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A benzenediamine derivative fc-99 attenuates lupus-like syndrome in MRL/lpr mice related to suppression of pDC activation

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ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease with prominent chronic inflammatory aspects. Plasmacytoid dendritic cells (pDCs), which are the principal interferon-α (IFN-α)-producing cells, have known to be critically involved in SLE pathogenesis. Our previous research demonstrated that a benzenediamine derivative FC-99 possessed anti-inflammatory activities. However, the effects of FC-99 on SLE have not been investigated to date. In this study, we found that FC-99 attenuated lupus-like pathological symptoms and lupus nephritis as well as the expression of pro-inflammatory cytokines in kidneys of MRL/*lpr* mice. FC-99 also decreased both the total IgM, total IgG and anti-dsDNA IgG levels in sera and the activation of B cells in the PBMCs and spleens of MRL/*lpr* mice. Moreover, FC-99 inhibited the abnormal activation and number of pDCs from PBMCs and spleens and levels of IFN-α in MRL/*lpr* mice. Notably, FC-99 significantly suppressed the expression of IFN-inducible genes in peripheral blood mononuclear cells (PBMCs) and spleens from MRL/*lpr* mice. As expected, *in vitro* experiments demonstrated that FC-99 decreased both the activation and IFN-α production of pDCs and inhibited IRAK4 phosphorylation in pDCs upon TLR7 and TLR9 stimulation. We further comfirm that the inhibition of FC-99 on B cell activation depended on level of pDCs-secreting IFN-α. These data indicate that FC-99 attenuated lupus-like syndrome in MRL/*lpr* mice related to suppression of pDC activation, especially pDCs-secreting IFN-α. This study suggests that FC-99 may be a potential therapeutic candidate for the treatment of SLE.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder with diverse clinical manifestations including acute and chronic inflammation and tissue damage [30,33]. To date, SLE therapy primarily depends on unspecific immunosuppressive or immunomodulatory agents, such as nonsteroidal anti-inflammatory drugs, corticosteroids, antimalarials, cyclophosphamide, mycophenolate mofetil, or certain biological compounds. However, these agents still exhibit some defects including the limited curative effects against highly active forms of SLE and severe side effects. Therefore, a new therapeutic approach is still necessary for improving the efficiency of SLE treatment.

Dendritic cells (DCs) are important antigen-presenting cells in the maintenance of peripheral self-tolerance and avoidance of autoimmunity and the deregulation of DCs can lead to autoimmunity [40]. Abnormalities in the numbers, phenotypes, activation and functions of DCs have also been reported in SLE patients and murine models of lupus [10,11]. Activated DCs often showed the elevated expression of co-stimulatory molecules (e.g., CD86 and CD40), which reflects their increased activation state [8,46,5,29]. Moreover, DCs are classically divided into two main subsets, i.e., conventional DCs (cDCs) and plasmacytoid DCs (pDCs) [25]. cDCs

Abbreviations: FC-99, (*N*1-[(4-methoxy)methyl]-4-methyl-1); SLE, systemic lupus erythematosus; DCs, dendritic cells; PBMCs, peripheral blood mononuclear cells; mDCs, myeloid DCs; pDCs, plasmacytoid DCs; IFN, interferon; IFNAR1, interferon-alpha/beta receptor1; MLN, mesenteric lymph nodes; BMDCs, bone marrow-derived dendritic cells; Q-PCR, real-time quantitative PCR; TLR, Toll-like receptor; IRAK4, interleukin-1 receptor-associated kinase 4; IL-12, interleukin-12; H&E, hematoxylin and eosin.

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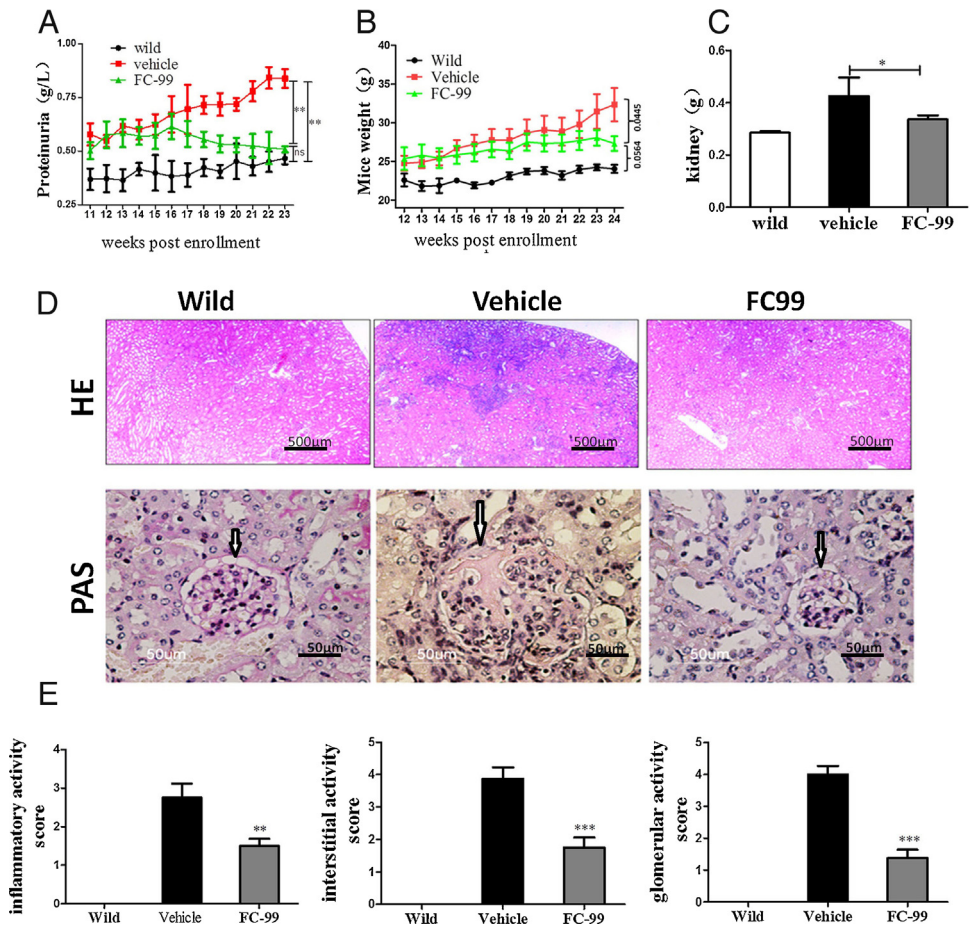

Fig. 1. FC-99 attenuates lupus-like pathology in MRL/lpr mice (A) The body weights of all mice were monitored weekly. (B) Proteinuria was determined weekly in each experiment using an automated turbidimetric method. (C) The weight of the kidney was determined at 24 weeks of age upon sacrifice. (D) Kidney sections from vehicle-treated and FC-99-treated MRL/lpr mice showed histologic differences. Hematoxylin and eosin (H&E) staining revealed an uneven renal cortical surface with inflammatory infiltrates in the vehicle-treated MRL/lpr mice. Periodic acid-Schiff (PAS) staining confirmed the expansion of glomeruli in vehicle-treated MRL/lpr mice, with enlarged glomeruli, distension of tubular lumina, protein casts, and either epithelial or endothelial deposits. Arrows indicate proliferating glomeruli. (E) The scores assigned to the staining images. Data represent the mean scores ± SEM. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

mainly function as antigen-presenting cells and produce interleukin (IL)-12, while pDCs have the ability to produce huge amounts of type-1 interferon (IFN). It is well known that abnormallly activated pDCs trigger the misregulated activity, growth and differentiation of B-cells through IFN-α [24].

Clinical data have especially revealed that IFN-α is elevated in the serum of SLE patients and plays a pivotal role in SLE pathogenesis [6,19]. It was reported that IFN-α increased the functional competence of DCs and promoted adaptive B cell immunity [19,18,4]. Moreover, some studies have reported that the predominant representation of IFN-inducible genes is correlated with disease in the peripheral blood mononuclear cells (PBMCs) of SLE patients [46,31,36]. The abnormal activation of pDCs and the expression of IFN-α-regulated molecules may contribute to the breach of lymphocyte self-tolerance [19]. Importantly, following the abnormal activation of pDCs through either the Toll-like receptor (TLR)-7 or TLR-9 pathways, IFN-α production can act in an autocrine-paracrine manner via the IFN-α receptor (IFNAR) to amplify the ongoing proinflammatory response [6]. Therefore, blocking the production of IFN-α by pDCs is a possible approach for treating lupus and other autoimmune syndromes.

Fumigaclavine C (FC) was isolated from an endophytic fungus of the salinity-tolerant medicinal plant Cynodon dactylon (Gramineae). Based on the bioactivity of the FC bioactivity backbone, we designed and synthetized a series of small molecule FC derivatives. Our previous studies have showed that a FC derivative, the benzenediamine derivate FC-99 (Supplementary Fig. S1), possessed the protective effects against experimental sepsis injury and experimental colitis in our previous studies [13,16]. Moreover, FC-99 was found to directly interacted with interleukin-1 receptor-associated kinase 4 (IRAK4) [13], which is a pivotal molecule in the activation pDCs through the TLR-7 or TLR-9 pathways [21]. Notably, manifold studies have reported that TLR-7 and TLR-9 expression levels were significantly increased in lupus patients compared to age-matched controls [26]. Wild-type mice of different genetic backgrounds treated with either the topical TLR-7 agonist imiquimod or R848 developed SLE, which could be prevented by the *in vivo* depletion of pDCs by a specific antibody to protect mice against the autoimmunity induced by imiquimod [49]. These findings indicate the pDCs are critical for SLE pathogenesis. Thus, we propose that FC-99 may improve SLE progression by suppressing the activation of pDCs.

MRL/*lpr* mice develop a systemic autoimmune disease resembling human SLE that is characterized by elevated levels of auto-antibodies (IgG, anti-sand, anti-dsDNA and IgM), and develop immune complex-type nephritis, lymphadenopathy, splenomegaly and vasculitis [45,1,44]. In the present study, we found that FC-99 attenuated lupus-like pathological symptoms in MRL/*lpr* mice. Moreover, FC-99 inhibited the abnormal activation and numbers of pDCs and IFN-α levels in MRL/lpr mice. Of Note, FC-99 also sig-