Asthma, hypersensitivity pneumonitis and cough

0038
Cell activation and cytokine release ex vivo – Limits and chances of the whole blood assay with fresh human blood

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Background: Whole blood assay (WBA) is a suitable tool to quantify pyrogenic or proinflammatory mediator release induced by cell activation in response to different stimuli, e.g. extracts from environmental- or workplace-related dust samples. For this purpose cryo-preserved blood is an appropriate instrument. However, WBA may also provide an insight view into features of innate immunity from individuals. In this case the WBA is based on human fresh blood obtained from single subjects and the ex vivo cytokine release is measured after stimulation.

Aim: The aim of the study was to evaluate the reproducibility of results obtained with WBA if fresh human blood from the same individual was collected and stimulated on different dates. These data should serve as basis to assess effects of individual ex vivo cytokine release related to different exposure circumstances.

Methods: On six different days blood was collected from 16 healthy volunteers (96 blood samples in total). Ex vivo stimulation for 18 hours was performed with each individual blood using five different endotoxin concentrations. Cytokine release (IL-1β and IL-8) was quantified with specific immunoassays in the cell-free supernatant.

Results: A dose-response relationship between endotoxin and cytokine concentration measured in the cell-free supernatant existed repeatable for all blood donors. Maximum cytokine release was measured after stimulation with 100 or 1000 pg/ml endotoxin, respectively. The median coefficient of variation (CV) of the repeated whole blood assays performed with the blood of the 16 subjects stimulated with five different endotoxin concentrations was 52 % for IL-8 and 29 % for IL1-β. Reproducibility of IL1-β release after stimulation with 40 pg/ml endotoxin was 99.1 % (median, range 45 to 400 %; CV 23.7%) Furthermore, blood donors can be classified into high and low responders according to the ex vivo reactivity of their blood. This characteristic was reproducible over time for IL1-β. Finally, an individual normal range of cytokine release based on the six repeated tests was calculated for each subject. Values above or below of the interval median +/- 2 x MAD (median absolute deviation) will be interpreted as probable changes of innate immunity.

Conclusion: With this experimental setting of repeated blood collection from different healthy donors on different days it was possible to evaluate the variability of the outcome of the whole blood assay. Taking the intra- und intervariability of whole blood assay with fresh blood as an ex-vivo test into consideration it is possible to describe an individual range of reactivity which may help to determine effects of in-vivo exposure.