Immunotherapy of invasive fungal infection in hematopoietic stem cell transplant recipients

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Despite the availability of new antifungal compounds, invasive fungal infection remains a significant cause of morbidity and mortality in children and adults undergoing allogeneic hematopoietic stem cell transplantation (HSCT). Allogeneic HSCT recipients suffer from a long lasting defect of different arms of the immune system, which increases the risk for and deteriorates the prognosis of invasive fungal infections. In turn, advances in understanding these immune deficits have resulted in promising strategies to enhance or restore critical immune functions in allogeneic HSCT recipients. Potential approaches include the administration of granulocytes, since neutropenia is the single most important risk factor for invasive fungal infection, and preliminary clinical results suggest a benefit of adoptively transferred donor-derived antifungal T cells. In vitro data and animal studies demonstrate an antifungal effect of natural killer cells, but clinical data are lacking to date. This review summarizes and critically discusses the available data of immunotherapeutic strategies in allogeneic HSCT recipients suffering from invasive fungal infection.

Keywords: invasive fungal infection, allogeneic hematopoietic stem cell transplantation, immunotherapy, granulocyte, T cell, natural killer cell

Invasive fungal infection remains a significant cause of morbidity and mortality in children and adults undergoing allogeneic HSCT. In the majority of invasive infections are due to A. fumigatus, followed by A. flavus, A. niger, and A. versicolor (Morgan et al., 2005; Pagano et al., 2007). In addition, recent studies report on an increasing incidence of infections due to Mucorales which are usually caused by the genera Rhizopus, Rhizomucor, Mucor, and Lichtheimia (Cuenca-Estrella et al., 2009). Although the definitive diagnosis of invasive fungal infection in the population of allogeneic HSCT recipients is difficult to establish, two recent studies report on an overall incidence of 3.4 and 7.8% of proven and probable invasive fungal infections, respectively; however, it is important to note that a considerable variability across institutions has been reported, and incidence rates may vary between 0.9 and 13.2% (Dvorak et al., 2005; Pagano et al., 2007; Kontoyiannis et al., 2010).

Despite the availability of new antifungal compounds such as broad-spectrum triazoles or the new class of echinocandins, morbidity and mortality of invasive fungal infections are still unacceptably high. In this respect, recent analyses report on mortality rates between 36% (combined analysis for autologous and allogeneic HSCT) and 67% (analysis for allogeneic HSCT from unrelated donors) due to invasive aspergillosis (Mikulska et al., 2009; Nefyotis et al., 2009). In patients with mucormycosis, 1-year mortality exceeded 70% (Dvorak et al., 2005; Pagano et al., 2007; Kontoyiannis et al., 2010).

Although pharmacological prophylactic measures (e.g., the institution of antifungal prophylaxis with posaconazole) and improved therapeutic approaches have decreased morbidity and mortality of invasive fungal infections, experts agree that the integrity of host defenses remains the mainstay of defense. Allo-
With *A. fumigatus* major complications (O’Donghaile et al., 2012). In addition, antigen (HLA) alloimmunization have been reported as potential (acute respiratory distress syndrome, ARDS) and human leukocyte described granulocyte transfusions as safe, fever and chills may be seen in up to 20% of transfusions, and pulmonary reactions (acute respiratory distress syndrome, ARDS) and human leukocyte antigen (HLA) alloimmunization have been reported as potential major complications (O’Donghaile et al., 2012). In addition, a randomized phase III study evaluating the effect of granulocyte transfusions in 74 neutropenic patients, most of them after allo- genic HSCT (*n* = 39), failed to demonstrate a significant benefit of this approach (Sendel et al., 2008). The administration of granulocytes did not improve survival rates until day 100 in patients with fungal (*n* = 53) and other infections. The authors attributed the lack of efficacy to procedural obstacles, which is supported by the observation that neutrophil reconstitution was equal in the treatment and control arm indicating that the granulocyte transfusions administered at the schedule and dose used were not sufficient to accelerate a lasting peripheral blood neutrophil reconsti- tution. Therefore, the results of large prospective randomized trials assessing the effect of high dose granulocyte transfusion in neutropenic patients, such as the ring study (NCT00627393) are urgently needed to prove or refute the empirical evidence of the benefit of granulocyte transfusion in this setting.

**T CELLS AND INVASIVE FUNGAL INFECTION**

In contrast to a relatively rapid recovery of cells of innate immunity such as granulocytes, both the absolute levels and function of B and T lymphocytes remain abnormal for many months (Byrich et al., 2001; Peggs and Mackinnon, 2004). T cells are known to possess such antigenic properties of *Aspergillus* and materials and reagents for clinical manufacture (Gaudard et al., 2012). In this protocol, cells were expanded with a cocktail of IL-2, IL-7, and IL-15, which resulted in a 30-fold increase in cell numbers over 21 days of culture. Generated cells were predominantly effector and central memory CD4+ T cells, which produced Th1 and Th17 cytokines and expanded upon re-stimulation. Interestingly, the functionally active anti-*Aspergillus* Th1 cells elicited limited cross-reactivity with other *Aspergillus* species and fungi (Beck et al., 2006). As compared to unselected CD4+ T cells, the gener- ated anti-*Aspergillus* Th1 cells exhibited reduced allosreactivity in vitro (Tramsen et al., 2009).

Recently, a simple, robust and clinically applicable procedure of generating anti-*Aspergillus* T cells was reported using an envi- ronmental strain of *A. fumigatus* and materials and reagents for clinical manufacture (Gaudard et al., 2012). In this protocol, cells were expanded with a cocktail of IL-2, IL-7, and IL-15, which resulted in a 30-fold increase in cell numbers over 21 days of culture. Generated cells were predominantly effector and central memory CD4+ T cells, which produced Th1 and Th17 cytokines and expanded upon re-stimulation. Although most groups have employed lyastes from *Aspergillus* isolates for the generation of protecting anti-*Aspergillus* T cells (Perruccio et al., 2005; Tramsen et al., 2009; Khanna et al., 2011; Gaudard et al., 2012), the use of antigen extracts may be con- sidered problematic from a regulatory standpoint. On the other hand, whereas the immunogenic antigens of viruses such as aden- ovirus, cytomegalovirus (CMV) or Epstein-Barr virus (EBV) are well described, the antigenic properties of *A. fumigatus* are rather complex and only a few of the hundreds of (glyco)proteins of...
the fungus reported in the literature have been characterized at a molecular and biochemical level (Lange, 1999). It has recently been demonstrated that different fungal components are endowed with a distinct, yet overlapping, capacity to activate protective and non-protective T المسند responses in mice and humans (Bozza et al., 2009). In addition, an immunodominant epitope derived from A. fumigatus cell wall glucanase Crf1, restricted to three common main histocompatibility complex class II alleles, HLA-DRB1*03, -04, and -13, has been identified which induces cross-reactive CD4+ T المسند immunity not only against A. fumigatus, but also against C. albicans (Stuehler et al., 2011). However, beside the restriction to donors with certainHLA-alleles, it is important to note that the Aspergillus cell wall is a highly dynamic structure with the ability to adapt to different environments. Depending on the specific environment, each of the fungal morphotypes may exhibit different biological features, which in turn, can influence the pathogenicity of the pathogen and may result in the differential expression of antigens on its surface. Therefore, the fungal pathogen could easily escape from adoptive immunotherapy using antifungal T المسند recognizing only a single antigen, whereas an approach using a fungal extract which includes multiple antigens for generating antifungal T المسند might decrease this risk. To this end, the specific advantages and limitations of the use of a lysate from Aspergillus isolates and of single or multiple antigens have to be evaluated, and further studies will show which will be the most feasible approach in the clinical setting.

Unfortunately, in the clinical setting, no distinct fungal pathogen can be isolated and characterized in the majority of patients with a suspected invasive fungal infection; this, however, is considered to be a prerequisite for the determination of the antigen in order to generate pathogen-specific T المسند cells. In addition, imaging studies such as computed tomography (CT) scan or the detection of a fungal antigen such as galactomannan, does not prove an infection due to a specific fungal pathogen. Lastly, a considerable number of patients suffer from coinfection with several species or genera of fungi. Therefore, similar to the approach in antiviral immunotherapy using T المسند populations that target CLR, the generation of multi-specific T المسند which target a variety of different clinically important fungi such as Aspergillus spp., Candida spp., and Mucor spp. would be of major advantage.

We recently reported on an approach of generating multi-specific human antifungal T المسند after simultaneous stimulation with cellular extracts of A. fumigatus, C. albicans, and Rhizopus oryzae (Teismen et al., 2013a). These generated cells consisted of activated memory T المسند cells and reproducibly responded with IFN-γ production to a broad-spectrum of medically important fungal pathogens, such as A. fumigatus, A. niger, Penicillium chrysogenum, C. albicans, C. tropicalis, M. circinelloides, Rhizomucor pusillus, Rhizopus oryzae, Rhizopus microsporus, and Rhizopus microsporus-oligosporus. Upon re-stimulation, the generated T المسند cells proliferated and enhanced antifungal activity of phagocytes, and showed reduced alloreactivity as compared to the original cell fraction. The ultimate goal would be to generate antifungal T المسند against all medically important fungi.

Various antifungal agents have been shown to influence the function of the host immune response. For instance, early studies demonstrated that itraconazole suppresses random movement and chemotaxis of neutrophils (Vaddhakul et al., 1990), and liposomal amphotericin B and amphotericin B-deoxycholate show different immunoregulatory effects on human peripheral blood mononuclear cells (Beyes et al., 2000). In addition, liposomal amphotericin B suppresses specific activity of cytotoxic CD8+ T المسند in the setting of murine listeriosis (Ketschmar et al., 2011). In contrast, we recently reported that various concentrations of commonly used antifungal compounds such as amphotericin B-deoxycholate, liposomal amphotericin B, fluconazole, voriconazole, posaconazole, and caspofungin did not significantly influence the secretion of IFN-γ and tumor necrosis factor-alpha (TNF-α) by human anti-Aspergillus and human anti-Candida T المسند cells (Teismen et al., 2013b). The proliferation of these cells was slightly decreased by posaconazole at high concentrations only, which, however, did not reach statistical significance. These data suggest that antifungal T المسند can be safely administered together with commonly used antifungal compounds.

ANTIFUNGAL ACTIVITY OF NATURAL KILLER CELLS

Since natural killer (NK) المسند are able to kill tumor cells in vitro, there is increasing interest in using NK المسند as adoptive immunotherapy against malignancies in HSCT recipients. In contrast to adoptively transferred donor-derived T المسند, which are associated with the risk of GvHD, NK المسند are usually well tolerated and may even mitigate GvHD (Passweg et al., 2006). In addition to the antitumor effect, NK المسند exhibit cytotoxicity against virus-infected cells and activity against bacteria such as Staphylococcus aureus and against various parasites (Biron, 1997; Lacke et al., 2004; Small et al., 2008). There is also growing evidence of in vitro and animal studies that NK المسند play an important role in the host response against fungal pathogens. For example, in vitro data demonstrate that NK المسند are able to damage Aspergillus spp. and Rhizopus oryzae (Bouzani et al., 2011; Schmidt et al., 2011, 2012). Importantly, hyphae of both fungi are damaged by both freshly isolated and IL-2 pre-stimulated NK المسند, whereas conidia are not affected (Bouzani et al., 2011; Schmidt et al., 2011, 2012). The in vitro data on the damage of Aspergillus spp. by NK المسند are supported by animal studies (Morrison et al., 2003; Park et al., 2009). For example, in neutropenic mice suffering from pulmonary aspergillosis, the depletion of NK المسند by antibodies resulted in a greater than twofold increase in mortality and markedly reduced clearance of the pathogen from the lungs (Morrison et al., 2003). Similarly, depletion of NK المسند reduced lung IFN-γ levels and subsequently increased fungal load, whereas the transfer of activated NK المسند from wild-type, but not from IFN-γ-deficient mice resulted in greater pathogen clearance from the lungs, which supports the importance of functionally active NK المسند in the antifungal host response (Park et al., 2009). If further studies evaluating the effect and side effects of adoptively transferred NK المسند to an immunocompromised host with invasive fungal infection will demonstrate a benefit, NK المسند might become an interesting tool in immunotherapeutic antifungal strategies.

CONCLUDING REMARKS

Invasive fungal infections, in particular infections due to Aspergillus, Candida, and Mucormycetes, are still a major
cause of morbidity and mortality in HSCT patients. These patients suffer from long lasting defects of the host immune response, and despite the availability of new antifungal drugs, mortality in this patient population is unacceptable high. Over the last decades, our knowledge of the immunopathogenesis of invasive fungal infections has greatly advanced, and the role of different factors, such as the modes of phagocytosis, T cells and NK cells have been elucidated. Data of in vitro experiments and animal studies have provided us with important information to augment host immunity, which resulted in growing interest in the clinical application of immunotherapeutic approaches in HSCT recipients suffering from invasive fungal infections. Although further information obtained from preclinical in vivo models is crucial, only clinical trials will ultimately demonstrate the impact of specific cell populations in the prevention or treatment of invasive fungal infections in the transplant recipient. These clinical trials have to address important questions such timing of intervention, type and dosing of adoptively transferred cells, and eligible patients. The latter most likely depends on the host’s unique genetic background, which might have important impact on susceptibility, clinical course, and outcome of invasive fungal infections. In addition, it is important to note that patients suffering from invasive fungal infection are a heterogeneous population, not only regarding the underlying pathogen, but also regarding affected organs as well as antifungal pre-treatment. Therefore, it will be difficult to prove a clinical benefit of a specific immunotherapeutic strategy in a sufficiently powered number of patients. In order to design meaningful clinical trials, international, multi-center collaboration is required, which hopefully will improve the outcome in immunocompromised patients suffering from invasive fungal infection.

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