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Research Paper

Mesenchymal Stem Cells Control Complement C5 Activation by Factor H in Lupus Nephritis

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ABSTRACT

Lupus nephritis (LN) is one of the most severe complications of systemic lupus erythematosus (SLE) caused by uncontrolled activation of the complement system. Mesenchymal stem cells (MSCs) exhibit clinical efficacy for severe LN in our previous studies, but the underlying mechanisms of MSCs regulating complement activation remain largely unknown. Here we show that significantly elevated C5a and C5b-9 were found in patients with LN, which were notably correlated with proteinuria and different renal pathological indexes of LN. MSCs suppressed systemic and intrarenal activation of C5, increased the plasma levels of factor H (FH), and ameliorated renal disease in lupus mice. Importantly, MSCs transplantation up-regulated the decreased FH in patients with LN. Mechanistically, interferon-α enhanced the secretion of FH by MSCs. These data demonstrate that MSCs inhibit the activation of pathogenic C5 via up-regulation of FH, which improves our understanding of the immunomodulatory mechanisms of MSCs in the treatment of lupus nephritis.

1. Introduction

Lupus nephritis (LN) is one of the most common and severe complications in patients with systemic lupus erythematosus (SLE) [1]. Nearly 50–60% patients develop renal involvement in the first 10 years after disease onset [2], and approximately 25% LN patients gradually progress into end stage renal failure within 10 years due to continuous disease activity [3]. There is increasing evidence that uncontrolled activation of the complement system plays a crucial role in the pathogenesis of LN [4–6]. Components of the complement system, including the classical pathway, alternative pathway, and the lectin pathway, were found to be accumulated in renal tissues of LN patients [7–9]. Notably, deregulation and abnormality of complement regulatory proteins have also been observed in LN patients [10, 11]. Additionally, preclinical studies and clinical investigations have shown that C1q knockout mice or individuals with defective C1q genes developed SLE-like autoimmune diseases [12, 13], and that C3 deficiency aggravated proteinuria in lupus mice [14]. Obviously, further studies are needed to identify which component is a causal factor for LN. Several studies reported that application of anti-C5 monoclonal antibody improved renal outcomes in lupus mice and ameliorated clinical symptoms of LN patients refractory to conventional therapies [15–18], which suggests that limiting the cascade on the C5 level or selectively blocking activated C5 might be an effective therapeutic strategy for the treatment of LN.

Mesenchymal stem cells (MSCs) have multiple-lineage differentiation potentials and a wide range of immunoregulatory functions [19]. We have treated >100 patients with refractory SLE, including patients with LN, using allogeneic mesenchymal stem cells transplantation (MSCT), and achieved good clinical efficacy [20–23]. However, the exact underlying mechanisms of the MSCs-mediated therapeutic effects remain to be determined. Few data are available regarding the effects of MSCT on the complement system [24]. In particular, it has yet to be elucidated whether MSCT influences the function of the complement system in LN. LN is a type I interferon-driven autoimmune disease manifested with chronic inflammation [25]. Although inflammatory factors affect the immunomodulatory effects of MSCs [19, 26], it is still unknown whether this immunoregulatory activity could be affected by interferon-α (IFN-α) in the context of LN with extensive complement activation.

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In this study, over-activated C5 was found in lupus patients and mice, and we demonstrated that MSCT attenuated glomerulonephritis in lupus mice via inhibiting the extensive activation of C5. MSCs produced Factor H (FH) upon IFN-α stimulation in vitro and, importantly, MSCT up-regulated the amount of circulating FH in vivo.

2. Materials and Methods

2.1. Patients and Healthy Controls

66 patients with SLE, and 40 age- and sex-matched healthy blood donors were recruited. All patients fulfilled the 1997 revised criteria of the American College of Rheumatology for SLE [27]. Renal involvement of SLE patients was clinically diagnosed through persistent abnormal urine tests [28]. We also calculated the disease activity score ranging from 0 to 105 for each patient [29]. Renal tissues of 40 patients with LN and 9 patients with nephrectomy were obtained from our department. The severity of LN was assessed according to the abbreviated version of the International Society of Nephrology/Renal Pathology Society classification [30]. The present study was approved by the Ethics Committee at Drum Tower Hospital. Informed consent was obtained from all of the participants prior to their enrollment in this study.

2.2. Human Umbilical Cords–Derived MSCs Isolation and Culture

Human MSCs were isolated and expanded from umbilical cords obtained from normal deliveries after receiving informed consent according to a standard protocol [22]. Unless otherwise stated, human umbilical cords–derived MSCs from passages 4–10 were used for all the following experiments.

2.3. Treatment of Mice

Female MRL/lpr mice of C57BL/6 background (B6.lpr) were obtained from the Chinese Academy of Military Medical Experimental Animal Center (Beijing, China) and were maintained at the Animal Laboratory Center of Drum Tower Hospital. After two weeks of adaptive breeding, B6.lpr mice were randomly allocated into three groups (8 per group), with one group receiving infusion of MSCs (1×10^6) via the tail vein, one group intraperitoneally injected with C5a receptor antagonist (C5aRA, Merck)1 mg/kg three times per week, and a control group intraperitoneally injected with an equal volume of 0.5%DMSO [31]. Levels of proteinuria and creatinine were periodically monitored. After 16 weeks of treatment, mice were sacrificed and perfused. All animal protocols were approved by the Animal Care and Use Committee at Drum Tower Hospital.

2.4. Kidney Histopathology

Kidneys were fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into 3 μm sections. Tissue sections were stained with HE, PAS, PASM and Masson's trichrome. The glomerulonephritis and perivascular cell infiltration were blindly evaluated by two renal pathologists according to a semi-quantitative criterion [32].

2.5. Immunohistochemistry

For detection of C5a (1:100, Abcam) and C5b-9 (1:50, Abcam) in renal biopsies, 3 μm slides were incubated with 0.5 mg/ml proteinase K (VETEC) for 10 min at 37 °C. For detecting C5a (1:100, Usbio), C5b-9 (1:100, Abcam), and MBL (1:50, Abcam) in mouse kidney cross-sections, 3 μm slides were immersed in citrate buffer (0.01 M, pH 6.0) and then a heat-mediated antigen retrieval procedure was performed. Slides ereincubated with the above primary antibodies overnight at 4 °C. The secondary antibody (GENETECH) was incubated for 30 min at 37 °C. Negative controls were included each time. The positive signals in the glomeruli were quantified as the mean optical density by the Image Pro Plus 6.0 (Media Cybernetics, Silver Spring, MD).

2.6. Immunofluorescence

Mouse kidney tissues were OCT-embedded, snap-frozen, and cut into 3 μm sections. For direct immunofluorescence, sections were labeled with TRITC-conjugated goat anti-mouse IgG and FITC-conjugated donkey anti-mouse IgM (both 1:100 diluted, Jackson ImmunoResearch), FITC-conjugated goat anti-mouse IgA (1:100, Abcam), and FITC-conjugated rat anti-mouse C3 (1:50, Santa Cruz) for 30 min at 37 °C. For indirect immunofluorescence, sections were incubated with the anti-mouse C1q antibody (1:50, Abcam) and rabbit anti-mouse properdin (1:10, Abcam) overnight at 4 °C. After washing off the primary antibodies, sections were stained with FITC-conjugated goat anti-mouse IgG (1:800, MultiSciences Biotech) or Alexa Fluor 488-conjugated donkey anti-rabbit IgG (1:100, ThermoFisher). Negative controls were included each time. Immunofluorescence staining intensity of glomeruli was scored on a scale of 0–3 under an Axio Observer A1 inverted fluorescence microscope (Carl Zeiss) [32].

2.7. Western Blotting

Frozen kidneys were homogenized and lysed in radioimmunoprecipitation assay buffer with a halt protease inhibitor mixture (Cell Signaling Technology) and PMSF (Beyotime Biotech.) according to the manufacturer's instructions. Anti-mouse C1q (1:50, Abcam), properdin (1:500, Abcam) and GAPDH (1:1000, Cell Signaling Technology) antibodies were utilized to probe the blots according to standard procedures.

2.8. In Vitro Study

MSCs were re-suspended in low glucose DMEM/F12 (Gibco) supplemented with 0.2% BSA (Biosharp). 10^4–10^5 MSCs were seeded into 24- or 12- well plates and different concentrations of IFN-α1b (KEXING BioTech, China) were added into each well. Cell lysates and culture supernatants were harvested at 0, 12, 24, 48, and 72 h.

2.9. Real-Time Quantitative PCR

Transcripts of human FH were detected via AceQ qPCR SYBR Green Master Mix (Vazyme) according to the manufacturer's instructions. Primer sequences were as follows: FH: 5′-TCTGCATGTTGGCCTTCCT GTC-3′ (forward), 5′-CTTCCTTGTAAATCTCCACCTG-3′ (reverse); GAPDH: 5′-GAAGGTGAAGGTCGGAGTC-3′ (forward), 5′-GAAGATGGT GATGGGATTTC-3′ (reverse).

2.10. Umbilical Cord Mesenchymal Stem Cells Transplantation (UC MSCT)

UC MSCs were prepared by the Stem Cell Center of Jiangsu Province (Beike Bio-Technology). All of the infused UC MSCs were derived from passages 2–5, with rigorous purification and quality control as we previously described [22]. Cells (1×10^6/kg of body weight) were administered by intravenous infusion, which was approved by the Ethics Committee at Drum Tower Hospital and registered at http://ClinicalTrials.gov (identifier: NCT00698191).

2.11. Enzyme-Linked Immunosorbent Assay (EILSA)

Quantification of human C5a, soluble C5b-9 (BD Pharmingen), and FH (Biolegend) were determined in the plasma or supernatants of MSCs. Levels of anti-dsDNA antibody (SHIBAYAGI), C3 (Abcam), C5a

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