

Immunocytochemical Detection of Lipid Peroxidation in Phagosomes of Human Neutrophils: Correlation with Expression of Cytochrome b in X-linked Chronic Granulomatous Disease

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Stimulation of phagocytes with a variety of particulate and soluble stimuli induces microbicidal responses which include the generation of toxic oxygen metabolites such as superoxide anion, hydrogen peroxide, and hydroxyl radicals. Although the neutrophils' oxidant producing system is designed to restrict oxidant production to the smallest possible region where the pathogen is located, some of the reactive oxidants inevitably leak into surrounding areas where they have the capacity to inflict tissue damage at sites of inflammation. Oxidants generated by activated neutrophils can react with a number of tissue targets, including polyunsaturated fatty acids in cell membranes, to form toxic metabolites. 4-Hydroxynonenal (4-HNE), a major lipid peroxidation product generated by free radical attack on ν -6 polyunsaturated fatty acids, is a marker for lipid peroxidation and is a candidate for one of these toxic metabolites. Its toxicity results from the fact that it is extremely reactive with protein sulfhydryls forming sulfoether adducts that can inhibit protein function.

In this study, we demonstrated the accumulation of 4-HNE in neutrophil phagosomes containing heat-killed *Staphylococcus aureus* using immunocytochemistry and electron microscopy. Specific polyclonal rabbit anti-4-HNE adduct antibodies and rabbit polyclonal anti-gp91-*phox* antibodies were used to label HNE adducts and neutrophil flavocytochrome b (the terminal component of the NADPH oxidase), respectively, in cryofixed, molecular distillation-dried human neutrophils. The neutrophils were purified from the peripheral blood of a normal control, a male patient with homozygous X-linked, gp91-*phox*-deficient chronic granulomatous disease (CGD), and his heterozygous mother. No 4-HNE could be detected in flavocytochrome b-deficient cells of homozygous or heterozygous CGD cells. However, in gp91-*phox*-positive cells from both the normal and heterozygous CGD carrier, significant 4-HNE labeling was observed, primarily in the phagosomes. When data from single- and double-labeled cells were combined, the frequency distribution of the labels in phagosomes supported this observation, showing that neutrophils from the heterozygous CGD carrier were 71% HNE-positive and 56%

gp91-*phox*-positive, while cells from the normal father were >97% positive for both 4-HNE and gp91-*phox*. These results confirm the nitroblue tetrazolium tests of 100% and 60±2% positive for the father's and mother's cells, respectively. Thus, the results show that 4-HNE-protein adduct antibodies are useful and accurate probes of the occurrence of lipid peroxidation *in vivo*. We conclude that 4-HNE-protein adducts are generated as a result of NADPH oxidase activity in the phagosomes of human neutrophils. Since *S. aureus* does not contain polyunsaturated fatty acids of its own our results also suggest that the source of the 4-hydroxynonenal must be the phagocyte membrane, defining one more weapon in its arsenal of microbial products. This system will be used to examine the targeting of superoxide by cytochrome b in inflamed and damaged tissue and may be a general marker of oxidative damage in a variety of tissues.

Recent Publications

Linner, J.G., Buescher, E.S., Siemsen, D.W., **Dratz, E.A., Quinn, M.T., and Jesaitis, A.J.** Electron microscopic immunocytochemical co-localization of superoxide generating components and targets in resting and stimulated human neutrophils. *In: Molecular Basis of Oxidative Damage by Leukocytes. Proceedings of Montana State University/Keystone Symposium on the Molecular Basis of Oxidative Damage by Leukocytes* (A.J. Jesaitis and E.A. Dratz, eds.) CRC Press, Boca Raton, pp. 277-281, 1992.