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Clinical-scale isolation of the total *Aspergillus fumigatus*–reactive T–helper cell repertoire for adoptive transfer

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Abstract

Background aims. Evidence of the criticality of the adaptive immune response for controlling invasive aspergillosis has been provided. This observation is supported by the fact that invasive aspergillosis, a grave complication of allogeneic stem cell transplantation, occurs long after myeloid reconstitution in patients with low T-cell engraftment and/or on immunosuppressants. Adoptive T-cell transfer might be beneficial, but idiosyncrasies of *Aspergillus fumigatus* and the anti-*Aspergillus* immune response render established selection technologies ineffective. *Methods.* We developed a Good Manufacturing Practice (GMP)-compliant protocol for preparation of *A. fumigatus*–specific CD4+ cells by sequentially depleting regulatory and cytotoxic T cells, activating *A. fumigatus*-specific T-helper cells with GMP-grade *A. fumigatus* lysate, and immunomagnetically isolating them via the transiently up-regulated activation marker, CD137. *Results.* In 13 full-scale runs, we demonstrate robustness and feasibility of the approach. From 2×10^9 peripheral blood mononuclear cells, we isolated 27×10^3–318×10^3 *Aspergillus*-specific T-helper cells. Frequency among total T cells was increased, on average, by 200-fold. Specific studies indicate specificity and functionality: After non-specific *in vitro* expansion and re-stimulation with different antigens, we observed strong cytokine responses to *A. fumigatus* and some other fungi including *Candida albicans*, but none to unrelated antigens. *Discussion.* Our technology isolates naturally occurring *Aspergillus*-specific T-helper cells within 2 days of identifying the clinical indication. Rapid adoptive transfer of *Aspergillus*-specific T cells may be quite feasible; the clinical benefit remains to be demonstrated. A manufacturing license as an advanced-therapy medicinal product was received and a clinical trial in post-transplantation invasive aspergillosis patients approved. The product is dosed at 5 × 10E3/kg T cells (single intravenous injection), of which at least 10% must be *A. fumigatus*–specific.

Key Words: *aspergillosis, cell therapy, GMP-compliant protocol, prospective isolation, T-helper cells*

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Introduction

The prolonged immunodeficiency after allogeneic hematopoietic stem cell transplantation continues to pose a clinical challenge. It predisposes the patients for infections, often originating from opportunistic microbes requiring control through functional T-cell responses. Among the agents most commonly associated with severe infections in the later post-transplant period are viruses of the Herpes and Polyoma families, adenovirus, and fungi, such as *Aspergillus* spp., most commonly *A. fumigatus*. Adoptive transfer of T cells specific for cytomegalovirus (CMV), Epstein-Barr virus and adenovirus has demonstrated clinical efficacy, and standard methods are available for their isolation.

In theory, unmodified donor lymphocyte apheresis products containing low frequencies of T cells reactive for each individual antigen are suitable to confer adoptive immunity because they can expand after antigen contact. However, the risk of graft-versus-host disease and the necessity of counteracting it with immunosuppressants (which will concurrently suppress any ongoing adaptive T-cell response) typically outweigh the potential benefits. Therefore, for adoptive transfer, antigen-specific T cells are typically enriched to minimize the number of co-transfused potentially allo-reactive T cells. Adoptive transfer of enriched virus antigen-specific T cells has thus taken center stage clinically [1–5].

Several techniques for isolation of antigen-specific T cells have been successfully applied. In principle, they are based on either antigen-driven *in vitro* expansion or prospective isolation. The advantages and disadvantages of the approaches have been debated elsewhere [6–8]; both appear to be feasible and active clinically. For prospective isolation of virus-specific T cells, selection based on antigen-induced interferon (IFN)-γ secretion or fishing for antigen-specific T cells with multimerized human leukocyte antigen complexes have both been successfully applied [2,9–12]. With respect to generation of *A. fumigatus*–specific T cells, however, neither method is aptly suitable: *A. fumigatus* is a complex pathogen with approximately 10,000 genes but apparently lacks one or a few immunodominant epitopes [13]. Moreover, unlike virus-specific T cells, *A. fumigatus*–reactive T cells do not respond with a predominant effector cytokine response, excluding cytokine secretion–based isolation. Also, the frequency of fungus-specific T cells is low (typically below 1:200 CD4+ T cells) [14,15]. Therefore, an alternative process had to be developed for direct Good Manufacturing Practice (GMP)-compatible enrichment of *Aspergillus* antigen-specific T cells. The one advantage over some of the viral antigens is that *A. fumigatus*–specific T cells are present in every healthy donor, likely because of the ubiquitous prevalence of the fungus in our environment [13,15]. Therefore, in recipients of allogeneic grafts, the original stem cell donor will essentially always be suitable as T-cell donor. In keeping with its predominant role as an environmental antigen, a significant fraction of *A. fumigatus*–responsive T cells are regulatory in nature [15]; for adoptive transfer to invasive aspergillosis (IA) patients these would be counter-productive and must therefore be removed before transfer.

To address these challenges, a completely new, fully GMP-compliant approach for prospective isolation of *A. fumigatus*–specific T cells was developed. After immunomagnetic depletion of CD8+ and Treg cells from unstimulated apheresis products, the residual cells are stimulated using a soluble whole *A. fumigatus* lysate. Antigen-activated cells expressing CD137 are subsequently immunomagnetically enriched. By using this approach, the small numbers of conventional *A. fumigatus*–specific T-helper cells can be directly isolated with high efficiency and purity. Preliminary evidence of functionality and specificity is provided by experiments showing that the cells can be expanded *in vitro* and display antigen-specific reactivity against several human-pathogenic *Aspergillus* species, some cross-reactivity against *Candida albicans*, but no unspecific responsiveness. The results of the process development and method validation are presented here. A manufacturing authorization was obtained and a clinical trial with transfer of a single dose of up to 3000/kg *A. fumigatus*–specific T-helper cells with the intent of eliminating IA is currently enrolling.

Methods

Apheresis material

Healthy volunteer donor steady-state leukapheresis products served as the starting material. Collections were performed with routine clinical apheresis equipment from TerumoBCT, following national guidelines as outlined in standard operation protocols [16]. Cells were collected with written informed donor consent upon approval of the ethics committee of Goethe University Medical Center (permit number 401/12).

Isolation of A. fumigatus–reactive T-helper cells

Erythrocytes and granulocytes were depleted from fresh apheresis products, using density centrifugation. Apheresis product was layered over Ficoll