Introduction

- Neutrophils are essential for killing microorganisms and have a significant role in the regulation of inflammatory responses. Microenvironment, receptor signaling, and metabolism all play key roles during neutrophil activation.
- Stimulated neutrophils activate membrane-associated NADPH oxidase (NOX2) resulting in a powerful oxidative burst during which a large amount of oxygen is consumed generating ROS. Generation of ROS is critical for effective microbial immunity but also play a significant role modulating inflammatory responses.
- In this study, neutrophil activation in the presence of cytokines known to be expressed within microenvironments in normal and disease states was evaluated.

![Image of neutrophil activation assay](Image)

**Experimental Approach**

**Method:**
The Agilent Seahorse XF Neutrophil Activation Assay provides a direct, non-invasive, real-time detection and quantification of neutrophil activation by measuring oxygen consumption rate (OCR) of the cells. This assay also provides a simultaneous measurement of proton efflux ratio (PER) an indicator of glycolysis.

![Image of XF assay](Image)

**Results and Discussion**

**Mitochondrial respiration is not required for oxidative burst in neutrophils**

![Graph of OCR and ROT](Image)

**Extended treatment with GM-CSF results in higher basal PER and enhancement of activation by PMA**

![Graph of PER and ROT](Image)

**Cytokine priming leads to enhanced neutrophil activation**

![Graph of cytokine activation](Image)

**TNFα alone induced a small oxidative burst**

![Graph of TNFα activation](Image)

**Conclusions**

- The XF neutrophil activation assay is a specific, quantitative and kinetic assay performed on live cells in real time. The XF analyzer is used to quantify oxygen consumption rate (OCR) as a direct non-invasive measure of neutrophil activation. The simultaneous measurement of PER provides further insight into how neutrophils meet the energy demands upon activation.
- Pretreatment and serial administration of 2-DG demonstrates the requirement of glycolysis to meet energy and substrate demands of oxidative burst. Neutrophils stimulated in the presence or absence of mitochondrial inhibitors Rot/AA indicates mitochondrial respiration is not required.
- TNFα alone induced a small oxidative burst within 1h of treatment, as observed in real time using the XF analyzer. This acute effect appeared to reduce the ability of neutrophils to respond to subsequent PMA activation within 1h.
- Treatment with GM-CSF and IL1β alone had no acute effect, but primed neutrophils for enhanced response to PMA. GM-CSF pretreatment for 18h not only enhanced PMA induced oxidative burst, but increased basal PER indicative of an increased basal glycolysis, signifying the cells were metabolically poised for NOX2 activation.
- XF neutrophil activation assay provides a simple and direct way to examine the effect of modulators such as drug treatments and microenvironment on oxidative burst (ROS production) and metabolic effects through parallel measurement of glycolysis.

**References**


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