Transplant Immunology 26 (2012) 212–219

Contents lists available at SciVerse ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Vav1 GEF activity is required for T cell mediated allograft rejection

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ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form 9 March 2012
Accepted 9 March 2012

Keywords:
Vav
Allograft rejection
RhoGTPase
T cells

ABSTRACT

The GDP exchange factor (GEF) Vav1 is a central signal transducer downstream of the T cell receptor and has been identified as a key factor for T cell activation in the context of allograft rejection. Vav1 has been shown to transduce signals both dependent and independent of its GEF function. The most promising approach to disrupt Vav1 activity by pharmacological inhibition would be to target its GEF function. However, the contribution of Vav1 GEF activity for allogeneic T cell activation has not been clarified yet. To address this question, we used knock-in mice bearing a mutated Vav1 with disrupted GEF activity but intact GEF-independent functions. T cells from these mice showed strongly reduced proliferation and activation in response to allogeneic stimulation. Furthermore, lack of Vav1 GEF activity strongly abrogated the in vivo expansion of T cells in a systemic graft-versus-host model. In a cardiac transplantation model, mice with disrupted Vav1 GEF activity show prolonged allograft survival. These findings demonstrate a strong requirement for Vav1 GEF activity for allogeneic T cell activation and graft rejection suggesting that disruption of Vav1 GEF activity alone is sufficient to induce significant immunosuppression.

1. Introduction

Since the introduction of potent immunosuppressants such as calcineurin inhibitors and improved immunological matching, the risk of acute transplant rejection has been reduced considerably. However, despite a wide range of immunosuppressive agents, severe episodes of rejection still occur and chronic allograft rejection still poses a significant problem [1,2]. In view of both the nonimmune toxicities and the immunodeficiency complications caused by prolonged immunosuppression, there is a strong need to develop more specific immunosuppressive therapies [3,4].

T cells are central players in the process of transplant rejection and are involved both in the acute and chronic rejection phases, presenting an important target for immunosuppressive drugs. They drive graft rejection by direct and indirect mechanisms including apoptosis induction by cytotoxic T cells, cytokine release by T helper cells and by promoting T-dependent alloantibody responses [1]. Activation of allograft-specific T cells is induced by antigen presenting cells such as dendritic cells from both the donor and the host. Binding of MHC–allopeptide complexes to the T cell receptor together with concurrent costimulation triggers intracellular signal cascades leading to the activation and expansion of alloreactive T cells [5].

The members of the Vav family of guanine nucleotide exchange factors (GEFs) are central signaling molecules downstream of antigen receptors, and their deficiency severely affects antigen receptor signaling, lymphocyte development, activation and proliferation [6]. While Vav2 and Vav3 show a broad expression, Vav1 is primarily expressed in hematopoietic cells. Upon T cell receptor (TCR) engagement, Vav1 is phosphorylated and recruited to a TCR-proximal signaling complex including LAT, SLP76, GADS and phospholipase C γ1 (PLCγ1). Vav1 has been shown to integrate various different signal transduction pathways downstream of the TCR and costimulatory receptors leading to gene expression, cytoskeletal reorganization and proliferation [7]. Mice deficient for Vav1 show defects in thymic T cell development and activation of peripheral T cells [8]. T cells lacking Vav1 show reduced Ca^{2+}flux, defective activation of extracellular signal-regulated kinase (ERK), Protein kinase C (PKC), the serine–threonine kinase Akt and T cell-APC conjugate formation [9–13].

Vav proteins contain a Dbl homology (DH) domain, which together with the adjacent plekstrin homology (PH) and C1 domains confers GEF activity toward the Rho-family GTPases Rac, Cdc42 and RhoA [14] [15]. In addition, they contain SH2 and SH3 domains which may mediate the GEF-independent functions of Vav. Phosphorylation of regulatory tyrosines in the acidic domain relieves the autoinhibitory interactions resulting in formation of the open, active conformation and activation of its GEF activity [16,17]. The relative contribution of the GEF-dependent and GEF-independent function of Vav1 for T cell signal transduction and activation still remains unclear. Conditional deletion of Rac1 and Rac2 resulted in a developmental block at the

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doi:10.1016/j.trim.2012.03.003

pre-TCR stage, resembling the phenotype of Vav1-deficient mice [18]. In addition, impaired T cell development in Vav1-deficient mice can be rescued by overexpression of constitutively active Rac1, indicating that Vav1 transduces pre-TCR signals via Rac1 [19]. The recent development of knock-in mice carrying a GEF-deficient Vav1 mutant (L334A, K335A, Vav1$^{AA/AA}$) allowed to distinguish between GEF-dependent and -independent effects in primary cells for the first time. Analysis of these mice showed that the GEF activity of Vav1 is required for thymic development of T cells and some but not all signal transduction events like activation of Akt and integrin activation. Importantly, despite being dispensable for Ca^{2+} flux and ERK activation, the GEF activity of Vav1 is required for T cell activation and proliferation [20].

As a central player in T cell activation, Vav1 has been linked to several immune-mediated diseases including common variable immunodeficiency syndrome and multiple sclerosis [21,22]. We have previously shown an important role for Vav1 in alloreactive T cell responses and transplant rejection in a cardiac allograft transplantation model, demonstrating the immunosuppressive potential of Vav1 inhibition [23]. Targeting Vav1 activity by small molecules is difficult due to its several functions fulfilled by distinct domains. Blocking Vav1 adapter functions, which comprise multiple protein–protein interactions over large areas is difficult using small molecular weight inhibitors. Thus trying to disrupt the interactions between Vav1 and the downstream GTPases and hence its GEF function seems to be the more feasible approach. However, it is not clear if disruption of Vav1 GEF function alone is sufficient to induce immunosuppression. To address this question, we have used the GEF-deficient Vav1$^{AA/AA}$ mice to analyze the contribution of Vav1 GEF function to allogeneic T cell activation and transplant rejection. We show that the GEF function is required for allogeneic T cell activation and proliferation both in vitro and in vivo. Vav1$^{AA/AA}$ mice show prolonged allograft survival in the cardiac transplantation model indicating an important role for Vav1 GEF function in transplant rejection.

2. Material and methods

2.1. Mice

Mutant C57BL/6 mice carrying the GEF-inactivating mutation L334A/K335A in the *Vav1* gene (Vav1$^{AA/AA}$) along with wild-type (WT) littermates have been described previously [20]. Animals were used between 8 and 12 weeks of age. Vav1$^{AA/AA}$ or C57BL/6 WT female control mice were used as recipients of fully MHC-mismatched

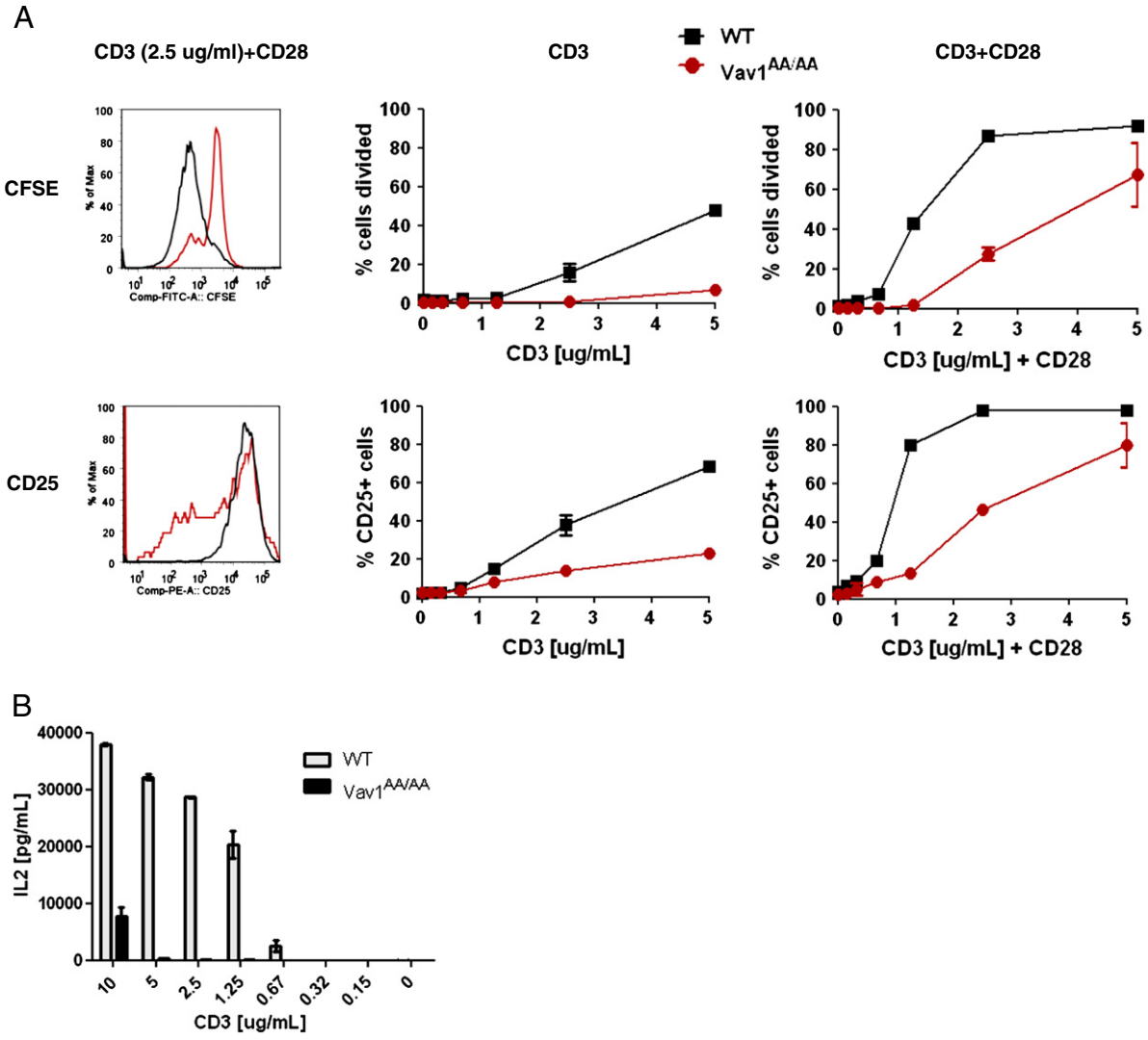

Fig. 1. Vav1 GEF function affects T cell proliferation and activation. Purified T cells from WT or Vav1$^{AA/AA}$ mice were seeded on plates coated with different concentrations of anti-CD3 antibody with or without soluble anti-CD28 antibody (1 μg/ml). After 72 h, percentage of cells that had divided at least once was measured by CFSE dilution, and activation was measured by staining for surface CD25 (A). IL-2 secretion in the supernatant of cells stimulated with anti-CD3 and anti-CD28 was measured by ELISA (B). Data are shown as mean ± standard deviation (SD).