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Microglia activation induced by serum of SLE patients

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ABSTRACT

To investigate the potential involvement of microglia in the neuropathology of systemic lupus erythematosus (SLE), we examined whether SLE patient sera could activate BV2 microglia in vitro. Exposure to SLE patient sera resulted in morphological changes in the microglia, an increase in MHC II and CD86 protein expression, and an obvious release of nitric oxide and proinflammatory cytokines. However, the SLE sera did not induce a specific change in the production of immunoregulatory cytokines. Inactivating complements or neutralizing proinflammatory cytokines in the SLE sera did not suppress microglial activation. Our results highlight the potential role of microglia in neuroinflammation in SLE patients.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that is characterized by widespread immunologic abnormalities and multi-organ involvement, including the skin, joints, kidney, and the peripheral and central nervous systems (CNS) (Ruiz-Irastorza et al., 2001). CNS involvement in SLE may occur at any time during the course of the disease and cause neuropsychiatric syndromes of systemic lupus erythematosus (NPSLE), which are diverse and range from depression to psychosis, seizures, and stroke. NPSLE has been reported to occur in 15% to 47% of SLE patients and is a major factor contributing to morbidity and mortality in these patients (Hanly, 2005; Hanly et al., 2007; Hanly et al., 2008; Zhou et al., 2008). To date, the pathophysiology of most NPSLE cases has not been well determined.

Accumulating evidence suggests that some immunological factors, such as autoantibodies and cytokines, in SLE patient sera may contribute to the pathogenesis of NPSLE (Fragoso-Loyo et al., 2008, Ho et al., 2016, Kozora et al., 2010, Okamoto et al., 2010, Zhou et al., 2008). For example, DeGiorgio, et al. reported that administration of SLE patient sera elicited cognitive impairments in mice (DeGiorgio et al., 2001). These authors further demonstrated that circulating anti-*N*-methyl-D-aspartate (NMDA) receptor antibodies in SLE patients were capable of causing neuronal damage and memory deficits if they breached the brain-blood barrier (BBB) (Kowal et al., 2006). However, most previous studies have focused on the dysfunction or damage of neurons in the CNS, and the effects of circulating factors on other cell types, such as glia, have not been investigated.

Microglia are tissue-resident macrophages in the CNS. As immune cells, microglia function as first-line responders under inflammatory conditions. At steady state, resting microglia express low levels of major histocompatibility complex (MHC) class II molecules and co-stimulatory molecules on their surfaces and are mostly involved in the maintenance of CNS homeostasis (Olson and Miller, 2004; Ponomarev et al., 2011; Satoh et al., 1995). When activated, microglia transform into amoeboid cells and acquire the ability to proliferate, migrate, and produce proinflammatory cytokines, chemokines, and neurotoxic factors(Kreutzberg, 1996; Merson et al., 2010). Reports have suggested that microglial activation is a key event in most neuroinflammatory and neurodegenerative disorders (Bhasin et al., 2007, Heppner et al., 2005, Jebelli et al., 2014, Lee et al., 2015, Merson et al., 2010). Considering the important immunological functions of microglia, we believe that microglia may play a critical role in the pathologies of NPSLE. However, no evidence has shown the involvement of microglia in NPSLE.

Therefore, we examined whether SLE patient sera could activate microglia. After treating BV2 microglia with sera from SLE patients or healthy controls in vitro, we evaluated the activation status of the microglia based on morphological changes, nitric oxide (NO) production, co-stimulatory molecule expression, and cytokine secretion. We used a mouse microglial cell line (BV2) in this study rather than a human cell line because BV2 cells have functions similar to primary microglia (Horvath et al., 2008), and obtaining human-derived cell lines is difficult due to ethical and legal issues. Our results provided evidence for

Abbreviations: SLE, systemic lupus erythematosus; CNS, central nervous system; MHC, major histocompatibility complex; NO, nitric oxide; iNOS, inducible NO synthase; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; sICAM-1, soluble intercellular adhesion molecule-1; NPSLE, neuropsychiatric syndromes of systemic lupus erythematosus; APC, antigen presenting cell

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Table 1
The general profile of the SLE patients.

	SLE(n = 18)	Healthy(n = 18)
Age, yrs	30.7 (2.2)	29.6 (3.0)
Gender, female	15 (83.3%)	14 (83.3%)
Disease Duration, yrs	2.3 (0.6)	NA
SLEDAI	4.1 (0.4)	NA
Serology		
C3, g/L	0.4 (0.1)*	1.1 (0.1)
C4, g/L	0.1 (0.01)*	0.3 (0.01)
ESR, mmH_2O	45.0 (3.9)*	1.2 (0.9)
CRP, mg/L	4.0 (0.6)*	1.9 (0.1)
IgG, g/L	13.9 (1.6)*	10.5 (0.6)
ANA positivity	18 (100%)	NA
Anti-dsDNA Abs positivity	8 (44.4%)	NA
Anti-Sm Abs positivity	13 (72.2%)	NA
Anti-U1RNP Abs positivity	11 (61.1%)	NA
Anti-histone Abs positivity	9 (50.0%)	NA
Anti-Rib-P Abs positivity	9 (50.0%)	NA
AnuA positivity	7 (38.9%)	NA
APL positivity	1 (5.6%)	NA

Values are mean (SD) or n (frequency).
NA, not applicable; SLDAI; Systemic Lupus Erythematosus Disease Activity Index; ESR, Erythrocyte sedimentation rate; CRP, C-reaction protein; IgG, Immunoglobulin G; ANA, Anti-nuclear antibodies; Anti-dsDNA Abs, Anti-double stranded DNA antibodies; Anti-Sm Abs, Anti-Smith antibodies; Anti-U1RNP Abs, Anti-U1 ribonucleoproteins (RNP) antibodies; anti-Rib-P Abs, anti-ribosomal P antibodies; AnuA, Anti-nucleosome antibodies; APL, Anti-phospholipid antibodies.
* $p < 0.05$ vs healthy group, t-test.

the first time that circulating sera from SLE patients could activate microglia and shed light on the potential roles of microglia in the neuropathology of NPSLE.

2. Materials and methods

2.1. Serum collection and processing

Sera were obtained from 18 hospital patients who fulfilled the American College of Rheumatology (ACR) classification criteria for SLE (Hochberg, 1997) from September 2015 to February 2016. The clinical profiles of the SLE patients are characterized in Table 1. Sera from the SLE patients and 18 healthy age- and gender-matched subjects were prepared by centrifugation at 3000 rpm for 10 min in a clinical centrifuge and then stored at − 80 °C prior to use. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University.

2.2. Cell culture preparations and morphological examination

The immortalized mouse microglial cell line (BV2) was originally obtained from the Cell Resource Centre, Peking Union Medical College. Briefly, the cells were cultured in 75-cm^2 flasks with DMEM (high glucose) supplemented with 10% FBS, 100 units/ml of penicillin and 100 μg/ml of streptomycin and maintained in a 5% CO_2 incubator at 37 °C. For subculture, the cells were removed from the culture flask with a scraper, re-suspended in the culture medium and sub-cultured in 96-well (0.3×10^6–0.8×10^6 cells/well) or 6-well (1.0×10^6 cells/well) plates for the experiments.

The cell morphology was observed using the Leica DMI3000 B microscope linked to the DP Controller software (Olympus Corporation, Tokyo, Japan) for image processing. Representative bright field images were obtained using a 40 × objective lens.

2.3. Nitric oxide measurement

Production of nitric oxide (NO) was determined by measuring the level of accumulated nitrite, a metabolite of NO, in the culture supernatant using Griess reagent (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. In this study, cells were cultured in DMEM (high glucose) without phenol red (HyClone, Logan, UT, USA) and treated with 5% sera from SLE patients or healthy volunteers. Serum-free medium was used as the blank control. Twenty-four hours later, aliquots (100 μl) of culture media were transferred to test tubes and incubated with 100 μl of reagent A [1% (w/v) sulphanilamide in 5% phosphoric acid] for 10 min at room temperature in the dark. This step was followed by incubation with 100 μl of reagent B [0.1% (w/v) N-1-napthylethylenediamine dihydrochloride] in water for 10 min at room temperature in the dark. After mixing, 200 μl of the purple/magenta solution was transferred to a 96-well plate, and the absorbance at 540 nm was measured within 10 min in the SpectraMax Plus 384 microplate reader (Molecular Devices Corporation, USA). A sodium nitrite dilution series (0–100 μM) was used to generate the nitrite standard reference curve.

2.4. Cell viability

Cell viability was determined using the MTT [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay protocol. Briefly, BV2 cells were seeded into 96-well plates at a density of 3000 cells/well. After 24 h of incubation, 5% SLE patient or healthy control sera were added to the plates. Twelve or 24 h later, 25 μl of MTT dye (5 mg/ml) was added to each well. The cells were incubated for 4 h at 37 °C. After dissolving the crystalscon with 150 μl of DMSO, the optical density (OD) was determined at 490 nm in the SpectraMax Plus 384 microplate reader (Molecular Devices Corporation, USA). The cell viability was represented as the OD ratio of each group to the control group and was expressed as the mean OD ratio ± SEM.

2.5. Cell proliferation assay

The trypan blue exclusion assay was used to examine cell proliferation. BV2 cells were treated with 5% healthy control or SLE patient sera for 12 or 24 h. The cells were stained with 0.4% trypan blue dye (Gibco, Paisley, UK) and then counted using a haematocytometer. The cellular proliferation results were expressed as the percentage of cell counts in the experimental wells relative to the counts in the control wells.

2.6. Cytokine assay

Supernatants of microglia cultured under different conditions were collected from several experiments and centrifuged at 10,000 × g for 10 min at 4 °C to remove cell debris. The supernatants were stored in aliquots at − 80 °C prior to use. Interleukin (IL)-1β, tumour necrosis factor (TNF)-α, IL-6, IL-10, transforming growth factor (TGF)-β1, interferon (IFN)-γ and soluble intercellular adhesion molecule (sICAM)-1 were measured in the culture supernatants by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA). All treatments were completed at least six times, and the data were expressed as the mean pg/ml ± SEM.

2.7. Western blotting

Cellular proteins were extracted from the BV2 cells using RIPA buffer with 1 mM phenylmethylsulfonyl fluoride (PMSF) (Beyotime, Jiangsu, China). The protein concentration in the lysate supernatant was measured using a BCA kit (Beyotime, Jiangsu, China) according to the manufacturer's instructions. Proteins (15 μg) in the cell extracts were denatured with sodium dodecyl sulphate (SDS) sample buffer and separated using 10% SDS-polyacrylamide gel electrophoresis (PAGE). The proteins were transferred to a polyvinylidene fluoride (PVDF) microporous membrane (Millipore, Bedford, MA, USA) and blocked with