This work was supported by grants from the American Cancer Society, and the NIH (GM55611-01).

REFERENCES
10. M. T. Huang et al., Blood 106, 1636 (Sep 1, 2005).