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The control of oxygen radical production in the neutrophil phagosome

Oliver Nüsse
University Paris-Sud, France

Phagocytosis is a central element of the immune response against microbial infection. Phagocytes, macrophages and neutrophils, engulf the microbe and deliver reactive oxygen species (ROS) and degrading enzymes into the phagosome. Resistance to the phagocyte's attack and survival inside phagosomes by certain pathogens cause a major threat to the host. The mechanisms of this microbial resistance to phagosomal killing are not fully understood. To analyze the maturation of the phagosome, new methods are under development for time resolved measurement of the key biochemical changes (enzyme activities, pH, ROS) inside the phagosome. Fluorescence microscopy provides new possibilities to investigate the phagosome and the phagocytes with high spatial and temporal resolution. The phagocyte NADPH oxidase is responsible for ROS production in the phagosome. The active enzyme complex is formed from by assembly of 2 membrane proteins (gp91^{phox} and p22^{phox}) and several cytosolic proteins (p40^{phox}, p47^{phox}, p67^{phox} and rac). We employ flow cytometry and video imaging to investigate the kinetics of NADPH oxidase assembly and the phagosomal ROS production. Subunits of the oxidase are tagged with fluorescent proteins and expressed in neutrophil-like PLB985 cells. These experiments indicate that the duration of phagosomal ROS production is similar to the time of phagosomal accumulation of presence of p67^{phox}. In contrast, p47^{phox} and rac are present for much shorter periods. The assembly of the NADPH oxidase appears to be under control of multiple anionic phospholipids. The latter could be a target for bacterial resistance mechanisms.

Biography

Oliver Nüsse has completed his Ph.D. at the Free University Berlin, Germany in 1991. After a post-doctoral period at Harvard Medical School in Boston and assistant professorship at the University of Geneva, he became Professor for immunology in Nancy, France. In 2003, he moved to the University Paris-Sud, Orsay, France to become Professor for cell biology. He has published more than 25 papers in reputed journals on the topic of signal transduction in neutrophils.

oliver.nusse@u-psud.fr