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Research Paper

Effect of Interferon-alpha in systemic lupus erthematosus (SLE) serum on the differentiation and maturation of dendritic cells derived from CD34$^+$ hematopoietic precursor cells☆

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Abstract

Objective: To study the effect of interferon-alpha IFN-α in the serum of SLE patients on the differentiation and maturation of dendritic cells (DCs) derived from CD34$^+$ hematopoietic precursor cells (HPCs). **Methods:** Serum samples from SLE patients and normal controls were collected and the concentration of IFN-α detected by ELISA. CD34$^+$HPCs were purified from cord blood by a magnetic cell sorting system (MACS), and cultured to differentiate to DCs. Normal serum, normal serum with exogenous IFN-α, SLE serum with raised levels of IFN-α, or SLE serum with anti-IFN-α neutralizing antibody was added to the culture medium. The phenotype of DCs was analyzed by flow cytometry (FCM) and the capacity of DCs to stimulate allogenic T lymphocyte proliferation was evaluated in a mixed lymphocyte reaction by the Cell Counting Kit-8. Cytokine production was assessed by ELISA. **Results:** Serum levels of IFN-α were significantly higher in SLE patients than in normal controls and this correlated positively with disease activity. Cultured in SLE serum with raised levels of IFN-α, CD34$^+$ HPCs could differentiate into DCs that expressed higher levels of HLA-DR, CD80 and CD86, and showed an enhanced allogenic T-cell stimulatory capacity, while producing lower levels of IL-12 and higher amounts of IL-10 compared with those DCs cultured in normal serum. **Conclusion:** Increased levels of IFN-α in SLE serum promotes the differentiation and maturation of DCs derived from CD34$^+$ HPCs and could contribute to the pathogenesis of SLE.

Keywords: lupus erythematosus, systemic; dendritic cells; interferon-alpha; serum

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, characterized by a loss of tolerance to self-antigens and persistent production of autoantibodies that cause damage to multiple organ systems[1]. However, the exact pathogenesis of SLE remains unknown.

Dendritic cells (DCs) are the strongest antigen presenting cells (APCs). They intervene both as the initiators and regulators of the immune response [2,3]. Many studies have shown substantial phenotypic and functional abnormalities in DCs from SLE patients[4,5]. The unabated DC activation may drive the autoimmune response in SLE. However, the basis of such anomalies, whether it is DC-intrinsic defects, or triggered by a specific environmental stimulus remains to be determined.

Our previous study found that the high levels of IL-6 in SLE serum had an inducing effect on the differentiation and maturation of DCs[6]: IL-6-treated DCs displayed a mature phenotype and an enhanced allogenic T-cell stimulatory capacity. However,

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besides IL-6, numerous abnormalities of the cytokine network have been described in SLE patients [7]. For example, a marked increase in serum IFN-α levels has been seen in the majority of SLE patients [8]. We therefore questioned whether IFN-α present in SLE serum might be involved in the aberrant development of DCs and influence the immune balance.

The objective of this research was to investigate the effect of IFN-α in SLE serum on the differentiation and maturation of DCs derived from $CD34^+$ hematopoietic precursor cells (HPCs).

MATERIALS AND METHODS

Reagents

The IFN-α ELISA Kit was the product of R&D Company (Minneapolis, MN, USA). IL-6, IL-10 and IL-12p40+p70 and IFN-γ ELISA Kits were purchased from Diaclone (Besancon, Cedex, France). The direct CD34 progenitor cell isolation kit and human CD3 Microbeads were provided by Miltenyi Biotec (Bergisch Gladbach, Germany). The recombinant cytokines, GM-CSF, IL-4, TNF-α and IFN-α were obtained from PeproTech (Rocky Hill, NJ, USA). Rabbit anti-human IFN-α was supplied by Invitrogen (Carlsbad, CA, USA). HLA-DR-PE, CD80-PE, CD86-PE, CD83-PE, CD1a-FITC and CD34-FITC murine anti-human monoclonal antibodies (mAbs) were provided by Ebioscience (San Diego, CA, USA). Cell Counting Kit-8 was obtained from Dojindo Laboratories (Kumamoto, Japan), and LPS were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Patients and serum samples

Fifty SLE patients (47 females, 3 males; mean age 32.74 years) were recruited from the department of rheumatology, the First Affiliated Hospital of Nanjing Medical University. All patients fulfilled the revised American College of Rheumatology criteria for SLE [9]. Disease activity of the patients was assessed using the SLE Disease Activity Index (SLEDAI) [10]. Routine laboratory tests included CRP, complement components (C3 and C4), anti-dsDNA antibodies, full blood count, and urinary analyses. Anti-dsDNA IgG antibodies were measured by immunoblotting. Serum concentrations of complement components C3 and C4 were determined by nephelometry. Thirty sex- and age-matched healthy volunteers were used as controls. After informed consent, 5 ml of whole blood was obtained from each patient and each control. Blood was centrifuged at 2 000 rpm for 10 minutes and serum was separated for the determination of cytokines by ELISA. The sera of SLE patients with raised level of IFN-α, but normal levels of IL-6 and IL-10, were chosen and pooled.

Cell separation and DC generation

Fresh sterile cord blood samples were collected and heparin used as an anticoagulant. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. $CD34^+$ HPCs were immunomagnetically sorted using the Direct CD34 Progenitor Cell Isolation Kit with the purity greater than 90%, and then cultured in RPMI 1640 containing 15% FCS at 1.5×10^5/ml in the presence of 100 ng/ml GM-CSF, 20 ng/ml TNF-α and 40 ng/ml IL-4[11]. During the differentiation process from $CD34^+$ HPCs into DCs, 10% human serum was added to the cultural medium. Cells were divided into four experimental groups: ① Normal control group (normal serum was added); ② Exogenous IFN-α group (normal serum with exogenous rhIFN-α added); ③ SLE serum group (SLE serum with raised levels of IFN-α added); ④ IFN-α neutralized group (SLE serum with anti-IFN-α neutralizing antibody added). Fifty percent fresh medium, cytokines and sera were added twice weekly. At 14th day, DCs were harvested for identification and comparison.

Analysis of DC phenotype

The harvested DCs were centrifuged at 1 500 rpm for 10 min and resuspended in PBS. These cells were then incubated with anti-HLA-DR-PE, anti-CD80-PE, anti-CD86-PE, anti-CD83-PE, anti-CD1a-FITC mAbs or isotype controls for 30 min at 4℃. After washing twice with PBS and fixation in 1% paraformaldehyde, cells were analyzed using a FACScan flow cytometer (BD Biosciences, USA). Data analysis was performed by CellQuest software (BD Biosciences, USA).

Mixed lymphocyte reaction (MLR) Assay

To determine allostimulatory capacity, DCs were harvested and co-cultured with a constant number of allogenic $CD3^+$ T cells (2×10^5 per well) in 96-well microtiter plates at four ratios of DCs to T cells (1:10, 1:20, 1:50 and 1:100). $CD3^+$ T cells were purified from peripheral blood mononuclear cells (PBMCs) of healthy volunteer by a magnetic cell sorting system (MACS) using human CD3 Microbeads. The cells were incubated at 37°C, 5%CO_2 for 96 h. For the last 2 h of co-culture, 20 ml CCK-8 solution was pulsed in each well. Finally, the optical density (OD) was measured at 450 nm using a microplate reader. Tests were performed in triplicates and results expressed as a stimulating index (SI): $SI = (OD_{experimental}-OD_{blank}) / (OD_{negative\ control}-OD_{blank})$.

Determination of cytokine production by DCs

DCs were seeded at a concentration of 5×10^5/ml in