Immunotherapy of invasive fungal infection in hematopoietic stem cell transplant recipients

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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

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.
with *A. fumigatus* major complications (O’Donghaile et al., 2012). In addition, antigen (HLA) alloimmunization have been reported as potential complications including invasive fungal infection (Mousset et al., 2005; Grigull et al., 2006; Atay et al., 2011). Although the studies suggested that adaptive immunity, in particular CD4+ cells, has an important function in the host response against fungi. For example, non-neutropenic patients with advanced AIDS have a high risk for invasive aspergillus (Denning, 1998). 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 some graft-versus-malignancy effect and to play an important role in the defense against viral pathogens (Bader et al., 2004; Peggs and Mackinnon, 2004). However, there is a growing body of evidence suggesting that adaptive immunity, in particular CD4+ T lymphocytes, has an important function in the host response against fungi. For example, non-neutropenic patients with advanced AIDS have a high risk for invasive aspergillus (Denning, 1998). In addition, in the transplant setting, the median time of invasive aspergillus infection has been observed to be around 8 weeks (range 3–64 days), 77 days (range 0–2219 days), and 173 days (range 0–2254 days) after HSCT, respectively, a time, when neutropenia and mucositis have generally resolved, but adaptive immune responses are still hampered (Hamza et al., 2004; Neofytos et al., 2009). An early study demonstrated that the presence of CD4+ T lymphocytes, which secrete interferon-gamma (IFN-γ) upon stimulation with *A. fumigatus* antigens indicating a T helper type 1 (Th1) cell response, correlated with a favorable outcome (Hebart et al., 2002).

It has also been shown that for up to 1 year after HSCT, transplant recipients have a significantly reduced number of functionally active anti-*A. fumigatus* T cells compared to healthy controls, and lymphopenia is associated with a higher risk of death of transplant recipients with invasive fungal infection (Beck et al., 2008; Mikulska et al., 2009). These data provide the rationale of adaptively transferring in vitro manufactured anti-*A. fumigatus* T cells for prevention or for treatment of Aspergillus infections. In the first clinical trial, ten haploidentical transplant recipients with evidence of invasive aspergillosis (e.g., pulmonary infiltrates, positive galactomannan test) received anti-*A. fumigatus* T cells in the dose range of 1 × 10^6 to 1 × 10^7 per kg body weight between 17 and 37 days after transplantation (Perruccio et al., 2005). In all 11 patients, galactomannan positivity disappeared within 6 weeks of infusion of anti-*A. fumigatus* T cells whereas it persisted in the 13 controls not receiving immunotherapy for as long as monitored. In addition, 9 out of the 10 patients who had received anti-*A. fumigatus* T cells cleared invasive aspergillosis, whereas this was seen in only 7 out of the 13 controls. Anti-*A. fumigatus* T cell clonese were prepared by using heat-inactivated conidia for stimulation followed by limiting dilution which involved complex cell manipulation.

Consequently, there was much interest in improving the techniques for the clinical scale-generation of anti-*A. fumigatus* T cells. Tramsen et al. (2009) used an Aspergillus extract for stimulation; pathogen-specific antifungal cells were selected by means of the IFN-γ secretion assay and expanded. Out of a total of 1.1 × 10^6 white blood cells from a leukapheresis product, a median number of 2 × 10^5 CD4+ T cells were obtained within 13 days. The cultured anti-*A. fumigatus* cells exhibited almost exclusively a memory acti-vated Th1 cell phenotype. Upon re-stimulation, the generated cells produced IFN-γ, but not interleukin (IL)-4 or IL-10, indicating a Th1 population. In addition, the cells were not end-differentiated T cells, since they proliferated upon re-stimulation. Interestingly, the functionally active anti-*A. fumigatus* Th1 cells elicited limited cross-reactivity with other Aspergillus species and fungi (Beck et al., 2006). As compared to unselected CD4+ T cells, the generated anti-*A. fumigatus* T cells exhibited reduced alloreactivity in vivo (Tramsen et al., 2009).

Recently, a simple, robust and clinically applicable procedure of generating anti-*A. fumigatus* T cells was reported using an environmental 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 lysates from *Aspergillus* isolates for the generation of protecting anti-*A. fumigatus* 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 considered problematic from a regulatory standpoint. On the other hand, whereas the immunogenic antigens of viruses such as adenovirus, 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...
We recently reported on an approach of generating multi-specific Aspergillus cell wall glucanase Crf1, restricted to three common fungal species or genera of fungi. Therefore, similar to the approach not prove an infection due to a specific fungal pathogen. Lastly, a patient with a suspected invasive fungal infection; this, however, did not reach statistical significance. These data suggest that antifungal T cells can be safely administered together with commonly used antifungal compounds.

**ANTIFUNGAL ACTIVITY OF NATURAL KILLER CELLS**

Since natural killer (NK) cells are able to kill tumor cells in vitro, there is increasing interest in using NK cells as adoptive immunotherapy against malignancies in HSCT recipients. In contrast to adoptively transferred donor-derived T cells, which are associated with the risk of GvHD, NK cells are usually well tolerated and may even mitigate GvHD (Passweg et al., 2006). In addition to the antitumor effect, NK cells exhibit cytotoxicity against virus-infected cells and activity against bacteria such as Staphylococcus aureus and against various parasites (Biron, 1997; Lieke et al., 2004; Small et al., 2008). There is also growing evidence of in vitro and animal studies that NK cells play an important role in the host response against fungal pathogens. For example, in vitro data demonstrate that NK cells 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 cells, 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 cells 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 cells 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 cells reduced lung IFN-γ levels and subsequently increased fungal load, whereas the transfer of activated NK cells 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 cells in the antifungal host response (Park et al., 2009). If further studies evaluating the effect and side effects of adoptively transferred NK cells to an immunocompromised host with invasive fungal infection will demonstrate a benefit, NK cells 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 challenge in the treatment of patients with cancer and other immune deficiencies. 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 (Beys et al., 2000). In addition, liposomal amphotericin B suppresses specific activity of cytotoxic CD8+ T cells in the setting of murine listeriosis (Kretschmar 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 cells (Teammen 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 cells can be safely administered together with commonly used antifungal compounds.
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 fungal antifungals, morbidity and mortality in this patient population is unacceptably high. Over the last decades, our knowledge of the immunopathogenesis of invasive fungal infections has greatly advanced, and the role of different cell types, the roles of phagocytes, NK cells and NK cell subsets, CD8 T-cells and CD4 T-cells 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 heterogenous 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.

REFERENCES


Lehrnbecher et al. Antifungal immunotherapy

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