Effect Of Sample Processing On The Analysis Of IL4, IL5, And IL13 In Sputum Supernatants

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Introduction

The analysis of cytokines and mediators in sputum supernatants may be affected by endogenous proteases and the processing of sputum with dithiothreitol (DTT) during homogenization. Sputum extraction with PBS (Bafadhel, Respiration 2012), dialysis (Erii, ARJCCM 2008) or the addition of protease inhibitors (Kelly, ERJ 2001) has been suggested to improve the recovery of mediators from sputum supernatants. Depending on the antibodies used, the effect of these interfering factors may also vary with the analytical systems. Here we report our experience with the MesoScale Discovery (MSD)-platform for the analysis of interleukin (IL)4, IL5, and IL13.

Methods

In contrast to published data we observed higher levels of IL4, IL5 and IL13 in DTT treated compared to PBS extracted samples in pre-tests with the MSD system. Therefore we used pooled DTT-treated sputum samples from healthy subjects supplemented with or without protease-inhibitor (PI) for spiking experiments using recombinant IL4, IL5, and IL13 in two concentrations. In addition, we analysed sputum from 26 asthmatic subjects at baseline and after allergen challenge (ClinicalTrials.gov Identifier: NCT0174376). Selected sputum plugs were processed as previously described (Biller et al. Resp Med 2011), first washed with PBS and then homogenized with DTT.

The DTT treated portion was taken for supernatant measurements. The 10-plex ECL assay (MesoScale Discovery, USA) was used for cytokine analysis. The samples were measured in duplicates and a CV < 15% were accepted.

Results

The median recovery of the 1000 and 20 pg/mL spike from diluent was 78%/100% for IL4, 17/18% for IL5 and 60/112% for IL13, respectively. The recovery from spiked DTT treated sputum samples was 25/24% for IL4, 17/9% for IL5, and 38/2% for IL13. PI did not significantly affect these values. Using DTT-treated sputum samples we were able to show a significant increase for IL5 and IL13, but not for IL4, in sputum obtained after allergen challenge compared to the patients' baseline sputum.

Conclusion

Despite the low recovery in the spiking experiments, which do not fulfill standard acceptance criteria, it was possible to detect the expected allergen-induced increase in IL5 and IL13 in DTT-treated sputum supernatants. Thus, the time consuming extraction of sputum samples can be avoided for this type of analyses.

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