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Nitric Oxide Shifts Neutrophil Interactions with Bacteria from Phagocytosis to Long-Range Extracellular Binding by Membrane Tubulovesicular Extensions

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Nitric oxide (NO) is shown to enhance the bactericidal activity of phagocytes, thus contributing to host defense against multiple bacterial infections. Human neutrophils can develop dynamic extraordinarily long and thin membrane tubulovesicular extensions (TVE, cytonemes, membrane tethers) serving as temporarily adhesive organelles [Galkina SI, Sud'ina GF, Ullrich, V (2001) Exp Cell Res 266: 222-228] and NO was found to induce TVE formation [Galkina SI, Molotkovsky JG, Sud'ina GF, Ullrich, V (2005) Exp Cell Res 304: 620-629]. NO-induced TVE consist of membrane tubules and vesicles of the uniform diameter (160-240 nm) filled with neutrophil cytoplasm. In length TVE can reach several neutrophil diameters during 20 min. We suggested that inducing TVE formation NO can alter neutrophil-bacteria interactions.

Using scanning electron microscopy we compared human neutrophil interactions with *Salmonella enterica* serovar Typhimurium in control conditions, in the presence of nitric oxide donor diethylamine NONOate and in the presence of NO-synthase inhibitor L-NAME. In the presence of a NO donor neutrophils bound and aggregated bacteria by a new mechanism using NO-induced TVE, which appear to be neutrophil secretory structures. Binding of bacteria by TVE leaded to shedding of TVE from the cells together with bound pathogens but not to phagocytosis of bacteria. In contrast, inhibition of NO-synthase activity by L-NAME stimulated phagocytosis of bacteria by neutrophils.

Our data revealed that NO could enhance neutrophil bactericidal activity shifting neutrophil-bacteria interactions from phagocytosis to extracellular binding by TVE. The latter have some advantages of phagocytosis. TVE strongly widen the area within which neutrophil could capture bacteria. TVE could deliver neutrophil bactericides "packed in membrane" to bound bacteria without dilution. Following lysis of TVE could release neutrophil bactericides nearby bound and aggregated bacteria. At least bacteria do not enter the host cells, where they can survive and multiply.

Keywords: human neutrophil, Salmonella enterica serovar Typhimurium, membrane tubulovesicular extensions, phagocytosis