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Patch-clamp and live fluorescence imaging analysis of the effect of potassium channel blockade on lipopolysaccharide-induced immune signaling

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Studies have shown that ion-channel function in immune cells can regulate pathogen induced signaling. Thus, ion-channels present a therapeutic target to regulate immune-cell function. At the cellular level, the use of electrophysiological recordings in combination with pharmacological agents has long been a touchstone to probe, with high temporal resolution, the functional expression of ion channels and their multivariate signaling pathways. Coupling this methodology with live-cell imaging of fluorescent reporter constructs to proteins involved in immune-signaling enables real-time elucidation of specific ion-channel-dependent signaling pathways. Determining the real-time dynamics of ion channel function in immune response is an important step in developing novel strategies for regulating immune response on demand when standard anti-pathogen therapies such as antibiotics and vaccinations fall short. Toward this end, we have tracked the real-time inflammatory response to *E. coli* derived lipopolysaccharide (LPS) in a macrophage-like cell-line with electrophysiology to measure potassium channel currents, and imaging of fluorescent reporter fusions to track Rel-A (a subunit of the transcription factor NFκB) and the cytokine TNFα promoter. In RAW264.7 cells, a 100 nM LPS challenge produces two waves of Rel-A translocation from the cytoplasm to the nucleus while gradually increasing the transcription of the TNFα promoter. Continuous exposure of LPS-challenged cells to the BK- and Kv-channel blocker tetraethyl ammonium modifies the short-term (<30 minutes) translocation dynamics of Rel-A and long-term (>4 hours) TNFα transcription in a dose-dependent manner.

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