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Transplantation of Aire-overexpressing bone marrow-derived dendritic cells delays the onset of type 1 diabetes

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ABSTRACT

Autoimmune regulator (Aire) plays an indispensable role in maintaining central immune tolerance by promoting the ectopic expression of tissue-restricted antigens (TRAs) in medullary thymic epithelial cells (mTECs) and dendritic cells (DCs), which lead to the deletion of autoreactive T cells or the induction of Tregs and consequently prevent autoimmune disease development. Curing autoimmune diseases has always been a challenge. DC-based immunotherapy represents a new and effective method to establish tolerance. We attempted to transplant Aire-overexpressing bone marrow-derived DCs (Aire-BMDCs) to treat type 1 diabetes (T1D) and to explore a new strategy for autoimmune disease treatment. We observed that the onset of T1D in recipient mice was delayed; insulin autoantibody (IAA) production was significantly decreased; the structure of islets was protected; and the degree of inflammatory infiltration was lower. Furthermore, we found that Aire-BMDCs can promote apoptosis and induce autoreactive $CD4^+$ T cell clonal anergy, inhibit Th1 and Th17 production, and induce Treg production. These results suggest that transplantation of Aire-BMDCs will be a manipulation and effective method for preventing or treating T1D.

1. Introduction

Autoimmune diseases are a varied group of immune-mediated disorders that are characterized by an invasive adaptive immune response to self-components that results in pathology and clinical disease, such as type 1 diabetes (T1D), multiple sclerosis, systemic sclerosis and rheumatoid arthritis. Over 60 autoimmune diseases have been reported [1]. Current clinical therapy mainly consists of the application of hormone and immune inhibitors. Although traditional treatment has improved the prognosis of autoimmune diseases, it cannot cure these diseases. Finding a cure for autoimmune diseases has become the main clinical challenge. An ideal treatment should involve the specialized deletion of autoreactive T cells and the induction of stable and lifelong immune tolerance, which could achieve the goal to control both the immune response to self-components and maintain resistance to foreign pathogens [2].

Dendritic cells (DCs) are professional antigen-presenting cells, which are key controllers of innate and adaptive immunity and are the central regulators of immune tolerance [3]. Autoimmune regulator (Aire) clearly controls the expression of many tissue-restricted antigens (TRAs) in DCs, leading to negative selection of self-reactive thymocytes and induction of T regulatory cells (Tregs) [4,5]. Recently, more attention has been paid to the exploration of effective methods for the prevention and treatment of autoimmune diseases, which are based on DCs [6]. Fortunately, some effect has been achieved. One study carried out intraperitoneal (i.p.) transfer of genetically modified embryonic stem cell-derived dendritic cells (ES-DCs) to protect against myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) [7]. However, we cannot ignore the fact that multiple autoantigens and the phenomenon of epitope spreading are involved in the pathogenesis of most human autoimmune disorders [8]. Thus, the role of DCs presenting only one kind of antigen is limited in the treatment of these diseases.

Thus far, Aire is the exact regulator of the expression of TRAs in thymic negative selection. The number of ectopic genes affected by Aire are approximately 200–1200 [4]. Previous studies have shown that ectopic expression of Aire can also regulate the expression of multiple TRAs [8,9,10]. Therefore, genetic manipulation of Aire, which plays an important role in regulating a variety of TRAs, is a more scientific and reasonable strategy for effective deletion of autoreactive T cells, inducing immune tolerance [11]. For the first time, HyunJa Ko et al. established chimeric mice by transplantation of Aire transfected bone

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marrow cells and found that Aire transduction up-regulates the expression of MOG and markedly delayed the development of EAE [8], but whether this treatment can be applied to other types of autoimmune diseases (such as T1D) is unclear.

Therefore, in the present study, we transplanted bone marrow-derived dendritic cells (BMDCs), which transduced Aire into streptozotocin (STZ)-induced T1D mice to investigate the effect of Aire-over-expressing BMDCs (Aire-BMDCs) on T1D. The results showed that transplantation of Aire-BMDCs delayed the onset of diabetes, decreased the level of insulin autoantibodies (IAAs), better preserved islet structure, and lowered the degree of inflammatory infiltration in the treatment group compared with the control group. Furthermore, Aire-BMDCs can promote the clonal deletion and clonal anergy of autoreactive $CD4^+$ T cells, inhibition of Th1 and Th17 cell production, and induction of Tregs. These results show that Aire-BMDCs can delay the occurrence of STZ-T1D by inducing tolerance. Our findings suggest that transplantation of Aire-BMDCs will be a more effective method of preventing or treating T1D, providing a new approach for the treatment of autoimmune diseases.

2. Materials and methods

2.1. Mice

C57BL/6 wild-type (B6) female mice were purchased from the Experimental Animal Center of Jilin University, and all mice were housed under specific pathogen-free conditions. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Jilin University (Approval No.: 2016-034, Approval date: January, 2017).

2.2. Generation of BMDCs

BMDCs were induced by BM cells from B6 mice. BM cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, rm. GM-CSF (20 ng/mL) and rm. IL-4 (20 ng/mL) for 6 days, and the medium was replaced on the third and fifth day. BMDCs were transduced with a lentiviral vector encoding the mouse Aire-green fluorescent protein (GFP) fusion gene for 72 h.

2.3. RNA isolation and quantitative real-time PCR

Total RNA was extracted from Aire and GFP cells using RNAiso™ PLUS (Takara, Japan) and dissolved in DEPC-treated water. The amount of total RNA was measured using an ultraviolet spectrophotometer (Biotek, USA). cDNA was synthesized from 1.0 μg of total RNA using M-MLV reverse transcriptase and oligo(dT) in a total volume of 20 μL according to the manufacturer's instructions (Takara, Japan). Real-time quantitative PCR (qPCR) was performed using an ABI PRISM 7300 sequence detection system (Applied Biosystems, USA) with SYBR Premix Ex Taq™ II (Takara, Japan) following the manufacturer's suggested protocol and using the following conditions: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. The results were analyzed using the formula 2-ΔΔC. The following primers were used: GAPDH (forward: 5′-GACTTCAACAGCA ACTCCCACTC-3′; reverse: 5′-TAGCCGTATTCATTGTCATACCAG-3′), Aire (forward,5′-ACCATG GCAGCTTCTGTCCAG-3′; reverse: 5′-GCAGCAGGAGCATCTCCAGAG-3′), Ins2 (forward: 5′-ACC TTCAGACCTTGGCACTG-3′; reverse: 5′-GCTGGGTAGTGGTGGGTCTA-3′), GAD65 (forward: 5′-TCAACTAAGTCCCACCCTAAG-3′; reverse: 5′-CCCTGTAGAGTC AATACCTGC-3′), GAD67 (forward, 5′-CTCAGGCTGTA TGTCAGATGTTC-3′; reverse, 5′-AAGCGAGTCACAGAGATTGGTC-3′), IA-2 (forward: 5′-GATTCCCTTGGGTTTGTAGTTC-3′; reverse: 5'TCCCTCCCTTCAGGTTTGA-3′), IGRP (forward: 5′-GTTCGGTAT TGACCTGCTGTG-3′; reverse: 5′-TTGATGAAGCGATAAAGT TGC-3′), RRAD (forward: 5′-ACAAGGGCAGCTTTGAGAAA-3′, reverse: 5'GCTGCTGATGTCTCG ATGAA-3′), Spna2 (forward: 5′-GACCGAATCCGTGGAGTTAT-3′; reverse: 5′-GCTGCTTATTTGC CTCTTTT-3′), Mog (forward: 5′-GGTATCCCATCCGGGCTTTAGT-3′; reverse: 5′-CATCTTGGT CCTTGCCATTTCG-3′), Nalp5 (forward: 5′-GTGGACAGAGAAGAGCAGTTTGGC-3′; reverse: 5′-TAGAGGGGGG ACACAACCATTGAC-3′), Lad1 (forward: 5′-CACCCACCCTGCTCAC CTAC-3′; reverse: 5′-GCCTGGAGACCTGACGGATT-3′), Atp4a (forward: 5′-CATTGCCTTTGCCA TCCAGG-3′; reverse: 5′-CTGGTAGT AGCCAAAACAGCC-3′), Spt1 (forward: 5′-TGCTCTTCTAC TTGTCACCATGA-3′; reverse: 5′-TGTTTGTCTCCGGGTCCT-3′), Rbp3 (forward: 5′-GCTGCCGA AGAGTTTGC-3′; reverse: 5′-TCACCGTGGTTGTCAATAAA-3), Dsg1a (forward: 5′-TGACAACAT CCCATACT-3′; reverse: 5′-TACATTCCGAACACCA-3′), Mup1 (forward: 5′-TCTGTGACGTATGA TGGATTCAA-3′; reverse: 5′-TCTGGTTCT CGGCC ATAGAG-3′).

2.4. Flow cytometry analysis

The cells were collected and counted, and 1×10^6 cells were suspended in 100 μL of PBS. The cells were incubated with PE-anti-CD40, PE-anti-CD80, PE-anti-CD83, PE-anti-CD86, APC-anti-MHC-II, PE-Cy7-anti-CD4, APC-anti-CD4, APC-anti-CXCR5, PE-anti-PD-1, PE-anti-CD11c and PE-Cy7-anti-CD11c (eBioscience, San Diego, CA, USA) on ice for 30 min and subsequently fixed with fixation/permeabilization concentrate with diluent (eBioscience) for 45 min. Alternatively, the cells were incubated with Fluo-3 AM for 30 min at room temperature for Ca^{2+} staining. Then, the cells were treated with 0.1% saponin (Sigma-Aldrich, St. Louis, MO, USA), PE-anti-IFN-γ, anti-phosphorylated-ERK, PE-anti-IgG, FITC-anti-IL-4, FITC-anti-IL-17A, and Alexa Fluor 647-anti-Foxp3 (eBioscience) at 4 °C for 40 min. Next, the cells were washed with PBS and resuspended in 2% paraformaldehyde for analysis using a BD FACS Calibur flow cytometer.

2.5. Streptozotocin (STZ)-induced type 1 diabetic mouse model

C57BL/6J female mice were treated with an intraperitoneal (i.p.) injection of STZ (60 mg/kg) dissolved in cold 0.1 M sodium citrate buffer solution once a day for five consecutive days [12]. The onset of diabetes was evaluated by measuring blood glucose levels in serum collected from the tail vein using an AccuChek system (Beyotime Biotechnology) and measuring insulin autoantibody (IAA) levels in serum with ELISA (Elabscience).

2.6. Adoptive transfer of BMDCs into mice

BMDCs infected with lentivirus were intravenously (i.v.) injected into the tail veins of 6-week-old mice (1×10^6 cells/mouse) once every 3 days and repeated three times. Two days after the first transfer of BMDCs, the mice were treated with STZ as described above and observed for the onset of diabetes by measuring blood glucose levels.

2.7. Immunohistochemical analysis

The pancreas was removed from the mice and fixed in formalin, embedded in paraffin, sectioned, and stained with H & E.

2.8. Statistical analysis

All experimental data are reported as the means, and the error bars represent the experimental standard errors. Statistical analysis was performed using Student's *t*-test. The statistical significance between two groups was set at $P < 0.05$.