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Lentivirus-mediated knockdown of FcγRI (CD64) attenuated lupus nephritis via inhibition of NF-κB regulating NLRP3 inflammasome activation in MRL/lpr mice

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ABSTRACT

Lupus nephritis, one of the most serious complications of systemic lupus erythematosus (SLE), has been confirmed in a large number of clinical surveys. Current studies have suggested that inflammatory situation is generally considered to facilitate the occurrence and development of lupus nephritis. Previous research found that Fcγ receptor I (FcγRI) was compulsory for several autoimmune and inflammatory diseases, and it might be involved in the treatment of lupus nephritis. Furthermore, the possible molecular mechanism of the role of FcγRI in lupus nephritis still needs a further study. In the present study, in order to evaluate the effect of FcγRI on kidney function in lupus-prone MLR/lpr mice, FcγRI knockdown was implemented utilizing FcγRI-RNAi lentivirus. We reported that the administration of FcγRI-RNAi lentivirus (1) mainly inhibited FcγRI expression on macrophage of the kidneys, lowered the levels of urinary protein and serum anti-dsDNA antibody and prevented the impairment of renal function; (2) reduced the renal inflammatory cytokines (IL-1β and IL-18); (3) decreased NF-κB p65 nuclear migration, suppressed NOD-like receptor protein 3 (NLRP3) inflammasome activation, and finally inhibited renal inflammation. Together, these results showed the role of FcγRI on macrophages to involve in renal inflammatory response, potentially via regulating the NLRP3 inflammasome-associated signaling.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic life-threatening autoimmune disease that related to environmental risk factors and genetic backgrounds. Loss of immune tolerance and excessive immune reaction against self-antigens, which involved in hyperactive B lymphocytes, T lymphocytes and monocytic lineage cells, are the most important characteristics of SLE.[1] Moreover, the vast accumulated nucleic acid-protein immune complexes in multiple tissues act as an obvious histopathological hallmark.[2] Irreversible organ damage of SLE covers the pathological changes in skin, joints, heart and even the central nervous system.[3] However, the renal manifestation, which is known as lupus nephritis, is a major cause of mortality in SLE patients.[4] Although the accurate mechanism of lupus nephritis remains to be fully clarified, a growing number of studies suggest that inflammatory reaction triggered by the deposition of immune complex is closely related to the duration and progression of lupus nephritis.[5,6]

Receptors for the Fc portion of IgG (FcγRs) play a vital role in the process of inflammatory response, autoimmunity and cancer incidence, there into, Fcγ receptor I (FcγRI/CD64) that is mainly expressed on immune cells acts as a member of the transmembrane proteins that known to bind monomeric IgG with high affinity.[7,8] Nevertheless, it has been newly reported that FcγRI preferentially interactions with immune complexes in spite of IgG pre-engagement.[9] Accumulating evidences suggested that FcγRI not only contributed to the clearance of immune complexes, but also involved in the regulation of inflammatory response and oxidative stress in a variety of immune-related disease models.[10] Elevated formation and deposition of immune complexes replace the monomeric IgG on FcγRI, aggravate inflammatory response, and then cause renal function impairment in lupus nephritis patients or

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animal models.[11] It was well known that tubules, dendritic cells and macrophages were all important contributors of chronic kidney disease and macrophage-related inflammasome activation played a particular role in development of renal diseases.[12] Hence, it is necessary to confirm whether the overexpression of FcγRI on macrophages of the kidneys is associated with inflammation and kidney injury in the development of lupus nephritis. Furthermore, previous studies have revealed that the productions of NOD-like receptor protein 3 (NLRP3)-associated inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-18 (IL-18) and tumor necrosis factor-α (TNF-α), were increased in the renal tissues of lupus nephritis patients or MLR/lpr mice.[13] The recent research also has elucidated that the relationship between increased expressions of FcγRI and inflammatory reaction is a possible target of alleviating lupus nephritis symptoms.[14,15] Therefore, the effects of FcγRI in the pathogenesis of lupus nephritis is necessary to be investigated.

Nuclear Factor-κB (NF-κB), firstly discovered in David Baltimore's laboratory in 1986, was found to exist in all types of cells and regulate the transcription of inflammation-related genes.[16] Under the normal condition, NF-κB exists in the cytoplasm in the form of inactive complexes. Once the ligand inhibitor of κB (IκB) is phosphorylated by IκB kinase (IKK) complexes, NF-κB is isolated and rapidly moved to the nucleus to participate in the transcription of inflammation-related genes.[17] Therefore, as one of the central components of inflammatory pathway, the activated NF-κB p65 leads to the production of pro-inflammatory cytokines, and this phenomenon has illustrated the pivotal role of NF-κB signaling in emergence and development of inflammatory and autoimmune diseases.[18] Besides, NLRP3 inflammasome pathway is also in charge of the activation of inflammatory progress.[19] NLRP3 is considered to oligomerize and recruit apoptosis-associated speck-like protein containing (ASC) and pro-caspase-1, thereby triggering the maturation of inflammatory factors, such as IL-1β and IL-18.[20] These events above suggest the role of NF-κB regulating NLRP3 pathway in inflammatory response that caused by immune complex deposition. Although there are some experimental evidences to demonstrate the significance of NF-κB and NLRP3 inflammasome signaling in kidney injury, whether FcγRI can modulate this pathway in the pathogenesis of lupus nephritis is still unknown.

In the present study, we hypothesized that FcγRI, a sensitive receptor for immune complexes on the surface of macrophages, was a potential target for alleviating lupus nephritis. For this purpose, we investigated whether FcγRI silencing could suppress renal inflammation reaction and protect the kidneys against serious pathological injury in MRL/lpr mice using FcγRI-RNAi lentivirus. Therefore, we assessed the clinical and pathological features of lupus nephritis, including proteinuria, blood urea nitrogen (BUN), creatinine, and anti-dsDNA autoantibody levels. At the meantime, the inflammatory cytokine contents, such as IL-1β and IL-18 in the renal tissues, were also measured. For the purpose of verifying the possible mechanisms of alleviating renal nephritis after FcγRI knockdown, we also examined the activation of NF-κB p65 and NLRP3 inflammasomes in the kidney of MRL/lpr mice.

2. Materials and methods

2.1. Drugs and reagents

The recombinant FcγRI-RNAi lentivirus and negative control lentivirus were obtained from Wanlei Biological Technology Co., Ltd. (Shenyang, China). IL-1β and IL-18 Enzyme-linked immunosorbent assay (ELISA) kits were respectively from R&D systems Inc. (Minneapolis, USA) and LifeSpan BioSciences Inc. (Seattle, USA). ELISA kit for anti-dsDNA antibody was from BioVision, Inc. (San Francisco, USA). Urine protein, BUN and creatinine kits were provided by Jiancheng Institute of Biological Engineering (Nanjing, China).

2.2. Animals and treatment schedule

Female MRL/MPJ-lpr/lpr (MRL/lpr) mice or MRL/MPJ mice at the age of 15 weeks were provided by Shanghai slake experimental animal Co., Ltd. (License no.: SCXK (Shanghai, China) 2012−0002). The MRL/lrp mice were randomly divided into two groups (n = 6 for each group): FcγRI-RNAi lentivirus group (FcγRI knockdown group) and negative control lentivirus group (Lupus nephritis model group). Meanwhile, the female MRL/MPJ mice at matched age (n = 6) served as the congenic controls (Control group). Depending on the grouping, the animals received an injection of 0.2 ml FcγRI-RNAi lentivirus ($1x10^9$ TU/ml), 0.2 ml negative control lentivirus ($1x10^9$ TU/ml), or phosphate buffered solution (PBS) from the tail intravenously once a week for three consecutive weeks. All the mice were kept in standard experimental environment with 12 h light−dark cycle and 45−55% humidity at temperature 22 ± 1 °C. All the animals were randomly maintained in the facility with water and food available. All the animal care were performed according to the Guide for Care and Use of Laboratory Animals. Laboratory procedures were approved by Ethics Committee of The First Affiliated Hospital of Dalian Medical University.

2.3. Sample preparation

As described previously, the urine samples were collected in metabolic cages for measuring the levels of urine protein.[21] Euthanized under deep anesthesia, the blood samples were harvested to detect BUN and creatinine levels respectively. The kidneys were carefully isolated and fixed in 4% paraformaldehyde or stored at −80 °C for the subsequent experiments.

2.4. Histopathological analysis

The obtained kidneys were fixed in 4% paraformaldehyde and embedded in paraffin blocks. For the histopathological examination, the renal sections (5 μm thick) were incubated with hematoxylin solution and counterstained with eosin. The microstructure changes in the kidneys were observed under light microscopy at 200 × magnification. As described in the reference, the severity of kidney injury was evaluated by a semi-quantitative scoring system and the scoring criteria are as follows[22]:

0 = normal;
1 = mild (cell proliferation and/or cell infiltration);
2 = moderate (cell proliferation and/or cell infiltration with membrane proliferation);
3 = severe (cell proliferation and/or cell infiltration, membrane proliferation, and crescent formation and/or hyalinosis).

2.5. Immunohistochemistry

The renal sections were deparaffinized with xylene and rehydrated with graded ethanol solutions, followed by being baked at 60 °C for 2 h. Subsequently, the samples were treated with 3% H_2O_2 for 15 min, goat serum for 15 min at room temperature, and then the primary antibody overnight at 4 °C. The primary antibodies were as follows: FcγRI (dilution 1:50, Santa Cruz Biotechnology), NLRP3 (dilution 1:100, Boster Biological Technology Co., Ltd.) and cleaved caspase-1 (dilution 1:100, Thermo Fisher Scientific). After washed with phosphate buffered saline, the renal sections were

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