

Reorganization of the Plasma Membrane of Human Neutrophils: Implication for Cell Activation and Superoxide Generation

Goutam Mukherjee, **Mark T. Quinn**, John G. Linner, and **Algirdas J. Jesaitis**

Superoxide (O_2^-) production by neutrophils is a major mechanism for microbial killing. It is stimulated by chemotactic peptides [e.g., formyl methionyl-leucyl-phenylalanine (fMLF)] and is normally transient in suspensions of cells. O_2^- increases in rate and duration after pretreatment of the cells with dihydrocytochalasin B (dhCB), an inhibitor of actin polymerization. Thus, effects of dhCB suggest a possible role for the membrane skeleton and plasma membrane organization in the regulation of the O_2^- generating system. Analysis of plasma membranes isolated from disrupted neutrophils by isopycnic sucrose density gradient sedimentation showed that there were no significant variations in the distribution of plasma membrane markers between control and dhCB-treated cells, while a significant redistribution of plasma membrane markers was observed in dhCB+fMLF-treated cells. Instead of being localized in 31-35% sucrose, as in former two groups, plasma membrane markers were broadly distributed over 25-50% sucrose in the dhCB+fMLF-treated cells. In addition, the stimulated cells were nearly ~80% degranulated. Analysis of the gradient fractions for membrane skeletal (actin and fodrin) and NADPH oxidase (cytochrome b and p47-*phox*) components in dhCB+fMLF-treated cells demonstrated that the distribution of fodrin, actin, cytochrome b, and p47-*phox* followed the broad distribution of plasma membrane markers, with an overall *eight-fold* increase in membrane-associated actin. Despite the broad redistribution of plasma membrane markers, the distribution of O_2^- generating activity remained confined to a narrow peak at ~50% sucrose. These results demonstrate that a heterogeneous surface membrane of higher density and a differential distribution of proteins and O_2^- generating activity is created after dhCB+fMLF treatment; however, domain structure is conserved in the new membrane, with only a subfraction of the reorganized plasma membrane containing all of the components necessary for active O_2^- generation. Our results support a role for plasma membrane lateral organization and participation of the membrane skeleton in the regulation of the O_2^- generating system. Current studies are examining the morphology of the surface at 30 nm resolution using scanning electron microscopy and immunocytochemistry.

Recent Publications

Quinn, M.T., Evans, T., Priscu, L.R., **Jesaitis, A.J.**, and Bokoch, G.B. Translocation of Rac correlates with NADPH oxidase activation: Evidence for equimolar translocation of oxidase components. *J.Biol.Chem.* **268**: 20983-20987, 1993.

Mukherjee, G., Quinn, M.T., Linner, J.G., and **Jesaitis, A.J.** Remodeling of the plasma membrane after stimulation of neutrophils with f-Met-Leu-Phe and dihydrocytochalasin B: Identification of membrane subdomains containing NADPH oxidase activity. *J. Leuk. Biol.* **55**: 685-694, 1994.