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Interferon Gamma in Egyptian children with LN by Flow Cytometery.

KEYWORDS

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ABSTRACT *Systemic lupus erythematosus (SLE) is a chronic, immune-mediated disease in which T and B cells cause systemic tissue injury. SLE is characterized by the production of high levels of auto-antibodies against nuclear antigens, resulting, at least in part, from a dysregulated T lymphocyte response to autoantigens. Results:the expression of INFɤ levels in T lymphocytes cells ofblood samples obtained from LN group was significantly decreased as compared to healthy control (9.24± 3.6and 14.05± 4.36 respectively; P ≤0.01).In conclusion:the low expression of INFɤ in T lymphocyte cells of LN patients may be a good marker in prognosis and diagnosis of LN .*

Introduction

Systemic lupus erythematosus (SLE) is a chronic,immune-mediated disease in which T and B cells causesystemic tissue injury [1]. T cell dysfunction has beendescribed at the cellular and molecular level in bothhumans and animal models of lupus [2–4]. Impairedin vitro T cell proliferation and altered cytokine production (e.g., IFN-_ production [5]) in response to foreignantigens, alloantigens (allogeneic major histocompatibilitycomplex (MHC) molecules), and mitogens has beenobserved [4]. In general, it is thought that CD4 cell activityis exaggerated, while CD8 cell activity is diminished,resulting in an imbalanced T cell functional repertoire, aTh1/Th2 cytokine imbalance, and B cell hyperactivity [2].However, it is not clear how functional and phenotypicaspects of antigen-specific CD8 cells compare in SLE.(B.R. Berner et al. / Cellular Immunology 235 (2005) 29–38)

A common and seriouscomplication of SLE is lupus nephritis, which plays a key role in theprognosis of SLE and is a risk factor for the development of endstagerenal failure. It occurs in about 15% of patients at diagnosisand in approximately 40% during the course of the disease.Until now there are no general accepted methods to reliablyidentify patients who are at risk of flares. Disease activity isassessed using a combination of clinical history, physical examination,organ specific functional tests, and serologic studies. To date,determination of complement levels and anti-dsDNA antibodiesare the serological parameters most commonly used to assess diseaseactivity in patients with SLE. Numerous investigations of otherimmunological tests as indicators of disease activity have been reported (Cytokine 60 (2012) 410–416)

Systemic lupus erythematosus (SLE) is a disease characterized by the production of highlevels of auto-antibodies against nuclear antigens, resulting, atleast in part, from a dysregulated T lymphocyte response to autoantigens. (Kammer et al., 2002; Kong et al., 2003).In SLE, multiple serological factors get altered. These includecytokines, often representing altered ratio of Th1/Th2 and thosereleased from different leukocytes; inflammatory peptides, proteinsand raised levels of circulating immune complexes. (V. Arora et al. / Molecular Immunology 44 (2007) 1722–1728)

TH1typecytokines such as IFN-ɤ, IL-2and TNF-β as well as TH2 type cytokines, such as IL-4,IL-6, IL-10 and IL-13 are known to be importantregulators of the immune system. (Nagy et al., 2000)

The aim of our present work was to study the CD4, CD8 and IFN-ɤ production of Tlymphocytes by flow cytometric analysisusing isolated lymphocytes fromblood samples of pediatric patients with lupus nephritis andhealthy donors.

2. Patients and methods

2.1. Patients and controls

The study cohort included 27 patients diagnosed as having lupus nephritis (% female and % male mean age 9.68±2.89 years according to the American College of Rheumatology (ACR) criteria ,Median (range) anti-dsDNA titers were 230 (3-1000 E/ml), median C3 and C4 were 0.55 g/l (0.05-1.03) and 0.14 g/l (0.04-0.30), and 11 healthy control subjects (% female and % male ; mean age9.98±2.10years) who had no history of autoimmune disease, infectious disease, malignancies or immunesuppressive therapy.The study was approved by Mansoura pediatric hospital, Mansoura University, and written informed constent was obtained all participants.

Materials

Monoclonal antibodies (mAb) and flow cytometry.

The following mAb and reagents were used in this study: fluorescein isothiocyanate (FITC)–conjugated or peridinin chlorophyll A protein–conjugated)–conjugated anti-CD8 mAb, FITC -conjugated anti-CD4 mAb, FITC -conjugated anti and antiINF-ɤmAb,

EDTA-blood and fresh urine samples were collected from patients. Urine samples from patients with signs of urinary tract infection were excluded.

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

Sample preparation for flow cytometry
The fresh urine sample was collected from each patients and control. For 3 ml urine was centrifuged at 2000 rpm for 20 min. The supernatant was removed and the pellet which contains lymphocyte was shaken. The lymphocytes from urine sample was Fixed with ice cold absolute alcohol 1ml for each tube and was preserved in +4c forever until analysis.

Isolation of peripheral blood: Peripheral venous blood samples were collected into tubes containing EDTA, and peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation for 20 min at 1500 rpm without braking using Ficoll-Paque Plus solution. The bands of cell were pipette carefully into another centrifuge tube filled up with phosphate buffer saline (PBS) or hanks solution and mixed. After centrifugation for 10min at 1800 rpm, lymphocyte sediment were fixed with ice cold absolute alcohol 1ml and preserved in +4C° until analysis.

This technique was applicable where the fluorochrome was directly linked to the primary antibody e.g.: phycoerythrin (PE) or fluorescein isothicyanate (FITC)- conjugate. 200 µl of lymphocytes were added in test tubes. The lymphocytes were washed with 1ml PBS, and then were centrifuged at 200 rpm for 5 minutes, the supernatant was discarded. The lymphocytes pellet was washed with PBS and mixed well. 3µl of required marker was added and was mixed well. The tubes were incubated at room temperature for 20 minutes. The cells were washed with 1ml PBS; then the tubes were centrifuged at 2000 rpm for 5 minutes. The supernatant was removed then 200ml of 4% paraformaldehyde were added for analysis by flow cytometer.

Flow cytometric analysis was performed with FACCalibur flow cytometer using Cell Quest software (Becton Dickinson, San Jose, CA). A total o10,000 to 20,000events were collected for each analysis. Lymphocyte gates were set on live cells using forward (FSC) and side scatter (SSC).

Statistical analysis:
Data were explored, processed and analyzed using the statistical package for the social science, windows version 16, USA (SPSS PC+ version 16 software). Variable with normal distribution were expressed as mean± SD. In these variables, the T test was applied for group differences.

RESULTS
We studied a total of 38 subjects. Their characteristics and clinical data are summarized in Table (1).

Table 1.Characteristics data of LN patients and control subjects

	Control	Lupus Nephritis patients
Case no.	11	27
Sex (male\Female)	5\6	7\20
Age /years	9.98±2.10	9.68±2.89
Anti-dsDNA	7.5±5.5	230±168.23

T-cell count discriminates between LN patients and control
Table (3) represents the flow cytometry markers of T-cells% from blood samples. $CD4^+$ T cells levels in blood samples obtained from LN patients were significantly elevated as compared to healthy control group (25.1± 8.43and 13.33± 3.97,respectively; p <0.001). The $CD8^+$ T cells levels in blood of samples obtained from LN group was significantly decreased as compared to healthy control (19.33±11.82and 30.02± 7.52respectively; P ≤0.01). Fig (2) showed the INFɤ levels in blood of samples obtained from LN group was significantly decreased as compared to healthy control (9.24± 3.6and 14.05± 4.36 respectively; P ≤0.01).

Table (3): Flow cytometery markers for T-cells% from blood samples of studied subjects included in the study (n=38)

	Control (n=11)	LN (n=27)
$CD4^+$	13.33± 3.97	25.1± 8.43***
$CD8^+$	30.02± 7.52	19.33±11.82***
INFɤ	14.05± 4.36	9.24± 3.6***

***** (P value <0.001)**

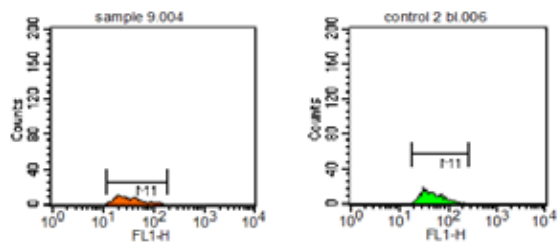

Fig (2) showed the% of blood cells stained with INFɤ FITC conjugated fluorescence in LN patients and control.

Discussion
IFN-ɤ has been long associated withlupus pathology but its precise role indisease pathogenesis has been unclear. IFN-ɤ promotes T cell proliferation from the naive stage andaugments Bcl-6 expression in Th cells and their precursors.These effects are likely to enhance the formation and possiblythe maintenance of Th cells (Lin et al., 2011).

The role of IFNɤ in the pathogenesis of SLE has been reported by Sau et al., (2012). However, there is contradictory data available about the dominance of Th1 and Th2 cytokines in SLE. Tucci et al. (2008)reported an increase level of serum IFNɤ in patients with SLE.

The elevation of IFNɤ level causes further inflammation and tissue injury in SLE patients .Increased level of IFNɤassociates with nephritis in SLE patients. The result is similar to the study of Tucci et al. where they suggested the increased production IFNɤ production in SLE patients. INFɤ are involved in cell mediated autoimmune disease , however, have been refuted by resent studied in several induced and spontaneous mouse module of SLE which showed that INF is a major effector molecule in this disease . These and additional finding review her , suggested this two cross talking cytokines can exert autoimmune disease promoting or disease inhibiting effect without predictability or strict adherent to the Th1 versus Th2 dualism. (Tseosiopoulos et al.,2001). `

In the present study, INFɤover express on the T cells was significantly decreased as compared to healthy control group (P≤ 0.01). This finding was disagreeing with that of (Koenig et al., 2012). According to the low expression of INFɤ in T lymphocyte cells of LN children in contrary to the high level of serum IFNɤ in patients with SLE.

Tcells with suppressor function were first described by

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(Gershon and Kondo 1971).Thereafter; several observations supported their role in immune regulation processes. In fact, at least two lines of evidence suggest the existence of $CD8^+$ T cells able to inhibit immune responses. First, the isolation of Ag-specific and alloantigen-specific T suppressor lymphocytes from mice and humans and, second, the identification and functional characterization of $CD8^+$ T lymphocytes which suppress autoimmune responses in mice with experimental autoimmune encephalomyelitis. (Filaciet al.,2001)

In the current study ,the $CD8^+$ T cells (%) in blood samples obtained from LN patients was significantly decreased as compared to healthy control group ($p<0.01$). This finding was in agreement with the study ofFilaciet al., (2001) who demonstrated that, the lack of suppressor T cell activity may play a role in the pathogenesis of disease.

Dinesh et al.,(2010) reported that, $CD8^+$Tregs in lupus patients have defective and/or reduced numbers, whileAlvaradoet al.,(2006) study found no difference in the number of $CD8^+$Tregs in SLE patients. A multiplicity of different $CD8^+$Tregs subsets appears to have different mechanisms of suppression, markers, and modalities of induction. This diversity is one of the field's most salient characteristics because there is so far no single molecule that can specifically identify$CD8^+$suppressor orTregs, either naturally occurring or induced.

In the present study, the surface markers $CD4^+$ T cells(%)in blood samples obtained from LN patients was significantly elevated as compared to healthy control group ($p<0.01$). This results are in agreement with those of Wanget al., 2013 and Tzifiet al., 2013.Tzifiet al., 2013 reported that increased numbers of $CD4^+$ T cells were correlated with increased disease activity and the production of anti-self-antibodies.

The finding result was disagreement with the data obtained fromMiyaraet al., (2005). Who reported that all active patients studied presented not only with a decreased proportion of Tregs among $CD4^+$ T cells, but also with decreased absolute number of the later cells, as compared with mean values obtained from healthy controls. These conflictions may be attributing to the population and race differences.

In conclusion: the low expression of INFɤ in T lymphocyte cells of LN patients may be a good marker in prognosis and diagnosis of LN .

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