Cytotherapy, 2013; 15: 344–351

International Society for Cellular Therapy
ISCT®

Clinical-scale generation of multi-specific anti-fungal T cells targeting *Candida*, *Aspergillus* and mucormycetes

LARS TRAMSEN[1], STANISLAW SCHMIDT[1], HALVARD BOENIG[2], JEAN-PAUL LATGÉ[3], CORNELIA LASS-FLÖRL[4], FRAUKE ROEGER[1], ERHARD SEIFRIED[2], THOMAS KLINGEBIEL[1] & THOMAS LEHRNBECHER[1]

[1]*Pediatric Hematology and Oncology and* [2]*German Red Cross Blood Donor Service Baden-Wuerttemberg-Hessen and Institute for Transfusion Medicine and Immunohematology, Johann Wolfgang Goethe University, Frankfurt, Germany,* [3]*Insitut Pasteur, Paris, France, and* [4]*Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria*

Abstract

Background aims. Invasive fungal infections, in particular, infections caused by *Candida*, *Aspergillus* and mucormycetes, are a major cause of morbidity and mortality in patients undergoing allogeneic hematopoietic stem cell transplantation. Adoptive transfer of donor-derived anti-fungal T cells shows promise to restore immunity and to offer a cure. Because T cells recognize only specific epitopes, the low rate of patients in which the causal fungal pathogen can be identified and the considerable number of patients with co-infection with several genera or species of fungi significantly limit the application of adoptive immunotherapy. *Methods.* Using the interferon-γ secretion assay, we isolated multi-specific human anti-fungal T cells after simultaneous stimulation with cellular extracts of *Aspergillus fumigatus*, *Candida albicans* and *Rhizopus oryzae.* Cells were phenotypically and functionally characterized by flow cytometry. *Results.* Of a total of 1.1×10^9 peripheral blood mononuclear cells, a median number of 5.2×10^7 $CD3^+CD4^+$ T cells was generated within 12 days. This cell population consisted of activated memory T_H1 cells and reproducibly responded to a multitude of *Aspergillus* spp., *Candida* spp. and mucormycetes with interferon-γ production. On re-stimulation, the generated T cells proliferated and enhanced anti-fungal activity of phagocytes and showed reduced alloreactivity compared with the original cell fraction. *Conclusions.* Our rapid and simple method of simultaneously generating functionally active multi-specific T cells that recognize a wide variety of medically relevant fungi may form the basis for future clinical trials investigating adoptive immunotherapy in allogeneic hematopoietic stem cell transplantation recipients with invasive fungal infection.

Key Words: *adoptive immunotherapy,* Aspergillus*,* Candida*, invasive fungal disease, mucormycete, T cell*

Introduction

Invasive fungal disease (IFD) is a major cause of life-threatening infections in immunocompromised patients, in particular, in allogeneic hematopoietic stem cell transplantation (HSCT) recipients. Although invasive aspergillosis is the most common IFD in this setting, there is a substantial risk for infections with *Candida* or mucormycetes, and the incidence of these infections appears to be increasing (1–3). Despite major improvements in supportive care, such as the availability of new broad-spectrum anti-fungal compounds, mortality rates of IFD in this patient population remain unacceptably high reaching 90% (2,4). This high mortality might be due at least in part to the long-lasting immunosuppression in patients after HSCT (5). Most IFD after allogeneic HSCT occurs when neutropenia and mucositis have resolved but cellular immunity is still impaired. Although it has become clear that anti-fungal T_H1 cells play an important role in the host response against IFD, the importance of cytotoxic T cells in this setting is not well defined (6). Because the number of T_H1 cells is decreased for a prolonged period after allogeneic HSCT (7), there is growing interest in restoring adaptive immunity against fungal pathogens by infusion of donor-derived anti-fungal T cells. The clinical benefit of adoptively transferred anti-*Aspergillus* T cells was shown in 10 allogeneic HSCT recipients with invasive aspergillosis (8). Although adoptive immunotherapy is a promising approach, the

The results of this work were presented in part at the 51th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, USA, 2011.
Correspondence: **Thomas Lehrnbecher**, MD, Pediatric Hematology and Oncology, Children's Hospital III, Johann Wolfgang Goethe University, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany. E-mail: Thomas.Lehrnbecher@kgu.de

(Received 26 June 2012; accepted 11 November 2012)

ISSN 1465-3249 Copyright © 2013, International Society for Cellular Therapy. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcyt.2012.11.014

feasibility of this strategy is limited for several reasons. First, in most patients, no distinct fungal pathogen can be isolated, which is considered a prerequisite for generation of pathogen-specific T cells that recognize specific epitopes. Second, the detection of a fungal antigen, such as galactomannan, is not proof that an infection is due to a specific fungal pathogen. Lastly, a significant number of patients are co-infected with different genera or species of fungi (1,2). In our previous work, we characterized anti-fungal T_H1 cells against *Aspergillus fumigatus*, *Candida albicans* and *Rhizopus oryzae* (9–11). In the present study, we aimed to develop a simple and rapid protocol for the simultaneous clinical-scale generation of a T_H1 cell population that targets *Aspergillus* spp., *Candida* spp. and mucormycetes. This strategy may offer new perspectives for adoptive immunotherapy trials in the setting of HSCT.

Methods

Study subjects

For the isolation of multi-specific anti-fungal T cells and the preparation of monocyte-derived autologous antigen-presenting cells (APCs), 100 mL of peripheral blood (small-scale experiments) and buffy coats (clinical-scale runs; provided by the Institute for Transfusion Medicine and Immunohematology, Frankfurt, Germany) of healthy individuals were used. The protocol was approved by the local ethics committee.

Fungal antigens

Water-soluble cellular extracts of *A. fumigatus* (CBS 144-89), *C. albicans* (CA444) and *R. oryzae* (clinical isolate; University of Innsbruck, Innsbruck, Austria) were prepared as described previously (12). For testing specificity, cellular extracts of *Rhizopus microsporus, R. microsporus var. oligosporus, Rhizomucor pusillus, Mucor circinelloides*, *M. racemosus*, (all clinical isolates; University of Innsbruck), *C. torpicalis* (CBS94), *A. niger* (CBS73388), *penicillium chrysogenum* (IP1652) and *Alternaria alternata* (IP1563) were used. Extracts were tested for sterility and endotoxin concentration.

Generation and expansion of multi-specific anti-fungal T cells

The clinical-scale generation of anti-fungal T cells was performed as previously described with some modifications (13). A total of 1.1×10^9 peripheral blood mononuclear cells (PBMCs), obtained by Ficoll/Paque (Biochrom, Berlin, Germany) density gradient centrifugation, were suspended in 100 mL X-VIVO 10 Medium without phenol red (BioWhittaker, Verviers, Belgium), 100 IU/mL penicillin G, 100 μg/mL streptomycin (Invitrogen, Paisley, UK) and 10% heat-inactivated Good Manufacturing Practices-grade human serum (Center for Clinical Transfusion Medicine, Tübingen, Germany). PBMCs were stimulated for 16 h at 37°C with antigen extracts of *A. fumigatus*, *C. albicans* and *R. oryzae* (10 μg/mL each). Activated T cells were enriched using the clinical-scale CliniMACS Cytokine Capture System (IFN-γ) (Miltenyi Biotec, Bergisch Gladbach, Germany) on the CliniMACS device (Miltenyi Biotec) according to the manufacturer's instructions. The separation was performed in a closed system.

After enrichment, cells were evenly distributed onto two 25-cm^3 cell culture flasks (Corning Inc, Corning, NY, USA) and suspended in 10 mL of culture medium in the presence of 1×10^7 irradiated autologous PBMCs, which were obtained from the negative fraction after cell separation. The cells were cultured for 12 days, supplemented with recombinant human interleukin-2 (50 IU/mL; Chiron, Ratingen, Germany) on days 0, 3, 6, 9 and 11 and with fresh medium when necessary. Irradiated autologous monocytes served as APCs and were obtained from PBMCs of the negative fraction by adherence method as described previously (9). APCs were incubated with the respective antigens (7.5 μg/mL) overnight and added on days 5 and 8. Residual expanded anti-fungal T cells were aliquoted and cryopreserved in X-VIVO 10 Medium containing 10% human serum and 20% dimethyl sulfoxide (Sigma-Aldrich, Steinheim, Germany).

Before the clinical-scale runs, small-scale experiments were performed to optimize the methodology. Three of these small-scale experiments that were identical to the clinical-scale generation regarding human cell source, media, antigens and culturing procedures were included in the analysis. For the small-scale experiments, 1.1×10^8 PBMCs were stimulated with the fungal antigens and enriched using the IFN-γ CSA MiniMACS device (Miltenyi Biotec) and cultured in 2 mL of medium in a 24-well plate (Nunc, Wiesbaden, Germany).

Phenotypic and functional characterization of multi-specific anti-fungal T cells

Phenotype analyses were performed using the flow cytometer BD FACS Canto II (BD Biosciences, San Jose, USA) and monoclonal antibodies against CD3, CD4, CD8, CD14, CD19, CD45RO, CD56, CD62L, CD69, CD154, HLA-DR (all BD Biosciences) and 7-AAD (Coulter Immunotech, Marseille, France). In addition, the number of viable cells was assessed using trypan blue staining and conventional microscopy.

Assessment of intracellular cytokine secretion was performed by means of intracellular cytokine

Download English Version:

https://daneshyari.com/en/article/2171860

Download Persian Version:

https://daneshyari.com/article/2171860

Daneshyari.com