Structural and Functional Analysis of the Superoxide Generating System in Human Neutrophils

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Activation of human neutrophils with a variety of stimuli induces microbicidal responses which include the generation of toxic oxygen metabolites such as superoxide anion ($O2^-$) and hydrogen peroxide (H2O2). The induction of $O2^-$ production results from the activation of a latent plasma membrane electron transport system known as the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system. Activation of the NADPH oxidase system involves the interaction and/or assembly of several neutrophil components, some located on the plasma membrane and others in the cytosol. It has recently been established that one of the required components for NADPH oxidase activity is a GTP-binding protein Rac.

To further investigate the role of Rac in the NADPH oxidase system, studies were carried out to determine its subcellular distribution in resting and activated human neutrophils. In resting cells, Rac and an associated guanine nucleotide regulatory factor, GDP dissociation inhibitor (GDI), were located only in the cytosol, along with other known oxidase factors, p47-*phox* and p67-*phox*. After activation of neutrophils with phorbol myristate acetate or *f*MLF, Rac was translocated from the cytosol to the plasma membrane, and this translocation corresponded temporally with the translocation of p47-*phox* and p67-*phox* and with the generation of superoxide. GDI remained localized to the cytosol, suggesting activation of the oxidase involved dissociation of the Rac-GDI complex prior to Rac translocation. Determination of the quantities of cytosolic factors associated with the plasma membrane indicated that Rac, p47-*phox*, and p67-*phox* are translocated to the plasma membrane simultaneously in equimolar amounts, but that the membrane-associated cytochrome b was present at 3-4 fold molar excess. These findings suggest that Rac may play a role in assembly of the active NADPH oxidase complex.

Studies in my lab have also focussed on the role of a second low molecular weight ras-related GTP-binding protein, Rap1A, in the neutrophil NADPH oxidase. Studies were conducted to analyze the nature of the interaction between neutrophil cytochrome b and Rap1A, which we previously showed to be associated with purified cytochrome b. Using subcellular fractionation methods and immunocytochemical electron microscopic analyses with anti-cytochrome b and anti-Rap1A antibodies, we found that Rap1A co-localized with cytochrome b in the plasma membrane as well as in the specific granule compartment in resting neutrophils and that Rap1A was co-translocated to the plasma membrane or phagolysosome in activated cells. These studies provide evidence for a functional association of these two molecules in the intact cell. In addition, we found that purified Rap1A and cytochrome b could be reconstituted *in vitro* as a stoichiometric

complex with cytochrome b and that if Rap1A was phosphorylated prior to reconstitution, it was unable to complex with the cytochrome. Thus, the ability of phosphorylation to inhibit the Rap1A:cytochrome b interaction makes Rap1A a possible target for inhibitory regulation by the cAMP pathway in human neutrophils. Potentially, such a mechanism could regulate the ability of Rap1A to interact with macromolecules in other cells as well.

Recent Publications

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