Establishment of a whole blood ELISA to quantify T-cellular cytokine release in response to Aspergillus fumigatus antigens

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Objectives

Depending on the host immune status, the ubiquitous mould Aspergillus fumigatus can cause a range of disease manifestations from invasive infection to severe hypersensitivity syndromes. Reliable bio-effect monitoring tools to efficiently track the multifaceted T-cell response to Aspergillus antigens are lacking. Flow cytometry or ELISPOT have been established to monitor Aspergillus-specific T-cells as a supportive biomarker for environmental exposure and invasive aspergillosis yet are complicated by their time- and resource-intensive nature and pre-analytic difficulties [1,2]. Cytokine release assays are successfully used as a T-cell-driven diagnostic marker for tuberculosis and cytomegalovirus (CMV) infections and are easy to use with minimal hands-on time. We therefore sought to develop a T-cell optimized whole blood-based interferon gamma (IFN-γ) and interleukin (IL)-17 release assay for A. fumigatus antigens.

Methods

Figure 1
0.5 ml hiraparinized blood was injected into stimulation tubes containing 0.5 ml RPMI medium (1:1), costimulatory molecules CD28 and CD49d plus GM-CSF phosphoprotein (pp) 65, A. fumigatus mycelial lysate (50 µg/ml final conc.), or recombinant A. fumigatus antigens (30 µg/ml final conc.); Aspf4, Aspf6, or Crf1. Phytomenadione (PHA) was used as a positive control. After incubation for 24-26 h, plasma was obtained by centrifugation and analysed by IFN-γ and IL-17 ELISA. The cytokine concentration determined in the background tube was subtracted from antigen-reactive cytokine concentrations in the stimulation tube.

Optimization of stimulation conditions for whole blood T-cell ELISA and assay validation in immunocompromised patients

Figure 2
A: Whole blood from 10 healthy mold-exposed subjects [1] was stimulated with an A. fumigatus mycelial lysate and 1 µg/ml CD28, 1 µg/ml CD49d + 1 µg/ml CD49d, or no co-stimulatory factors. Friedman test and Dunn’s multiple comparison test. (B) Samples from 8 GMF seronegative (green) and 8 seropositive (red) stem cell transplant recipients (60-180 days post-transplant) were stimulated with pp65 or PHA. Medians (black bars), inter-quartile ranges (boxes).

Whole blood ELISA to detect Aspergillus-specific T-cell activation in subjects with occupational mold exposure

Figure 3
Whole blood from 10 organic farmers (red) and a control group of 10 non-occupationally exposed subjects (green) was stimulated with A. fumigatus mycelial lysate as well as antigenic proteins Crf1, Aspf4, or Aspf6. IFN-γ (A) and IL-17 (B) concentrations were determined by whole blood ELISA. Medians (black bars), and inter-quartile ranges (colored boxes) are shown. Mann-Whitney test.

Technical and intra-individual variation of whole blood T-cell ELISA

Figure 4
Whole blood from 4 highly mold-exposed healthy subjects was stimulated with A. fumigatus lysate and Aspf4 (black bars). The grey-colored boxes represent 15% and 30% coefficients of variation (CV). To determine the intra-assay CVs, a second tube was injected by the same operator (blue diamonds).

Robustness of whole blood T-cell ELISA pre-analytic delays

Figure 5
Whole blood from 4 highly mold-exposed healthy subjects was stimulated with A. fumigatus lysate (AfuLy) or Aspf4 either immediately or after a 24 h pre-analytic storage period at ambient temperature. The grey-colored error range represents a +/- 2-fold deviation compared with the immediately incubated samples (black bars).

Conclusions

- Optimized whole blood T-cell ELISA with enhanced co-stimulation is a fast, simple, and resource-efficient assay to track T-cell responses to A. fumigatus antigens, providing a differentiated representation of individual T-cell subsets by their signature cytokines.
- The whole blood ELISA protocol is technically reliable, reproducible, and robust to pre-analytic delays.
- The assay is currently undergoing clinical validation in different patient cohorts including chronic pulmonary aspergillosis.


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