Candida-reactive T cells for diagnosis of invasive *Candida* infection

Felix C. Koehler¹, Oliver A. Cornely^{1,2,3}, Hilmar Wisplinghoff^{4,5,6}, Astrid C. Schauss¹, Jon Salanton-Garcia², Helmut Ostermann⁷, Maren Ziegler⁴, Petra Bacher⁸, Alexander Scheffold^{8,9}, Regina Alex¹⁰, Anne Richter¹⁰, Philipp Koehler^{1,2}

¹Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany ²Department I of Internal Medicine, ECMM Diamond Center of Excellence in Medical Mycology, German Centre for Infection Research (DZIF), University of Cologne, Cologne, Germany ³Clinical Trials Centre Cologne (ZKS Köln), University of Cologne, Cologne, Germany ⁴Labor Dr. Wisplinghoff, Cologne, Germany ⁵Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany ⁶Institute for Clinical Microbiology, University Witten/Herdecke, Witten, Germany ⁷Department of Internal Medicine III, University of Munich, Munich, Germany ⁸Department of Cellular Immunology, Clinic for Rheumatology and Clinical Immunology, Charité – University Medicine Berlin, Berlin, Germany ⁹German Rheumatism Research Centre (DRFZ) Berlin and Leibniz Association, Berlin, Germany ¹⁰Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Objectives

Invasive *Candida* infection (ICI) is the most common fungal bloodstream infection. Blood and tissue cultures are the current gold standard diagnostic methods, however false-negatives remain a clinical challenge. Tissue culture and histology burden certain risks for the patient and are often contraindicated due to underlying medical conditions. *Candida*-reactive T cells were quantitated based on the upregulation of CD69/CD154 (CD40L) from peripheral blood as new diagnostic read-out for ICI. In a pilot study, we examined healthy donors and three patient cohorts with either proven ICI, suspected ICI, or high risk of ICI.

Methods

Candida cells were lysed mechanically by gentleMACS[®] dissociator. Peripheral blood mononuclear cells (PBMC) of patients and healthy donors were isolated by density gravitation. PBMCs were stained with 7AAD (Miltenyi Biotec GmbH, Germany and eBioscience, San Diego, CA, USA) and count of viable cells was examined on a MACSQuant[®] flow cytometer (all Miltenyi Biotec GmbH, Germany). 1×10^6 cells/100 μ l and well were seeded into 96 well flat-bottom plates. PBMCs were incubated overnight at 37°C with 5% CO₂. Cultured cells were stimulated with CD28 and CD40 pure antibodies and co-incubated with lysate of either *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* or *C. krusei* for 5h in 5% CO₂. Each *Candida* spp. resulted in a separate stimulation. Missing challenge with fungal lysate served as negative control, Staphylococcal Enterotoxin B (SEB) as positive control and cells were analyzed on a MACSQuant[®] flow cytometer.

Figure 1. CD69/CD154 (CD40L) upregulation for measurement of *Candida*-reactive T cells.

Figure 2. Flow Cytometry - Gating Strategy and detection of *Candida albicans*-reactive T cells. Cell frequency (%) and absolute cell (n) count. (A) Gating strategy. Negative control. (B) Detection of *Candida*-reactive T cells. From left to right. Negative control; positive control; antigen-stimulated probe.

Statistics

Cut-off values discriminating between healthy donors, disease control and patients with proven ICI were calculated by receiver operating characteristic analysis using IBM SPSS Statistics software (Version 23, IBM Corporation, NY, USA). For clinical evaluation, patient cohorts were grouped according to the 2008 European Organisation for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria.

Results

In a prospective pilot study, we determined the performance of the *Candida*-reactive lymphocyte assay in 26 patients, including 16 proven ICI and one patient with probable hepatosplenic candidiasis. Nine hematological high-risk patients served as disease control and 14 healthy donors as negative control. To examine the mean frequency and to calculate the cut-off value of *C. albicans*-reactive T cells in healthy individuals we included an additional cohort of 96 healthy blood donors.

Thirteen of 16 patients with proven ICI and one patient with probable ICI had elevated levels of *Candida*-reactive CD4⁺ T cells. Due to autofluorescence of cells we excluded three candidemia patients from analysis. In 10 of 12 proven ICI, T cell reaction matched the *Candida* spp. identified by conventional diagnostics. One histology proven ICI patient had no species identification by standard diagnostics.

Disease and healthy control patients of the pilot study cohort had no elevated *Candida*-directed T cells counts.

Figure 3. Frequencies of *Candida*-reactive T cells in healthy donors, disease control and patients with probable or proven ICI. Test positivity considered elevated levels of *Candida*-reactive T cells with simultaneously 3.05 fold increase of antigen-stimulated T cells compared to unstimulated T cells. Dashed lines show cut-off values for *Candida albicans*-reactive T cells (0.40%) and *Candida glabrata*-reactive T cells (0.22%). (A) Frequencies of *Candida albicans* CD69⁺/CD154⁺ T cells among CD4⁺ T cells. (B) Frequencies of *Candida glabrata* CD69⁺/CD154⁺ T cells among CD4⁺ T cells.

Conclusion

The *Candida*-reactive lymphocyte assay correctly identified the majority of ICI patients by species level. Autofluorescence of cells and insufficient cell count of T cells are limiting factors. The *Candida*-reactive lymphocyte assay has the potential to complement current diagnostic assays for invasive *Candida* infection, especially when blood cultures remain negative and when tissue cultures and histopathology are contraindicated.

Acknowledgments

Mario Assenmacher, Elisa Barth, Denis Hoffmann, Larisa Idrizovic, Stefan Körfigen, Tatjana Lammertz and Julia Milleck.