

The Structure and Function of Lipid Raft in fMLP-stimulated neutrophil[®]

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Neutrophil polarization is required for neutrophil chemotaxis. Chemotactic stimulation of neutrophils induces a complex sequence of events: morphological changes, asymmetric redistribution of many proteins and lipids, and reconstruction of cellular structure. The signaling proteins, such as PI3K, small G protein, ARP2/3, cofilin, profilin and etc., could direct the formation of lamellipodium, regulate the polymerization of F-actin and orchestrate the dynamic assembly of the front and the rear of polarized neutrophil. The exact point at which the signaling proteins could be associated is not known. However, a number of studies have shown that cholesterol- and glycolipid-enriched plasma membrane microdomains, so-called lipid rafts, are a recent focus of interest as organizers of signaling molecules. It was reported that neutrophil polarization was affected by the cholesterol in the membrane. Therefore, the change of lipid raft structure in fMLP-stimulated neutrophil was investigated by confocal laser scanning microscopy (CLSM or LSCM).

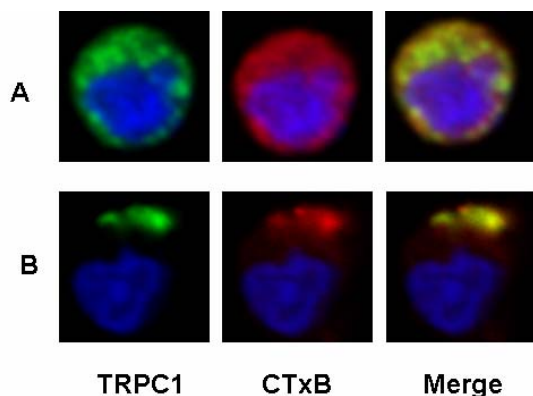


Fig 1. Co-localization of TRPC1 (green) with Lipid raft (red) in fMLP-stimulated neutrophil
A. control B. fMLP-stimulated neutrophil

Polymorphonuclear neutrophils (PMNs) were isolated from whole blood donated by healthy volunteers by Dextran sedimentation. Cholera toxin B(CTX-B). In addition, the neutrophils were simultaneously stained with DAPI (targeting DNA in the cell nucleus). Figure1 showed that the lipid raft was distributed around the edge of untreated neutrophil. After the stimulation of fMLP, lipid raft was localized on one edge of neutrophil, which indicated that there is the reconstruction of lipid raft in polarized

neutrophil. It was reported that TRPC1 was involved in the regulation of directed cell migration. In our study, TRPC1 was distributed uniformly in untreated neutrophil membrane, and there is co-localization of TRPC1 with lipid raft in fMLP-stimulated neutrophil. After the lipid raft was disrupted by methyl- β -cyclodextrin (M β CD), the polarized distribution of TRPC1 disappeared in fMLP-stimulated neutrophil. Our study suggested that translocation of TRPC1 to lipid raft is associated with neutrophil polarization.

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