Cell-stopping numbers

Douglas A. Lauffenburger
Departments of Chemical Engineering and Cell & Structural Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA

What physical properties of receptor/ligand bonds are responsible for arresting cells flowing in the bloodstream by the vessel endothelium? Since the identification by Lawrence and Springer (1) selectin-based adhesion bonds as uniquely crucial for rolling of neutrophil leukocytes on endothelial cells, a central issue has been to define the special nature of these adhesion-receptor/ligand interactions. Models attempting to elucidate the key properties of selectins mediating neutrophil rolling on endothelia have recently appeared in this journal (2, 3), assessing the significance of various system parameters including bond association and dissociation rate constants and the effect of fluid mechanical stress on bond disruption. To date, however, quantitative experimental data bearing on mechanistic aspects of this problem have been lacking.

Kaplanski et al. in the present volume (4) provide novel experimental information relevant to these biophysical modeling efforts, and to the more general field of receptor-mediated cell adhesion. They have determined effective receptor/ligand bond association and dissociation rate constants for neutrophils interacting with endothelial cells via selectin-mediated mechanisms. Using a very slow fluid velocity in a parallel plate flow chamber, Kaplanski et al. observed transient attachment and detachment events for individual cells. Quantitative analysis by a simple, probabilistic model was facilitated by the reasonable conclusion that the observed events in this case were probably mediated by a very small number of bonds.

Their observations on the initial arrest frequency of freely rolling cells yielded an estimate for the cell/substratum bond association rate constant, k_f, of about 0.04 sec-1. For cells rearrested following detachment k_f was increased to 0.4 sec-1 and the bond dissociation rate constant, k_r, was determined to be approximately 0.5 sec-1. Previous measurement by other investigators of binding of selectin in free solution to neutrophils yields an estimate of k_r smaller by one or two orders of magnitude. This suggests the possibility that the dissociation rate constant may be increased substantially in the presence of fluid mechanical stress. The smaller value for the association rate constant before initial arrest compared to later rearrests might represent binding under conditions of molecular strain due to the initially greater cell/substratum separation distance, though it could alternatively reflect fluid mechanical effects resisting cell/substratum encounter. Limitations in this work remain the uncertainties in precisely how many bonds are involved in the attachment/detachment dynamics and in the mode of bond disruption or distraction (that is, whether the bonds are reversibly dissociated or simply pulled from the cell membrane).

In a more general context, the findings by Kaplanski et al. provide an important step in addressing some central questions in physical modeling of cell adhesion: (*a*) what is the mechanical "strength" of a receptor/ligand bond? (*b*) what is the effect of applied stress on the average "lifetime" of a bond? and (*c*) how does the strength depend on the bond affinity? Essentially, what is the relationship between the mechanical and chemical properties of receptor/ligand bonds? Theoretical treatments addressing these questions have been offered, and most models incorporate these concepts in some manner. Bell (5) considered applied stress to increase the bond dissociation rate constant, and estimated bond strength both thermodynamically (by relating it to the equilibrium chemical energy change) and kinetically (by determining the force needed to disrupt bonds "instantaneously") to be on the order of microdynes. Dembo et al. (6) hypothesized that both the association and dissociation rate could be functions of the strain, or displacement of molecular separation distance, induced by applied stress. In both of these treatments the equilibrium bond chemical affinity is related to the effective mechanical strength, or the force needed for cell/substratum detachment.

Few experimental studies have previously been brought to bear on these questions. Bond strength has been determined indirectly from cell detachment experiments, typically falling into the microdyne range as expected. Data on how applied stress affects receptor/ligand binding parameters have not previously appeared, nor has substantial information on the relationship between chemical affinity and mechanical strength. This latter issue is confounded by the extreme difficulty of measuring binding parameters when the two molecular species are confined to apposed surfaces. This new work by Kaplanski et al. provides a first set of quantitative data on cell/substratum bond kinetics in the presence of applied fluid stress. Measurements of this type comparing binding parameters and the effects of applied stress for a set of receptor/ligand pairs with different chemical affinities could serve to test the fundamental theories by Bell and Dembo et al. concerning the critical relationship between bond mechanical and chemical properties.

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