Intracellular Nitric Oxide Production And Cellular Localization In Human Leukocytes

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(RATIONALE) Nitric oxide (NO) regulates a host of inflammatory and physiologic function of critical importance to lung function and disease. Since the half-life of NO in biological tissues is so short, NO activity is most often measured indirectly, such as with levels of nitric oxide synthase (NOS). More direct measurement of NO gas in living tissues could lead to a better understanding of NO mechanisms in health and disease. Our goal in this study is to examine NO presence in leukoctyes and to corroborate the findings with two different techniques: flow cytometry and fluorescence imaging.
(METHODS) Peripheral whole blood from 4 healthy donors was stimulated with PMA (phorbol 12-myristate 13-acetate, 20 ng/ml for 1hr). Leukocytes with and without PMA stimulation were incubated with intracellular NO fluorescent probe (DAF-2DA) and surface expression markers for lymphocyte (CD56), monocyte (CD14), and granulocye (CD16). NO production and cellular localization of NO in different leukocyte subtypes were examined using flow cytometery (BD ACCURI C6, California, USA) and confocal laser microscope (Olympus FluoView FV1000, Tokyo, Japan).
(RESULTS) The median (min, max) increase in NO fluorescence was demonstrated in all leukocyte subtypes following PMA stimulation; lymphocyte 0.2 (0.1, 0.5) to 2.2 (1.5, 8.5). Monocyte, 1.6 (0.1, 5.9) to 11.8 (2.2, 13.4), and granulocyte 1.0 (0.1, 2.3) to 7.7 (2.3, 23.1). Figure 1(A) demonstrates representative histograms of intracellular NO in unstimulated and stimulated granulocytes using flow cytometry. Figures 1(B) and 1(C) show a fluorescence imaging of NO (green) in granulocytes before (B, dim scattered dots) and after (C, bright dense pockets) PMA stimulation.
(CONCLUSION) Our study demonstrated that 1) we can successfully measure intracellular NO in whole blood leukocytes obtained from healthy subjects; 2) Whole blood PMA stimulation dramatically enhances NO production mainly in granulocytes and the pattern of change differs between other leukocyte sub types; and 3) fluorescence imaging provides additional spatial information of NO production in human leukocytes.

Figure 1. Intracellular NO in unstimulated and PMA-stimulated granulocytes. Data obtained from (A) flow cytometry and (B-C) fluorescence imaging using confocal laser microscope