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Immunomagnetic Isolation of Pathogen-Containing Phagosomes and Apoptotic Blebs from Primary Phagocytes

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ABSTRACT

Macrophages and polymorphonuclear neutrophils are professional phagocytes essential in the initial host response against intracellular pathogens such as *Mycobacterium tuberculosis*. Phagocytosis is the first step in phagocyte-pathogen interaction, where the pathogen is engulfed into a membrane-enclosed compartment termed a phagosome. Subsequent effector functions of phagocytes result in killing and degradation of the pathogen by promoting phagosome maturation, and, terminally, phago-lysosome fusion. Intracellular pathogenic microbes use various strategies to avoid detection and elimination by phagocytes, including induction of apoptosis to escape host cells, thereby generating apoptotic blebs as shuttles to other cells for pathogens and antigens thereof. Hence, phagosomes represent compartments where host and pathogen become quite intimate, and apoptotic blebs are carrier bags of the pathogen's legacy. In order to investigate the molecular mechanisms underlying these interactions, both phagosomes and apoptotic blebs are required as purified subcellular fractions for subsequent analysis of their biochemical properties. Here, we describe a lipid-based procedure to magnetically label surfaces of either pathogenic mycobacteria or apoptotic blebs for purification by a strong magnetic field in a novel free-flow system. *Curr. Protoc. Immunol.* 105:14.36.1-14.36.26. © 2014 by John Wiley & Sons, Inc.

Keywords: macrophages • primary cells • mycobacteria • phagosomes • apoptotic vesicles • isolation protocol

INTRODUCTION

The protocols described in this unit can be used to isolate bacteria-containing phagosomes or apoptotic blebs from different phagocytic host cells, such as primary macrophages and neutrophils, but also from macrophage cell lines of either human or murine origin. After isolation, phagosomes and apoptotic blebs can be characterized by various methods—e.g., flow cytometry, light and electron microscopy, enzymatic assays, western blot analysis, or mass spectrometry. The protocol described for the isolation of mycobacteria-containing phagosomes (Basic Protocol 1) is a useful tool to study how cytokine-mediated phagocyte activation, recruitment of humoral components during uptake, or the presence or absence of certain virulence factors of the pathogen alter phagosome biogenesis.

Basic Protocol 1 relies on the use of a lipid-based procedure to efficiently label the surface of the bacteria of interest. The protocol has been successfully used to isolate and characterize mycobacteria-containing phagosomes—however, the labeling

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