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CXCR3 blockade combined with cyclosporine A alleviates acute graft-versus-host disease by inhibiting alloreactive donor T cell responses in a murine model

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ABSTRACT

Chemotaxis of T cells to acute graft-versus-host disease (aGvHD) target tissues directed by chemokines and their receptors plays a key role in the pathogenesis of aGvHD. Blockade of lymphocyte migration by targeting chemokine receptors may be a viable strategy for the prevention and treatment of aGvHD, which is quite distinguishable from typical efforts to use immunosuppressive medications that have been associated with some side effects. CXCR3 and its ligands have been reported to be correlated with aGvHD pathogenesis. Using the small-molecule CXCR3 antagonist AMG487, we demonstrated that AMG487 combined with cyclosporine A (CsA) effectively alleviated aGvHD with a prolonged mean survival time and significantly inhibited the infiltration of inflammatory cells in aGvHD target tissues in a murine aGvHD model. In addition, AMG487 combined with CsA inhibited the activation, proliferation and differentiation of donor-derived T cells in the spleens. Further results showed that the concentrations of Th1 cells associated with pro-inflammatory cytokines such as IFN-γ and TNFα in serum were decreased. In addition, AMG487 treatment did not alter CXCR3 and CCR5 expression in donor-derived T cells but elevated the serum CXCL9 and CXCL10 levels. This novel and effective approach has the potential to develop a new clinical method to prevent and treat aGvHD.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has become widely used as a curative therapy for various life-threatening hematological malignancies and congenital immune deficiencies (Jenq and van den Brink, 2010). However, aGvHD remains one of the main factors that affect the long-term survival and quality of life of patients following allo-HSCT despite considerable advances in our understanding of disease pathogenesis (Choi and Reddy, 2014; Ferrara et al., 2009). Calcineurin inhibitor (CNI)-based therapies, such as CsA, remain the mainstay of immune suppression in mitigating the incidence of aGvHD after allo-HSCT (Ruutu et al., 2014). However, despite standard prophylaxis with these regimens, aGvHD still develops in approximately 40–60% of recipients (Johnston, 2008). Additionally, it has been associated with drawbacks, such as hepatotoxicity, neurotoxicity, and nephrotoxicity as well as other side effects (Cheng et al., 2011). Preventing aGvHD without intensive immune suppression would represent a major advance for allo-HSCT recipients (Davies and Gribben, 2012). Therefore, it is necessary to reduce frequent doses of immunosuppressants, and additional strategies to prevent and treat aGvHD are desperately needed (Moy et al., 2017).

Targeting chemokine receptors of alloreactive T cells is a novel approach for the prevention and treatment of aGvHD (He et al., 2008). Donor-derived T cells are infused into the recipients and recognize the recipients' antigens as foreign, resulting in activation, expansion and migration to the aGvHD target tissues to mediate the injury of host tissues (Ferrara et al., 1999). Chemokines and chemokine receptors play critical roles in directing the migration of alloreactive donor-derived T cells into aGvHD target tissues (Moy et al., 2017). Previous studies have demonstrated that the expression of CCR1, CCR2, CCR5, CCR6 and CCR7 in T cells in patients after allo-HSCT is associated with aGvHD (Jaksch et al., 2005; Yakoub-Agha et al., 2006; Yuan et al., 2015a).

Recently, the chemokine receptor CXCR3 and its ligands have also been shown to be correlated with aGvHD (Jaksch et al., 2005; Wysocki et al., 2005; Piper et al., 2007). CXCR3 is a chemokine receptor that is highly expressed on effector T cells and plays an important role in T-cell trafficking and function (Groom and Luster, 2011). It is rapidly induced in naïve T cells following activation and preferentially remains highly

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Table 1
Groups in this experiment.

Groups	Transplation regimens	Drug treatment
BM[a] group	TBI + 1 × 10^7 BM	PBS
aGvHD group	TBI + 1 × 10^7 BM + 1 × 10^7 SP[b]	PBS
AMG487 group	TBI + 1 × 10^7 BM + 1 × 10^7 SP	AMG487 (5 mg/kg/day)
CsA group	TBI + 1 × 10^7 BM + 1 × 10^7 SP	CsA (5 mg/kg/day)
Combination group	TBI + 1 × 10^7 BM + 1 × 10^7 SP	AMG487 + CsA

[a] BM: bone marrow.
[b] SP: spleen.

expressed in Th1 cells and CTL cells (Groom and Luster, 2011). Monokines induced by gamma interferon (MIG, CXCL9), IFN-γ inducible protein 10 (IP-10, CXCL10) and IFN-α inducible T cell α-chemoattractant (I-TAC, CXCL11) are the natural ligands of CXCR3 (Ma et al., 2017). A previous study showed that the infusion of donor-derived T cells derived from CXCR3-/- mice caused reduced gastrointestinal tract and liver damage (Duffner et al., 2003). The in vivo administration of anti-CXCR3-neutralizing antibody inhibits alloreactive T cell-mediated aGVHD (He et al., 2008). In addition, the downregulation of CXCR3 may be related to higher survival rates after allo-HSCT in a murine model (Qiao et al., 2016). The above experiments are based on the mouse model of aGVHD. In humans, the serum CXCL10 levels were elevated in allo-HSCT recipients who developed aGvHD (Piper et al., 2007; Ahmed et al., 2015). These results suggest that CXCR3 blockade may be an attractive strategy for aGVHD prophylaxis.

AMG487 is a selective CXCR3 antagonist that exhibits good oral bioavailability and robust in vivo biological activity in a preclinical model of cellular recruitment (Johnson et al., 2007; Henne et al., 2012) and is the only CXCR3 antagonist that has been reported to be evaluated in clinical trials (Andrews and Cox, 2016). It can inhibit CXCR3-mediated cell migration by inhibiting the binding of chemokines CXCL9, CXCL10 and CXCL11 to CXCR3.

We previously demonstrated that CCR5 antagonist, together with CsA, has a synergistic effect in a murine aGvHD model (Yuan et al., 2015b). Whether there is a synergistic effect of CXCR3 with CsA in the treatment of aGvHD has not been reported. Thus, our present study was aimed to investigate the immunoregulation role of CXCR3 blockade with CsA in a murine aGvHD model, determine whether it can prevent aGvHD, and clarify the underlying mechanisms.

2. Materials and methods

2.1. Mice

Male BALB/c (H2Kd) and female C57BL/6 ((H2Kb) mice were purchased from Vital River (Charles River, China) at 7–8 weeks of age. The mice were fed with acidified water containing gentamicin for 7 days post-transplantation. All of the animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Ethics Committee of Peking University First Hospital.

2.2. Reagents

CsA was purchased from North China Pharmaceuticals (Shijiazhuang, China), dissolved in a sterile saline solution to 1 mg/ml and administered by intraperitoneal injection (i.p.) once daily at 5 mg/kg. AMG487 was purchased from R&D Systems (Minneapolis, MN). The in vivo formulation of AMG487 was prepared in 20% of hydroxypropyl-β-cyclodextrin (Sigma, St. Louis, MO) as described previously (Walser et al., 2006)and was used to i.p. treat mice once daily at 5 mg/kg. Both drugs were administered within 24 h after transplantation and were given once daily for 7 days.

2.3. aGvHD induction in a murine model

Lethally irradiated mice underwent allergenic bone marrow transplantation, as previously described (Yuan et al., 2015b). Briefly, bone marrow cells and splendid mononuclear cells prepared by Ficoll gradient centrifugation were harvested from C57BL/6 mice. BALB/c mice received 7.5 Gy of total body irradiation (TBI) and then were intravenously injected with cell mixtures of 1 × 10^7 bone marrow cells and 1 × 10^7splenic mononuclear cells at 4–6 h after irradiation. aGvHD were assessed every 2 days by a clinical score, as published previously (including weight loss, activity, posture, skin integrity and fur texture)

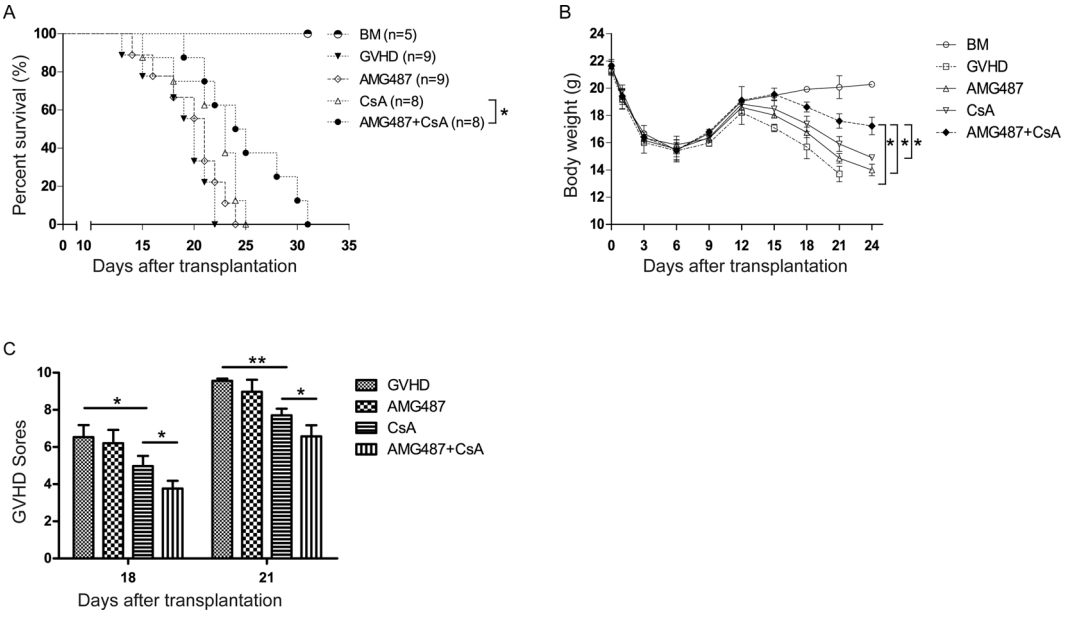

Fig 1. AMG487 combined with CsA effectively alleviates aGvHD. (A) Survival curves of each group. (B) Body weights of mice for 25 days after irradiation. (C) Degree of systemic scores of aGvHD. The results are representative of three different experiments. Bars represent means ± SD. *, p < .05; **, p < .01; ***, p < .001.