Immunotherapy for Invasive Aspergillosis in Immunocompromised Post-Engraftment Allogeneic Bone Marrow Transplant Patients

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Abstract. Invasive aspergillosis (IA) is a dangerous infection that is common in immunocompromised patients. IA is a major cause of mortality in bone marrow transplant (BMT) patients due to steroid-induced immunosuppression and chemotherapy-induced neutropenia. In normal individuals, Aspergillus is controlled by a Type 1 immune response. However, immunocompromised patients have a decreased ability to mount a Type 1 immune response. BMT patients are treated with glucocorticoids to suppress the Type 1 immune response which is associated with graft versus host disease (GVHD) toxicity. Therefore it is a complex problem to develop strategies to enhance Type 1 immunity without also causing GVHD. To overcome this problem, we propose that multiple intradermal injections of activated allogeneic Th1 memory cells will create a pool of alloantigen-specific Th1 memory cells in the circulation. Intradermal allogeneic injections are expected to be rejected and thus not cause GVHD. Additional intradermal allogeneic Th1 cell injections should activate the anti-alloantigen memory cells in circulation causing them to migrate to the sites of fungal infection and produce Type 1 cytokines. This Type 1 cytokine production in the microenvironment of the fungal infection should serve as an adjuvant to the stimulation of innate immune responses against the fungus and the development of Type 1 anti-fungal adaptive immunity.

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Abbreviations used: IA, invasive aspergillosis; BMT, bone marrow transplant; GVHD, graft versus host disease

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1. Introduction

The creation of a high titer of alloantigen-specific Th1 immune cells in the circulation of immunosuppressed patients and the subsequent reactivation of this memory pool is proposed to provide a novel strategy to eliminate and protect these patients from opportunistic infections. The proposed mechanism of this protection is based on the concept of “heterologous immunity”. Heterologous immunity is an immunological mechanism that occurs when the immune system is biased by a high frequency of memory T cells specific for a first infection; and, whereby the subsequent reactivation of these memory cells alters the host’s primary immune response to a second unrelated infection [1,2].

The focus of our hypothesis is the development of a treatment for Aspergillus infection in immunocompromised patients, especially patients post-allogeneic bone marrow transplant (BMT). However, we feel the same approach may also potentially benefit patients with viral infections, such as hepatitis B and C, as well as patients with advanced cancers.

Aspergillus spp is a ubiquitous fungus found in nature. It is commonly isolated from soil, plant debris, and the indoor air environment. Among all filamentous fungi, Aspergillus is the one most commonly isolated in invasive infections and Aspergillus fumigatus is the most common cause of invasive aspergillosis (IA) in the EU and USA [3].

IA is a fulminant and highly lethal infection that is common in immunocompromised patients [4,5]. The incidence of IA is increasing despite recent advances in therapy and IA remains a major cause of mortality in immunosuppressed patients [6]. IA is an especially serious problem following BMT procedures due to steroid-induced immunosuppression and chemotherapy-induced neutropenia. IA is the leading cause of both nosocomial pneumonia and death in recipients of allogeneic BMT [7,8].

IA infection is initiated upon inhalation of conidia (fungal spores) by immunocompromised patients. Conidia are efficiently cleared from the lungs in healthy individuals, but in immunocompromised patients they can germinate to form hyphae that invade the surrounding tissues, resulting in a severe and progressive pneumonia that can subsequently disseminate to other organs.

The antifungal agents approved for the treatment of IA have clinical response rates ranging from 33% to 52% [9,10]. Current therapies for IA are inadequate and include: voriconazole [11]; amphotericin B, which causes nephrotoxicity in 80% of patients [12]; liposomal amphotericin B which is a less nephrotoxic formulation [13], but can be hepatotoxic and is expensive; itraconazole which has many drug interactions [14]; surgical excision of infarcted tissue [15]; and caspofungin [16], recently approved by the US Food and Drug Administration as salvage therapy for IA patients refractory or intolerant to other therapies. Despite aggressive anti-fungal therapy with these agents, the prognosis for IA in BMT patients remains extremely poor with mortality rates of 90% or more [17,18].

Evidence from normal individuals, as well as in patients surviving IA demonstrate that protection from disease is correlated with a Type 1 immune response [19]. Immunosuppressed patients lack the ability to mount a Type 1 immune response making these patients particularly susceptible to opportunistic infections with these organisms.

In immunosuppressed patients refractory to standard treatments for IA, immunotherapy strategies that can stimulate Type 1 immunity are being developed for treatment of this opportunistic infection [20,21]. However, vaccination or adoptive transfer of immune cells to create Type 1 immunity is an especially difficult challenge in the post allogeneic BMT setting where patients are immunosuppressed to prevent lethal graft vs. host disease (GVHD).

GVHD is a lethal side effect of allogeneic BMT and is correlated with a "cytokine storm" of Type 1 cytokines [22]. GVHD is prevented by treatment with glucocorticoids which directly enhance Type 2 cytokine production and inhibit Type 1 cytokine production [23]. Therefore, the challenge in this setting is to elicit a Type 1 cytokine response to eliminate the lethal Aspergillus infection without also eliciting lethal
2. Hypothesis

Post-BMT patients with opportunistic Aspergillus infection can be treated with multiple intradermal inoculations with activated Th1 memory cells that are allogeneic to both the host and the graft. It is predicted that this treatment will elicit an anti-alloantigen Type 1 memory immune response that when reactivated by subsequent alloantigen injections will serve as an adjuvant for activation of both innate and adaptive anti-fungal immunity to clear Aspergillus infection. The allogeneic cell injections will be rejected by the host without causing GVHD.

3. Discussion

We have previously hypothesized that ex-vivo differentiated and expanded Th1 memory cells activated prior to infusion could elicit antitumor immunity in immunocompetent patients without pre-conditioning or GVHD toxicity; and without the requirement for a matched donor by a mechanism called the “Mirror Effect” [24]. We now extend this hypothesis to apply to immunosuppressed patients with opportunistic infection.

We suggest that administration of fully allogeneic cells in an immunocompromised host will not cause GVHD toxicity. The allogeneic rejection response remains intact in immunosuppressed patients, and therefore fully mismatched allogeneic cells would be expected to be rejected in this setting and not cause GVHD. For example, severely injured burn patients have profound immune dysfunction yet universally reject allogeneic skin grafts [25]. Immunocompromised HIV+ patients will also reject allografts [26,27]. Further, intradermal injection rather than intravenous infusion of the allogeneic should reduce the risk of engraftment and thus GVHD toxicity.

Intradermal injection of allogeneic cells (antigen) producing Th1 cytokines (adjuvant) is proposed to create anti-alloantigen Th1 immunity. Skin is a highly immunogenic organ populated by dendritic cells (DC), including epidermal Langerhans cells (LC) [28]. LC cells that reside in the skin play a key role in the initiation and regulation of the immune response throughout the body. LC will readily respond to an allogeneic cell inoculation and capture and process alloantigens resulting from the rejection response. The activated allogeneic Th1 memory cells that are inoculated will express Type 1 cytokines and express CD40L. These “danger” stimuli are expected induce the LC to mature and migrate through the afferent lymph vessels toward the T-cell areas of secondary lymphoid organs [29]. CD40 ligation of LC by CD40L expressing allogeneic Th1 memory cells is known to trigger enhanced LC IL-12 production [30,31] which is known to result in IL-12 dependent priming of allo-specific Th1 cells [31].

Multiple allogeneic cell injections of activated Th1 memory cells is expected to cause the development of an increasing pool of circulating alloantigen-specific Type 1 memory cells in the circulation that will become activated upon each allogeneic injection. One consequence of reactivating this memory T cell pool is the synthesis and secretion of large concentrations of the cytokines that they have been pre-programmed to synthesize [32]. We propose that reactivation of memory T cells that are programmed to produce type 1 cytokines, such as interferon-γ, are capable of priming for a Type 1 anti-fungal immune response via the “heterologous immunity” mechanism [33,34].

Heterologous immunity is the term used to describe the phenomenon by which memory T cells that were generated during an earlier infection are reactivated in response to a second, unrelated infection. When the immune system is biased by a high frequency of memory cells specific for a given pathogenic antigen, the activation of these cells during an unrelated pathogen infection can significantly enhance clearance of the unrelated infection [33]. The pathogenesis of viral infections in the lung has been shown to be related to the host experience with unrelated pathogens [34]. We propose that injection of allogeneic cells in a patient with a high titer of Th1 memory cells specific for the alloantigens will cause increased Type 1 cytokine release which will in turn serve to stimulate immunity against an opportunistic infection.
Activated memory cells are known to express chemokine receptors CCR5, CCR2 or CCR3 that stimulate the upregulation of adhesion receptors in the lung endothelium. [35]. This non-specific infiltration of activated Th1 memory cells producing Type 1 cytokines at the sites of fungal infection in the lungs are expected to have a potent stimulatory effect on local innate and adaptive immune cells responding to the fungus.

Activation of both innate and adaptive immune mechanisms is essential for host control of fungal infection. Effector mechanisms of the innate immune system are a major defense against IA [36]. Resistance to infection requires unimpaired innate anti-fungal activity of pulmonary phagocytic cells operating in a cytokine environment rich in Type 1 cytokines [37] as would be provided by the activated alloantigen-specific Th1 memory cells infiltrating the sites of fungal infection.

In normal individuals, resident alveolar macrophages ingest and kill resting conidia, while neutrophils attack hyphae germinating from conidia that escape macrophage surveillance [38]. The effectiveness of this immune response is evident from the observation that challenge, even with a large number of conidia, fails to cause disease in immunocompetent animals [39].

However, in immunosuppressed patients reduced numbers or impaired function of neutrophils are by far the best-characterized risk factors for IA [40]. Type 1 cytokine production as a result of the activation of alloantigen-specific Th1 memory cells infiltrating pulmonary lesions should serve to activate alternative anti-fungal innate effector cells. The Type 1 cytokines (predominantly IFN-γ, TNF-α, IL-1, IL-2, IL-12 and IL-18) produced as a result of the activation of alloantigen-specific Th1 memory cells should activate alternative innate immune effector cells such as NK cells and dendritic cells (DC), as well activate T-cells [41]. In turn, these cells should produce Type 1 cytokines which will create an autocrine and paracrine cytokine network serving to both maintain and enhance the production of Type 1 cytokines [42].

In immunocompromised hosts, recruitment of NK cells to the lungs has been shown to be an effective defense mechanism against IA [43]. DCs orchestrate the overall antifungal immune resistance in the lungs and were also found to be essential for the activation of Type 1 immune responses to Aspergillus [44].

Activated innate immune cells produce IL-12 and IL-18, which synergistically act in autocrine feedback loop to enhance the production of IFN-α [45, 46]. The production of IFN-α by activated NK cells functions in the priming process of Th1 cells, which in turn supports the expansion and effector function of CD8+ CTLs in the Type 1 adaptive immune response [47]. This cascade of immunological events triggered by activation of alloantigen specific Th1 immunity is expected to enhance cellular immune function against opportunistic infection in immunocompromised hosts.

DC are the innate immune cells recognized as initiators of the immune response to pathogens, including Aspergillus, and serve as a bridge between innate and adaptive immunity. DC have a primary role in surveillance for pathogens at the mucosal surfaces [48]. A dense network of DC has been described in the respiratory tracts [49].

Immature DC in the respiratory track recognize and phagocytose fungus. Upon phagocytosis and signaling from Type 1 cytokines, such as TNF-α, DC become activated and then migrate as mature DC to the lymph nodes [50,51]. Mature DC produce IL-12 and in turn activate naïve T-cells in the lymph nodes via presentation of fungal antigen in the context of MHC I and MHC II molecules, concurrent with the expression of co-stimulatory molecules. Type 1 cytokine production by DC promotes the development of a Type 1 adaptive immune response [52]. Type 1 immune responses have been shown to successfully control IA in patients with hematological malignancies [19].

The proposed mechanism of heterologous immunity for treatment of opportunistic infection by activation of resident allospecific Th1 memory cells causing a switch in existing Type 2 immunity to a resident infection to Type 1 immunity is supported by several observations. For example, the opposite shift occurs in in-
In conclusion, multiple intradermal infusions of activated Th1 memory cells, allogeneic to both the host and donor, will create a pool of Type 1 alloantigen-specific memory cells that when activated will traffic to the lungs and sites of active fungal infection. At the site of fungal infection, the activated memory cells will release Type 1 cytokines which will activate local NK cells and DC to eliminate fungal spores. The Type 1 inflammatory environment will serve as an adjuvant to the development of Type 1 adaptive immunity to clear the fungal infection and protect against recurrence. Since this approach is not specific for the fungal antigens, it could also be used to elicit protective immunity in immunocompromised patients to any pathogen susceptible to a Type 1 immune response.

References


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