TNF-α release and functional SNPs (single nucleotide polymorphisms)

BACKGROUND\textsuperscript{1,2}

TNF-α is one of the most important mediators of inflammation. The cell types secreting highest concentrations of TNF-α are monocytes or macrophages [1]. A strong inducer for TNF-α release/expression is bacterial endotoxin (LPS), stimulating a signaling cascade via TLR-4. The counterpart of TNF-α is lymphotoxin A (LTA, TNF-β), a product of lymphocytes which is similar to TNF-α and also guides lymphnode formation [2].

Earliest effects by TNF-α have been described in the context of tumor necrosis [1]. Later, TNF-α has been shown to promote tumor growth and today, chronically elevated TNF-α may support the manifestation or progression of malignancies. TNF-α is often responsible for wasting syndromes [2]. The responsiveness of a patient to secrete TNF-α is a measure for the intensity of an inflammatory response. Part of the intensity of the immune response is related to a single nucleotide polymorphism in the TNF-α promotor at position -308. The -308 SNP has been reported to influence the course of sepsis [3].

In our experiment we intend to find out, whether a promoter polymorphism of TNF-α affects the endotoxin response of patients with systemic inflammation (SIRS) or sepsis. In case you are interested to test your own blood sample, please take:

2.5 ml of heparin-anti coagulated whole blood and follow our whole blood stimulation protocol. gDNA can be isolated from 0.3ml of whole blood to sequence the TNF-α SNP by pyrosequencing.

References

PART I

Ex-Vivo Whole Blood TNF-α Secretion Assay - TruCulture®
(https://myriadrbm.com/truculture/)

TruCulture® is a commercially available test to quantify the amount of TNF-α secreted in a whole blood assay following stimulation with a standardized lipopolysaccharide (LPS). The amount of TNF-α produced, can be used as a marker of fitness, hyperactivation or suppression of a patient’s immune system. The amount of released cytokine is further dependent on a functional SNP mutation of the TNF-α gene.

Procedure: Heparinized whole blood is taken from patients treated on ICU (intensive care) almost every 2 h to test blood gases, pH, glucose, C-reactive protein and other markers. We may use the left-over blood samples for our Immunology Course.

Set-up of whole blood stimulation:

Experimental Setup

After incubation at 37 °C for 4 h, the supernatant is collected and tested for the amount of TNF-α released, by a fully automated ELISA (Immulite®).

Immulite 1000®:
Is a chemiluminescent Immunoassay System. The sample is pipette into the incubation vial. An automated robot adds the sample into another vial containing a polystyrene bead with anti-TNF-α antibodies. After washing, a secondary ALP-labelled antibody is added. Following incubation, a substrate is added and chemiluminescent photons are generated correlating with the TNF-α concentration of the original sample.
For clinical studies, a highly standardized system is used to test the immune competence of a patient. This ex-vivo whole blood stimulation is named: TruCulture® and has been invented by HOTScreen GmbH (formerly EDI GmbH) in Germany. The system is distributed by Myriad RBM in the U.S. It can be also used to test the effect of chemotherapeutics, toxins and nutritional substances on the immune system.

**Read Results** of the Immulite 1000® assay:

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<tr>
<th>KeyPat Id</th>
<th>No stimulus [pg/ml]</th>
<th>Stimulation with LPS [pg/ml]</th>
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**Results of SNP genotyping: Group Name:**

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<tr>
<th>Patient[name or number]</th>
<th>TNF-α genotype (-308)</th>
<th>[TNF-α] (Truculture)</th>
<th>IL-6 genotype (-174)</th>
<th>[IL-6] (Truculture)</th>
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