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Depletion of T lymphocytes ameliorates cardiac fibrosis in streptozotocin-induced diabetic cardiomyopathy

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ABSTRACT

T cell infiltration has been associated with increased coronary heart disease risk in patients with diabetes mellitus. Effect of modulation of T cell trafficking on diabetes-induced cardiac fibrosis has yet to be determined. Therefore, our aim was to investigate the circulatory T cell depletion-mediated cardioprotection in streptozotocin-induced diabetic cardiomyopathy. Fingolimod (FTY720), an immunomodulatory drug, was tested in wild-type (WT) C57BL/6 and recombination activating gene 1 (Rag1) knockout (KO) mice without mature lymphocytes in streptozotocin-induced type 1 diabetic model. FTY720 (0.3 mg/kg/day) was administered intraperitoneally daily for the first 4 weeks with interim 3 weeks then resumed for another 4 weeks in 11 weeks study period. T lymphocyte counts, cardiac histology, function, and fibrosis were examined in diabetic both WT and KO mice. FTY720 reduced both $CD4^+$ and $CD8^+$ T cells in diabetic WT mice. FTY720-treated diabetic WT mouse myocardium showed reduction in CD3 T cell infiltration and decreased expression of $S1P_1$ and TGF-β1 in cardiac tissue. Fibrosis was reduced after FTY720 treatment in diabetic WT mice. Rag1 KO mice exhibited no $CD4^+$ and $CD8^+$ T cells in the blood and CD3 T cells in the heart. Diabetic Rag1 KO mouse hearts appeared no fibrosis and exhibited preserved myocardial contractility. FTY720-induced antifibrosis was abolished in diabetic Rag1 KO mice. These findings demonstrate that chronic administration with FTY720 induces lymphopenia and protects diabetic hearts in WT mice whereas FTY720 increases cardiac fibrosis and myocardial dysfunction in diabetic Rag1 KO mice without mature lymphocytes.

1. Introduction

Myocardial fibrosis, one of the cardinal features of diabetic cardiomyopathy, contributes to increased ventricular stiffness which is accountable for contractile dysfunction in failing diabetic hearts [1,2]. Much progress on the cellular and molecular mechanisms of cardiac fibrosis has been achieved. Immune cells, cardiomyocytes, and endothelial cells have been proposed to contribute to the formation of fibrotic process by secreting pro-fibrotic mediators to modulate differentiation of fibroblasts to myofibroblasts [3]. Some neurohumoral factors, cytokines, and reactive oxygen species are recognized to play distinct but overlapping roles in the pathogenesis of fibrosis [4]. However, the underlying mechanism of fibrosis remains controversial.

Several patient-based studies have shown increased expansion of proinflammatory T cell subsets in diabetic patient blood that is correlated with adverse cardiac events including acute coronary syndrome [5,6]. A recent study on murine pressure-overload-induced heart failure has shown that T lymphocyte presence in injured cardiac tissue is associated with exacerbated fibrosis [7]. T lymphocytes can activate profibrotic cells and are involved in fibrosis perpetuation by secreting proinflammatory cytokines [8]. T cells infiltration in diabetic myocardium has been observed [9]. Although results in different approaches including neutralizing antibodies or genetic knockout of signaling protein of T cells have shown reduced fibrosis in murine heart failure models, T cell-based therapeutic interventions to protect hearts against diabetic injury need to be explored. Modulation of T cell trafficking and its effect on long term diabetic fibrogenesis are still not known clearly. Further, crosstalk between T cells and other cellular components in myocardial fibrosis has not been addressed. Fibrocytes, blood derived monocyte-lineage cells that secrete increased extracellular matrix

Abbreviations: KO, knockout; Rag1, recombination activating gene 1; S1P, sphingosine 1-phosphate; $S1P_1$, sphingosine 1-phosphate receptor 1; STZ, streptozotocin; TGF-β1, transforming growth factor beta 1; WT, wild-type.

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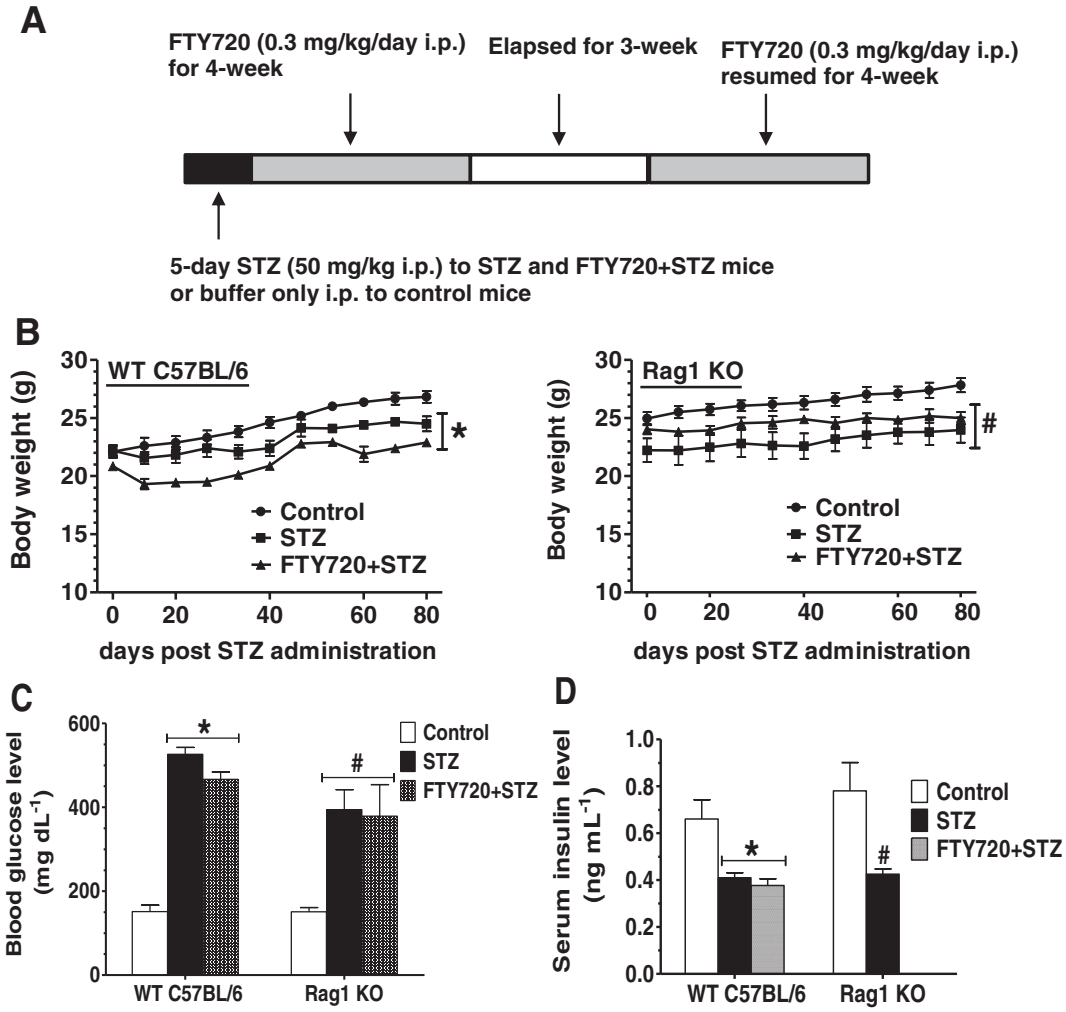

Fig. 1. General physiological parameters in study groups. (A) Schematic diagram of experimental protocol. Streptozotocin (STZ) or buffer was given for first 5 days. Fingolimod (FTY720) treatment was given at two phases for 4 weeks long each. Both WT and Rag1 KO mice were divided into three groups, Control, only sodium citrate buffer treatment mice; STZ, untreated diabetic mice; FTY720 + STZ, FTY720 treated diabetic mice. (B) Body weight (g) was measured twice each week during 11 weeks study period in all mice. (C) Blood glucose level (mg/dL) was measured in tail vein blood before STZ induction, 4 weeks and 11 weeks after STZ induction. (D) Insulin level (ng/mL) was measured by ELISA in serum at the end of 11 weeks. Data are expressed as means ± SEM, $n = 8$–10 per group. *$P < 0.05$ vs. control in WT mice, #$P < 0.05$ vs. control in Rag1 KO mice.

protein under chronic injury/inflammation or with appropriate external stimuli, e.g. transforming growth factor beta 1 (TGF-β_1), can differentiate fibroblasts into myofibroblasts [10,11].

Sphingosine 1-phosphate (S1P), a small bioactive lipid molecule, plays pivotal roles in many physiological processes, such as: cell migration, angiogenesis, cytoskeleton reorganization, and survival, by activating G protein-coupled S1P receptors or intracellular targets [12]. Clinical and experimental data have shown that circulating S1P is predictive for obstructive coronary artery disease and type 1 diabetes [13,14]. S1P and S1P receptor 1 ($S1P_1$) signaling have been implicated in inflammation-mediated myocardial injury [15]. Mature lymphocytes upregulate the expression of $S1P_1$ for their egress from lymphoid organs to circulation [16]. FTY720 down-modulates lymphocytic $S1P_1$ receptor, thus induces lymphopenia by sequestering them in secondary lymphoid organs [17]. FTY720 protects cardiac microvascular structure and function in diabetic rats [18,19]. However, the role of T cells in FTY720-induced cardioprotection against diabetic injury was unknown.

We hypothesized that modulation of T cell trafficking through $S1P_1$ functional antagonism could protect the heart from diabetes-associated fibrosis and cardiac dysfunction. In the present study, we investigated T cell trafficking modulation by using fingolimod (FTY720), an immunomodulatory drug, and recombination activating genes 1 (Rag1) knockout mice without mature lymphocytes as a genetic approach in streptozotocin-induced type 1 diabetic cardiomyopathy model by quantifying T lymphocytes in the heart and circulation, identification of CD34-bearing fibrocyte localization in cardiac tissue, profibrotic TGF-β1 expression, $S1P_1$ expression, heart histological study, fibrosis area measurement in heart sections, and *ex vivo* cardiac function evaluation.

Fig. 2. Flow cytometry analysis for $CD4^+$ and $CD8^+$ T cells in WT and KO mouse blood. (A) Representative images of dot plot and histogram of flow cytometry analysis in WT mouse blood. FTY720 treatment depleted both $CD4^+$ and $CD8^+$ T cells in the peripheral blood compartment as shown in lower right ($CD4^+$) and upper left ($CD8^+$) quadrants in dot plot and at M1 gate in histogram. (B) Rag1 KO mouse blood had no detectable $CD4^+$ and $CD8^+$ T cells as shown in dot and histogram images. (C) Number of both $CD4^+$ and $CD8^+$ T cells in WT mice blood after 11 weeks of STZ induction. FTY720 significantly reduced both subtype of T cells in blood as compared with control and STZ mice. Data are expressed as means ± SEM, $n = 6$–8/each group, **$P < 0.01$ vs. control and STZ.