A RISE IN IONIZED CALCIUM ACTIVATES THE NEUTROPHIL NADPH-OXIDASE BUT IS NOT SUFFICIENT TO DIRECTLY TRANSLOCATE CYTOSOLIC $p_{47}^{\text{phox}}$ OR $p_{67}^{\text{phox}}$ TO b CYTOCHROME CONTAINING MEMBRANES

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Abstract—Neutrophil production of reactive oxygen species is dependent on an assembly process that involves a translocation of the cytosolic NADPH-oxidase components ($p_{47}^{\text{phox}}$, $p_{67}^{\text{phox}}$, Rac2) to a b cytochrome containing membrane. Based on the fact that an intracellular Ca$^{2+}$ rise can activate the oxidase without any extracellular release of reactive oxygen species, we suggest that the oxidase can be assembled in a membrane distinct from the plasma membrane. Disintegrated cells were used to monitor Ca$^{2+}$ dependent membrane binding of neutrophil cytosolic proteins. Membranes containing the b cytochrome part of the oxidase, i.e., specific granules and plasma membranes/secretory vesicles, were used in the translocation experiments. Several cytosolic proteins were found to translocate to specific granules as well as the plasma membranes/secretory vesicles, one of them being annexin I. Using antibodies in the blotting assay against the cytosolic oxidase components $p_{47}^{\text{phox}}$ and $p_{67}^{\text{phox}}$, we could show that no Ca$^{2+}$ dependent translocation of these cytosolic proteins occur to neither of the b cytochrome containing membranes.

INTRODUCTION

The human neutrophil phagocyte is of great importance for eliminating invading micro-organisms, and for this purpose these cells are armed with bactericidal enzymes and a membrane-bound respiratory burst oxidase that generates reactive oxygen species (1, 2). This production is linked to activation of an electron transport chain which reduces oxygen by transferring electrons from NADPH. The system is composed of at least four different structural units, and one of them, an unusual b cytochrome (referred to as either cytochrome b$_{245}$ or b$_{558}$; 3, 4), is a membrane spanning heterodimer composed of an $\alpha$-subunit with a molec-
ular weight of 22kDa (p22phox) and a glycosylated β-subunit with a molecular weight of 91kDa (gp91phox). The large b cytochrome subunit has been suggested to contain the binding sites for flavine adenine dinucleotide (FAD) as well as for NADPH, and to serve as the final electron donor for reduction of molecular oxygen (5). In resting cells, the other three oxidase components (p47phox; p67phox and the GTP binding protein Rac2) reside in the cell cytosol, and when the cells are activated they translocate to a membrane (2, 6) that contains the b cytochrome (7). The neutrophil contains at least three types of granules (azurophil granules, specific granules and secretory vesicles), which store a variety of bactericidal products and mobilizable cell-surface receptors (8, 9). Subcellular fractionation studies, as well as ultra structural analyses, have shown that the b cytochrome of the oxidase is present in two (or three) different subcellular compartments, being embedded in the membrane of the specific granules (70–75%), as well as in the plasma membrane (5%) or in a plasma membrane derived secretory vesicle (20–25%; 10–12). Results using intact cells as well as a cell-free assay system indicate that an active oxidase can become assembled not only in the plasma membrane, but also in the membrane of the specific granules (13–15).

A number of different stimuli can initiate a respiratory burst activity in neutrophils. The regulatory mechanisms operating in translocating the cytosolic components to the b cytochrome containing membranes are only poorly understood, however, according to the present paradigm the oxidase is assembled exclusively in the plasma membrane or plasma membrane derived phagosomal membrane (16). Results obtained upon stimulation of intact neutrophils with a Ca2+-specific ionophore challenges, however, this paradigm (17). The neutrophil oxidase activity induced by an intracellular Ca2+ rise is (in contrast to what is seen with the chemoattractant FMLP or the PKC activator PMA) associated with a very low level of release of reactive oxygen species (18) and we have earlier shown that this activation involves the b cytochrome present in the granules but not that present in the plasma membrane (14). The metabolites generated by the NADPH-oxidase (i.e., O2 and H2O2) constitute an important part of the oxygen-dependent anti microbial arsenal of the neutrophil, but recent data suggest that the reactive oxygen species generated by the oxidase may act also as intracellular second messengers (19, 20), making intracellular production of reactive oxygen species a highly relevant process.

Taken together these results indicate that a rise in intracellular Ca2+ could be an important signal for the assembly of the oxidase in a granule (or granule related) membrane. The fact that translocation from cytosol to membrane of protein kinase C (β-PKC; 21) and grancalcin (a 28kDa cytosolic protein; 22,23) is directly regulated by Ca2+, clearly establishes that the Ca2+-dependent membrane binding capacity is not a characteristic exhibited exclusively by members of the annexin protein family (24). In the present report we have used an in vitro technique to determine Ca2+ induced translocation of cytosolic pro-