Real-time calcium transient measurement in mouse dendritic cells stimulated with LPS or ATP

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Method Article

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Abstract

Introduction

Protocol for calcium transients measurement in dendritic cells used in our Nature paper. Intracellular calcium concentration was determined by a fluorimetric ratio technique, an approach that overcomes possible problems of uncertainty related to the calibration or uneven distribution of fluorescent calcium indicators. A direct optical microscope \(Olympus, BX51) with a two-photon Ti-Sapph laser source \(720 nm wavelength; Mai Tai, SpectraPhysics) was used for indo-1 excitation. The fluorescence signals emitted by indo-1-loaded cells were digitized at 200 Hz and recorded every 0.5-0.8 s. The ratio of fluorescence emissions at 400 nm/bp to those at 500 nm/bp was recorded \(R400/500) and used as an index of intracellular calcium concentration. Data were then normalized to baseline. In some cases, cells were analyzed in calcium-free PBS or calcium-free PBS supplemented with 50 nM thapsigargin \(Sigma Aldrich).

Procedure

1.Seed 3 x 10^6 cells \(BMDCs or BMMΦs) in 60 x 15 mm non-treated cell culture dishes at a concentration of 10^6 cells/ml in the appropriate medium. 2.Incubate overnight \(37 °C – 5% CO2). 3.Gently wash cells three times with 3 ml of pre-warmed PBS to remove residual extracellular esterases contained in the medium. 4.Add 1 ml of 2 microM of the ratiometric probe indo-1 \(Molecular Probes, Eugene, OR) in PBS and incubate 20’ \(37 °C – 5% CO2) in the dark. 5.Gently wash cells three times with 3 ml of pre-warmed PBS to remove extracellular free probe. 6.Add 1,8 ml of pre-warmed PBS. 7:Start real-time imaging acquisition and superfuse the appropriate stimulus in 200 µl PBS.