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KINETIC MEASUREMENTS OF E-SELECTIN/CARBOHYDRATE LIGAND INTERACTIONS ON THE CELL SURFACE

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INTRODUCTION

E-selectin is a member of the selectin family of adhesion molecules that are important in processes such as leukocyte trafficking, thrombosis, inflammation, and ischemia-reperfusion [1]. Inducible on stimulated endothelial cells, E-selectin binds carbohydrate ligands expressed on leukocytes and some carcinoma cells. Selectin/carbohydrate ligand interactions initiate the multistep adhesion and signaling cascades in the recruitment of leukocytes from circulation to inflamed tissues and may also play a role in tumor metastasis. Kinetic properties of these interactions are essential determinants governing the tethering and rolling of blood-borne cells to the vessel wall. Extending our recently developed micropipette method [2], we have measured the kinetic rates of E-selectin/ligand interactions.

MATERIALS AND METHODS

A recombinant soluble E-selectin construct consisting of only the Lec-EGF domains was captured on the surface of human red blood cells (RBC) by an anti-E-selectin nonblocking monoclonal antibody (mAb) 1D6, which was in turn coated on the RBC by chromium chloride coupling. Site densities were measured by radioimmunoassay. Representing model leukocytes and carcinoma cells respectively, promyelocytic leukemia cell line HL-60 and colon adenocarcinoma cell line Colo-205 cells were used to present the carbohydrate ligands.

Separately aspirated by two micropipettes, a pair of E-selectin-coated RBC and ligand-expressing target cell were driven by a piezoelectric actuator to make repeated contacts with each other. Adhesion, if present at the end of a controlled contact, was detected by the membrane deflection of the RBC during its retraction. In addition, the number of isolated point-attachments linking the two cell membranes was enumerated by direct observation during their rupture when the RBC was being retracted. The contact duration t was kept constant in each series of 100 contacts to estimate the probability distribution p_n of point-attachments and their average $\langle n \rangle$ at that t but systematically varied over a range to measure the dependence of $\langle n \rangle$ on t. Two (for HL-60) and three (for Colo-205) P_a vs t curves were

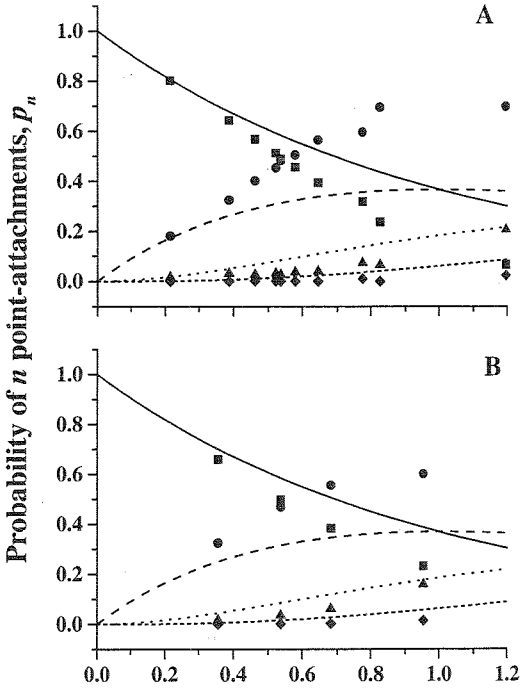

Fig. 1. Comparison between measured (*points*) and predicted (*curves*) probabilities of having zero (B, *solid curve*), one (J, *long-dashed curve*), two (H, *dotted curve*), and three (F, *short-dashed curve*) point-attachments on the average number of point-attachments for HL-60 (*A*) and

Colo-205 (*B*) data. Theoretical predictions were calculated using Eq. 1.

measured for different E-selectin densities. Binding specificity was confirmed by replacing 1D6 with irrelevant antibody, by replacing the E-selectin construct with a P- selectin construct that does not bind 1D6, by blocking with adhesion blockade mAb ES1, and by removing divalent cations using EDTA.

RESULTS

Although the two cells were pressed against each other to make a controlled contact of large apparent area (~3 μm^2), adhesions were only seen in a few (most often just one) spatially separated distinct points, which detached sequentially. These point-attachments were found to follow the Poisson distribution (Fig. 1).

$$p_n(\langle n\rangle) = (\langle n\rangle^n / n!)\exp(-\langle n\rangle) \quad (1)$$

This suggests that they are statistically independent and equivalent, most likely mediated by single bonds.

The average number of point-attachments $\langle n\rangle$ was found to proportional to the E-selectin density m_r. The $\langle n\rangle/m_r$ data increase with the contact time initially and then reach a plateau, as shown in Fig. 2.

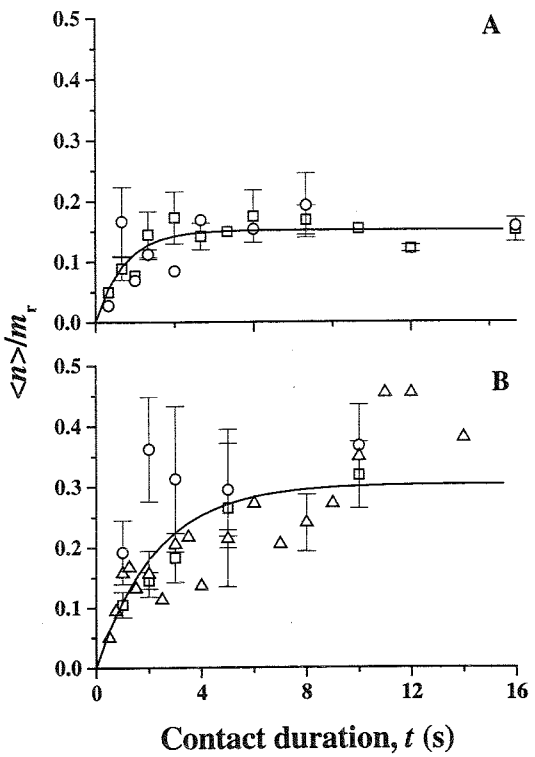

Fig. 2. Dependence of average number of point-attachments per E-selectin density, $\langle n\rangle/m_r$, on contact duration, *t*. Data (*points*) generated using different E-selectin densities are indicated by different symbols. HL-60 and Colo-205 data are shown in *A* and *B* respectively, which are presented as mean ± SEM of 1-5 pairs of cells of 100 cycles each. Curves are theoretical fits to the data based on Eq. 2.

A simple reversible kinetics model,

$$\langle n\rangle = m_r m_l A_c K_a^0 [1 - \exp(-k_r^0 t)] \quad (2)$$

was fit to the data, allowing one to estimate the reverse-rate k_r^0 and the per unit E-selectin density cellular binding affinity $m_l A_c K_a^0$. The latter is an aggregate parameter that lumps the molecular binding affinity K_a^0 with the contact area A_c and the ligand density m_l. The per unit E-selectin density cellular forward-rate was then calculated according to $m_l A_c k_f^0 = m_l A_c K_a^0 \times k_r^0$. These parameters are summarized in Table 1.

Table 1. Summary of kinetic constants (mean ± SEM)

Parameters \ Cells	HL-60	Colo-205
k_r^0, s^{-1}	0.92 ± 0.23	0.44 ± 0.10
$m_l A_c k_f^0$, $\mu m^2/s$	0.14 ± 0.04	0.13 ± 0.03
$m_l A_c K_a^0$, μm^2	0.15 ± 0.01	0.30 ± 0.03

CONCLUSION

The micropipette method has been successfully applied to the study of E-selectin/carbohydrate ligand interactions and extended from adhesion kinetic measurements to single-bond kinetic measurements. It has provided further validation not only to this useful experimental tool but also to its underlying mathematical framework – the probabilistic kinetics theory.

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REFERENCES

1. Lasky, L. A. 1995. Selectin-carbohydrate interactions and the initiation of the inflammatory response. *Annu. Rev. Biochem.* 64:113-139.
2. Chesla, S. E., P. Selvaraj, and C. Zhu. 1998. Measuring two-dimensional receptor-ligand binding kinetics with micropipette. *Biophys. J.* 75:1553-1572.