FEBS Letters 582 (2008) 3935–3940

# Involvement of Sema4A in the progression of experimental autoimmune myocarditis

Nobuhiko Makino[a,b,c], Toshihiko Toyofuku[b,c,*], Noriko Takegahara[b,c], Hyota Takamatsu[b,c], Tatsusada Okuno[b,c], Yukinobu Nakagawa[b,c,e], Sujin Kang[b,c], Satoshi Nojima[b,c,f], Masatsugu Hori[a,h], Hitoshi Kikutani[c,d,g], Atsushi Kumanogoh[b,c,*]

[a] *Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan*
[b] *Department of Immunopathology, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan*
[c] *World Premier International Immunology Frontier Research Center, Osaka University, 3-1 Ymada-oka, Suita, Osaka 565-0871, Japan*
[d] *Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan*
[e] *Department of Dermatology, Osaka University Graduate School of Medicine, 2-2 Yamadoka, Suita, Osaka 565-087, Japan*
[f] *Department of Pathology, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan*
[g] *CREST, Japan Science and Technology Corporation, Japan*
[h] *Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari-ku, Osaka 537-8511, Japan*

Received 2 October 2008; revised 23 October 2008; accepted 24 October 2008

Available online 31 October 2008

Edited by Masayuki Miyasaka

**Abstract** **Dilated cardiomyopathy often results from autoimmunity triggered by microbial infections during myocarditis. However, it remains unclear how immunological disorders are implicated in pathogenesis of autoimmune myocarditis. Here, we demonstrated that Sema4A, a class IV semaphorin, plays key roles in experimental autoimmune myocarditis (EAM). Dendritic cells pulsed with myosin heavy chain-α peptides induced severe myocarditis in wild-type mice, but not in Sema4A-deficient mice. In adoptive transfer experiments, $CD4^+$ T-cells from wild-type mice induced severe myocarditis, while $CD4^+$ T-cells from Sema4A-deficient mice exhibited considerably attenuated myocarditis. Our results indicated that Sema4A is critically involved in EAM by regulating differentiation of T-cells.**


*Keywords:* Autoimmune myocarditis; Semaphorin; Dilated cardiomyopathy; Autoimmunity

## 1. Introduction

Myocarditis is an inflammatory heart disease that can be initiated by infectious pathogens, which is a frequent cause of cardiac disease among young adults. Myocarditis often results from infections with enteroviruses such as coxsackievirus B3 or adenoviruses [1]. In addition, cardiotropic bacteria such as Borrelia and Chlamydia can induce myocarditis and heart failure. Cumulative evidence suggests that myocarditis is a precursor of dilated cardiomyopathy (DCM), the major cause of heart failure and heart transplantation [1]. It has been suggested that autoimmune responses to cardiac antigens that are exposed after heart damage play a crucial role in prolonged damage of the myocardium, which promotes the development of DCM [2]. In fact, many affected individuals develop heart antigen-specific autoantibody responses and immunosuppressive therapy can improve heart function in DCM patients who have no evidence of viral or bacterial genomes in heart biopsies. In addition, peripheral blood lymphocytes from DCM patients can adoptively transfer the disease to severe combined immunodeficiency (SCID) mice lacking T- and B-cells [3]. Collectively, these findings strongly suggest that post-infectious autoimmunity is involved in DCM development. However, it has not been fully elucidated how pathogenic autoimmunity is triggered in myocarditis, making it difficult to treat myocarditis by immunosuppression.

Experimental autoimmune myocarditis (EAM) is a model of post-infectious myocarditis. EAM can be induced in susceptible mouse strains by immunizing with self-peptides derived from the myosin α heavy chain (MyHC-α) together with a strong adjuvant [4], or by injecting MyHC-α-loaded dendritic cells (DCs) [5]. Collectively, these findings imply that immune tolerance is broken by damage during infection, resulting in the release of self-antigens, the activation of DCs, and the subsequent activation of autoreactive T-cells. Consistent with this hypothesis, it has been demonstrated that EAM is a T-cell mediated autoimmune disease that can be transferred by $CD4^+$ T-cells from EAM mice [5]. Traditionally, $CD4^+$ T-cells have been divided into two subsets, Interferon-γ (IFN-γ)-producing type 1 helper T-cells ($T_H1$) and interleukin (IL)-4-, IL-10-, and IL-13-producing $T_H2$ cells. It has been assumed that the $T_H1$/ $T_H2$ cytokine balance is important in the development of myocarditis [6]. Recently, this model has been challenged by the identification of the IL-17-producing $T_H17$ cell subset, which has been shown to be involved in various models of immune-mediated tissue injury [7]. In addition, it has been reported that immuno-regulatory cytokines such as IL-10

*Corresponding authors. Department of Immunopathology, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan. Fax: +81 6 6879 8332.
*E-mail addresses:* toyofuku@ragtime.biken.osaka-u.ac.jp (T. Toyofuku), kumanogo@ragtime.biken.osaka-u.ac.jp (A. Kumanogoh).

*Abbreviations:* DCM, dilated cardiomyopathy; SCID, severe combined immunodeficiency; EAM, experimental autoimmune myocarditis; MyHC-α, myosin α heavy chain; DCs, dendritic cells; IFN-γ, Interferon-γ; $T_H1$, type 1 helper T-cells; IL, interleukin; Tim-2, T-cell, immunoglobulin and mucin domain protein 2; EAE, experimental autoimmune encephalomyelitis; Treg, regulatory T-cells; Foxp3, forkhead box P3

0014-5793/$34.00 
doi:10.1016/j.febslet.2008.10.040

[8,9] and IL-13 [10] can ameliorate EAM development. In this context, the precise mechanism of $CD4^+$ T-cells in the development of EAM remains unclear.

Semaphorins were originally identified as axon guidance factors during neuronal development [11]. In recent years, semaphorins have emerged as important factors that have diverse and critical functions in other physiological processes, including heart morphogenesis, vascular growth, tumor progression, and immune cell regulation. In particular, cumulative evidence indicates that semaphorins are crucially involved in various phases of the immune response [12,13]. The class IV semaphorin subfamily member, Sema4A, regulates T-cell–mediated immune responses by interacting with its receptor Tim-2 (T-cell, immunoglobulin and mucin domain protein 2) which is expressed on $T_H2$ cells as a negative regulator [14–16]. Sema4A expression is specifically induced on the cell surface of $T_H1$ cells during helper T-cell differentiation. We previously reported that T-cell derived Sema4A is necessary for regulating T-cell differentiation. In fact, Sema4A-deficient mice have impaired $T_H1$-responses but rather enhanced $T_H2$-responses. Furthermore, anti-Sema4A antibody treatment attenuated the development of experimental autoimmune encephalomyelitis (EAE), which is a T-cell mediated disease. In this context, Sema4A is potentially a strong therapeutic target for T-cell mediated diseases.

In this study, we show that Sema4A-deficient mice are resistant to the development of EAM, and that a disregulated balance of helper T-cells is responsible for this resistance. Our findings suggest that Sema4A is a potential therapeutic target for autoimmune myocarditis.

## 2. Materials and methods

### 2.1. Mice

BALB/c and SCID mice were purchased from Nippon Clea (Japan). BALB/c Sema4A-deficient mice were generated as described previously [14]. Six- to ten-week-old mice were used for all experiments. Mice were maintained in a specific pathogen free environment. All experimental procedures complied with our institutional guidelines.

### 2.2. Induction of autoimmune myocarditis

Immature bone marrow-derived DCs were generated as described previously [17]. Immature DCs were pulsed overnight with 10 μg/ml of mouse MyHC-α (amino acids 614–629) (Ac-**R**SLKLMATLF-STYASAD**R**-OH; purity >95%; Biologica, Japan). Bold letters indi-

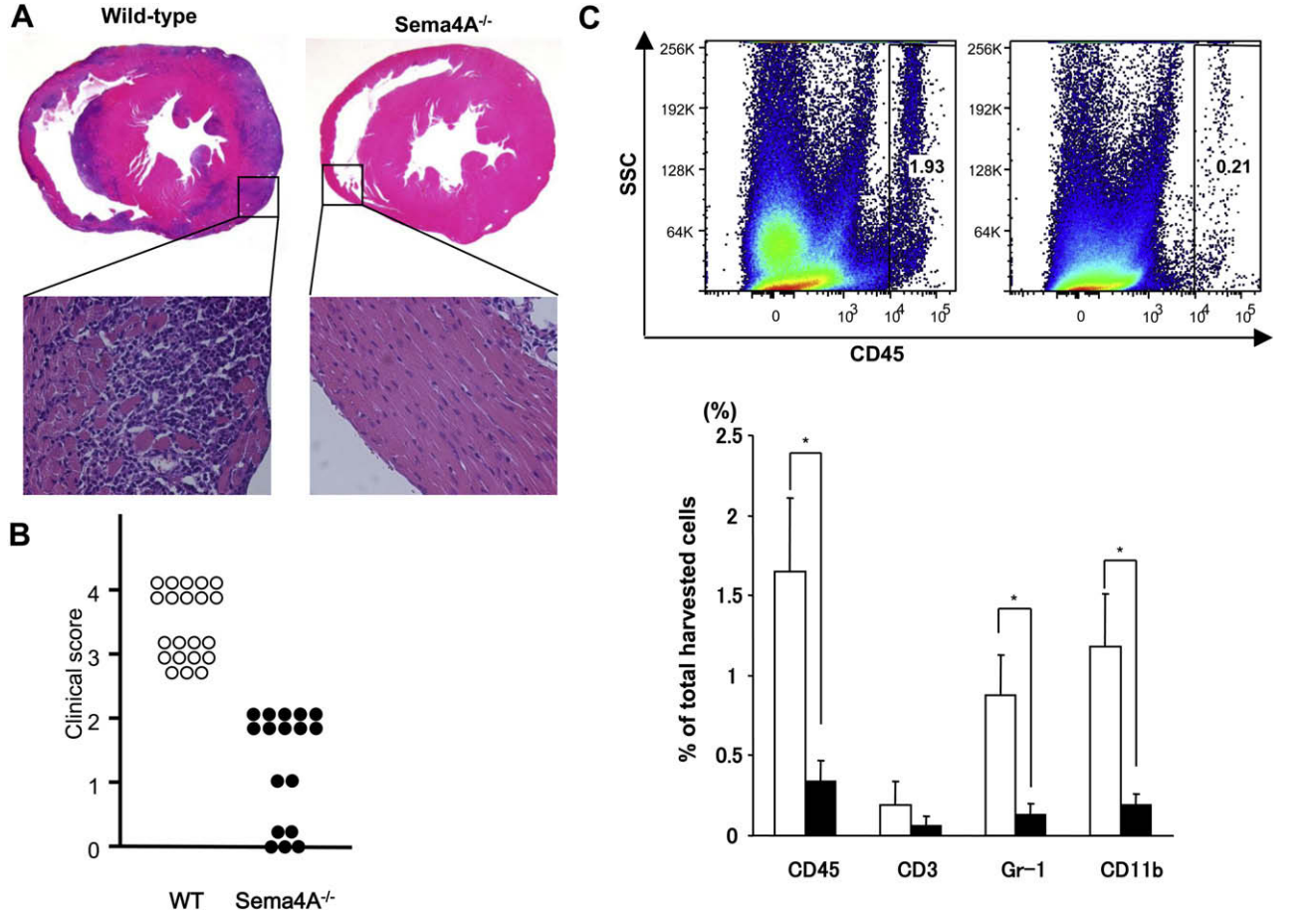


Fig. 1. Sema4A-deficient mice were resistant to EAM. (A) Autoimmune myocarditis was induced by immunizing with activated, MyHC-α loaded DCs. Severe myocarditis was observed in wild-type mice (left), while Sema4A-deficient mice were resistant to EAM (right). (B) Sema4A-deficient mice were resistant to EAM. Disease severity scores of individual wild-type (open circles) and Sema4A-deficient (closed circles) mice immunized with activated, MyHC-α loaded DCs. (C) The number of heart-infiltrating inflammatory cells was decreased in EAM-induced in Sema4A-deficient mice (upper). Heart-infiltrating cells were isolated from the hearts of wild-type (left) or Sema4A-deficient (right) mice 10 days after immunization with activated, MyHC-α loaded DCs. Cells were analyzed by flow cytometry, and a CD45/side scatter plot was used to determine the percentage of $CD45^+$ leukocytes (lower). The cellular composition of the heart infiltrate in immunized wild-type (white bars) or Sema4A-deficient (black bars) mice. The results are representative of five independent experiments.