FACS-based calcium mobilization assay

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Introduction

Several agonists induce the rapid increase in cytosolic free calcium (Ca^{2+}) concentration upon cell activation. E.g. the chemokines CXCL8 and CXCL7 cause Ca^{2+} release from intracellular pools and influx through the chemokine receptor CXCR2. In our work, we have identified the chemokine receptor CXCR2 as a functional receptor for the chemokine-like cytokine MIF, which acts as a non-cognate ligand of CXCR2 (ref. 1). As MIF binding to CXCR2 resulted in typical chemokine effects such as cell arrest and chemotaxis, we also investigated the Ca^{2+} mobilization as an early signal of MIF-induced cell activation.

Reagents

Neutrophils (obtained from healthy donors)

Fluo-4 AM

Assay buffer:

(130 mM NaCl, 4.6 mM KCl, 1 mM CaCl2, 5 mM glucose, 20 mM HEPES, pH 7.4)

Equipment

In order to record the change of cytosolic Ca^{2+} concentration over time, we employed the FACSAria System (BD Biosciences, San Jose, CA).

Procedure

1. Label peripheral blood-derived neutrophils (106 cells/mL) with fluo-4 AM (0.9 µM) in assay buffer for 45 min at 37˚C.
2. Wash and resuspend cells at 2×10^6 cells/mL.
3. Keep cell solutions at 37˚C.
4. Add stimulus to the cells, and directly record the change in mean fluorescent intensity (MFI) for 120 s using the BD FACSAria System. In a typical experiment, add the chemokine at its optimal concentration, e.g. as determined by chemotaxis assay. The MFI is a measure of the cytosolic Ca^{2+} concentration.
5. For determining desensitization, add a second stimulus at time 130 s and measure another 120 s.

6. Analyze experiments via FlowJo Software (Tree Star, OR).

Timing

2.5 h

Critical Steps

Pass cells through separation filters to avoid clogging.

As the Ca^{2+} concentration increases within seconds after addition of the stimulus, it is important to start the measure as fast as possible.

Troubleshooting

Neutrophils are often prepared in buffers containing EDTA, which keeps them from agglutinating. Be sure that all EDTA is removed, before starting the experiment.

Anticipated Results

See Bernhagen *et al.*, *Nat. Med*. 2007.

References

1. Bernhagen, J. et al. MIF is a non-cognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat. Med*. Epub ahead of print(2007).

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