Asian Biomedicine Vol. 5 No. 5 October 2011; 643-647 DOI: 10.5372/1905-7415.0505.085

Original article

Interferon Alpha mRNA level and subtypes in lesion and non-lesion from discoid lupus erythematosus patients without systemic lupus erythematosus

Jongkonnee Wongpiyabovorn[a], Kriangsak Ruchusatsawat[b], Yuthana Onganantapong[c], Wannasri Sintupak[c], Nattiya Hirankarn[a]

[a]*Department of Microbiology, Division of Immunology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330,* [b]*National Institute of Health, Department of Medical Sciences, Nonthaburi 11000,* [c]*Department of Medicine, Division of Dermatology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*

Background: Up-regulation of interferon alpha (IFN-α) in cutaneous lesions of lupus erythematosus (LE) has been proposed to play a role in the pathogenesis of cutaneous LE lesions of LE patients. IFN- α is composed of 13 subtypes of immunoregulatory cytokines that promote innate and adaptive immune responses. However, the precise biological properties may differ among subtypes.

Objective: To investigate the mRNA level of IFN-α and subtypes in cutaneous LE patients

Methods: We analyzed the mRNA level of IFN-α and subtypes in normal skin from 12 healthy female controls and compared it to normal skin and discoid lupus erythematosus (DLE) lesions from 16 DLE patients without systemic involvement (14 females and 2 males) by real-time PCR, cloning, and sequencing.

Results: Significant up-regulation of mRNA level of IFN-α was found in both lesional and non-lesional skin from DLE patients without SLE when compared to normal skin from healthy donors (P=0.05 and P=0.04, respectively). An analysis of the IFN-α subtypes from skin biopsies detected a reduction of IFN-α subtype 5 in DLE lesion compared to healthy control skin.

Conclusions: Our results indicate that IFN- α is upregulated in both lesional and non-lesional DLE in cutaneous LE patients. IFN-α subtype 5 is the main subtype of IFN- α expression in normal skin but declines in DLE.

Keywords: Cutaneous LE patients, DLE**,** interferon-alpha, mRNA, subtype

Lupus erythematosus (LE) is an autoimmune disease with a broad spectrum of manifestations in various organ systems. Cutaneous LE has been found in 73 to 85% of patients with systemic lupus erythematosus (SLE) [1]. Cutaneous LE lesions, ranging from chronic, sub-acute, to acute LE, have been classified into LE-specific and LE-nonspecific. Discoid lupus erythematosus (DLE) is the most common chronic cutaneous LE-specific manifestation. Cutaneous LE can occur to patient without systemic involvement or patients may have SLE without skin involvement. The prevalence of cutaneous LE was approximately two to three folds higher than SLE. Experimental models in animals suggested that the pathophysiology of cutaneous LE and SLE are different. Many therapies in LE can improve cutaneous or systemic disease independently. Both genetic and environmental factors influence humoral and cellular immune responses resulting in immune dysregulation in cutaneous LE [2, 3]. Environmental factors including ultraviolet radiation, viruses, hormones, medications, and stress have been suggested as initiating factors of cutaneous LE. Abnormal apoptosis, the presence of autoantibodies, and infiltration by plasmacytoid dendritic cells (PDCs), T cells as well as B cells are also important factors for the induction and maintenance of cutaneous LE in SLE patients.

Correspondence to: Jongkonnee Wongpiyabovorn, MD, PhD, Department of Microbiology, Division of Immunology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. Email: jongkonnee.w@chula.ac.th

Accumulation of PDCs, the natural IFN-α/β producing cells, results in an increased expression of IFN-α/β inducible protein. MxA has been demonstrated in cutaneous LE lesions from SLE patients [4-6]. The IFN-α subsequently induces activation of autoreactive T cells, CXCR3+ lymphocytes, CXCL9+ and CXCL10+ keratinocytes and endothelial cells in cutaneous LE, therefore bringing on peripheral intolerance and inflammation [7, 8]. However, up-regulation of IFN-α in DLE lesions has never been demonstrated in cutaneous LE patients without SLE. The IFN-α family consists of 13 subtypes encoded by 13 *IFNA* genes. The IFN-α proteins share 76 to 99% homology in their amino acid level [9]. All IFN-α subtypes bind to the same type I IFN receptor, IFNAR1 and IFNAR2, but with different binding sites and affinities. These may lead to different biological relevance and specific cellular responses [10-14]. The immunological properties of different IFN-α subtypes in various tissues remain to be explored. In this study, we analyzed IFN-α mRNA levels and subtypes in DLE lesions and compared results to normal skin from DLE patients without SLE and from healthy controls.

Materials and methods

Patients and controls

Punch skin biopsies of DLE lesion and normal skin were obtained from 16 DLE patients (14 females, 2 males) and 12 healthy subjects (12 females). All patients were clinically and pathologically diagnosed as DLE by a qualified dermatologist at King Chulalongkorn Memorial Hospital before being registered for this study. Patients with acute or subacute LE, other autoimmune diseases, viral infections and cancers were excluded. All patients were free from systemic therapies for at least four weeks or from topical skin therapies for at least two weeks before sample collection. Control subjects were normal recruited from elective cosmetic surgery cases at King Chulalongkorn Memorial Hospital. None had a personal or family history of autoimmune disease. The study was approved by the Ethics Committee of King Chulalongkorn University. All patients signed an informed consent.

Determination of mRNA Expression Level by Real Time RT-PCR

Total RNA was extracted from tissue specimens using Trizol reagent (Life technologies, Inc., California, USA) according to the manufacturer's instruction. To remove the contaminating DNA, the total RNA was treated with one unit of deoxyribonuclease (RQ1 RNase-free DNase; promega). Two g of the total RNA samples were reversely transcribed with oligo dT primer using an ImProm-II reverse transcriptase system (Promega). For Real Time PCR, 1 l of cDNA was amplified using universal IFN-α primer as previously described [15]. Real-time RT-PCR was performed in a Light Cycler machine (Roche Molecular Biochemicals, Indianapolis, USA) using QuantiTect SYBR Green I (Qiagen, Hilden, Germany), according to the manufacturer's instructions with a total volume of 20 l. 18s ribosomal RNA (rRNA) was used as a housekeeping gene and the expression level was determined using a Taqman hydrolysis probe as previous described [16].

Cloning and sequencing of IFNA PCR products

To identify IFN-α subtypes, the PCR products from universal IFN-α primer amplification were cloned using TOPO TA cloning kit (Invitrogen, California, USA). Clones from each insert were sequenced in ABI PRISM 310 Genetic Analyzer (Perkin Elmer, Massachusetts, USA) using the dye Rhodamine terminator cycle sequencing kit (Perkin Elmer, Massachusetts, USA).

Statistical analysis

IFN-α mRNA levels were compared between DLE lesions and normal skin from DLE patients and normal subjects. The significant difference in the independent and paired samples was determined by Mann Withney U tests and Wilcoxon Signed Rank tests, respectively using the SPSS software for windows 10.0 (SPSS Inc., Chicago, USA). A *P* value of <0.05 was considered to be significant.

Results

Up-regulation of IFN-α mRNA level in lesional and non-lesional skin from DLE patients

The IFN-α mRNA levels were measured in DLE lesions and normal skin from DLE patients and healthy subjects by real-time PCR using universal primers for all IFN-α subtypes as shown in **Figure 1**. The IFN-α mRNA level was significantly increased in DLE lesions (mean±SD=0.00463±0.00784) and normal skin from DLE patients (mean±SD=0.00468±0.00699) compared to normal skins from healthy donors (mean±SD=0.00130±0.00100) (P=0.05, P=0.04, respectively).

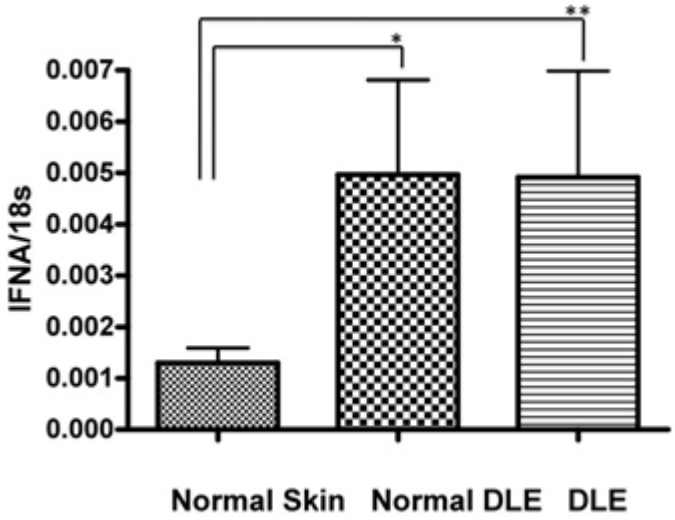

Figure 1. An analysis of IFN-α mRNA level in normal skin from healthy donors and normal skin and DLE lesions from DLE patients by real-time RT-PCR. IFN-α mRNA expression was normalized to 18S housekeeping gene. Error bars indicate the mean SD of each group. The stars (* and **) indicate significant differences of p=0.04 and p=0.05 between normal skin from DLE patients and DLE lesions compared to normal skin from healthy donors, respectively.

The difference in IFN-α subtype from DLE lesion and normal skin

The subtype of IFN-α was analyzed from normal skin of nine healthy donors and normal skin and DLE lesional skin from nine DLE patients. The PCR products of cDNA, amplified with universal primers for all IFN-α subtypes, were cloned and sequenced as shown in **Table 1**. A combination of different IFN-α subtypes was found in skin biopsies from healthy controls and DLE patients. Normal skin from nine healthy donors and from nine DLE patients revealed 54% (46 of 85 clones) and 52% (44 of 84 clones) sequence equivalent to IFN-α subtype 5, respectively. Whereas, DLE lesions from nine DLE patients revealed only 38% (33 of 86 clones) sequence equivalent to IFN-α subtype 5. In addition, IFN-α subtype 1 and 21 had a trend of increasing in DLE lesions compared to non-lesion and normal skin.

Discussion

We detected a significant increase of IFN-α mRNA levels in both lesional and non-lesional skin from DLE patients without SLE compared to normal skin from healthy subjects. A previous study by immunohistochemistry revealed a significant increase in IFN-α producing cells in both lesional and non-lesional skin of SLE patients [4]. It is not surprising that IFN-α producing cells are increased in non-lesional skin of SLE patients since SLE is a systemic disease. Our study demonstrated a significant up-regulation of IFN-α mRNA in normal skin form DLE patients without SLE. The up-regulation of IFN-α in normal skin from DLE patients without SLE may represent a role of IFN-α in the pathogenesis of DLE lesion.

The IFN-α family contains 13 subtypes that have different cell type origins and biological properties. IFN-α subtype 5 has been reported to be the solitary IFN-α subtype expressed in normal liver cells while IFN-α subtype 1, 5, and 21 are the major IFN-α subtypes expressed in normal peripheral blood mononuclear cells (PBMC) [15, 17-19]. Our study illustrated that the IFN-α subtype 5 is the most common subtype in normal skin and similar to normal liver. These observations suggested that IFN-α subtype 5 might be an important subtype in various tissues. In this study, a significant reduction of IFN-α subtype 5 and increase of other subtypes (1, 13, and 21) was observed in DLE lesions compared to normal skin from healthy donors and DLE patients. Interestingly, this finding is supported by a previous study that showed the reduction of IFN-α subtype 5 and the increasing of IFN-α subtype 1, 3, and 21 in PBMC after stimulation with SLE serum [19]. Previous experiments have demonstrated a significant reduction of IFN-α subtype 5 in liver cells of chronic hepatitis C patients which might be the result of infiltration of PBMC in chronic hepatitis liver cells

Table 1. An analysis of subtypes of IFN-α in normal skin from healthy donors and normal skin from DLE patients and DLE lesions from DLE patients

Samples	No. of clones tested (%)	α1,13	α2	α4	α5	α6	α7	α8	α10,14, 16	α21
Normal skin										
Total	85 (100)	6 (7)	8 (9.4)	4 (4.7)	46 (54.1)	1 (1.2)		8 (9.4)	8 (9.4)	4 (4.7)
Normal skin from DLE patients										
P1	9		3	2	1		1		2	
P2	10	1	3		4			2		
P3	14	1	3		9				1	
P4	7	1	1	1	1				2	1
P5	9	1			5			2	1	
P6	8		1		4			1	1	1
P7	9				7					2
P8	10				10					
P9	8	2	1		3			1		1
Total	84	6 (7.1)	12 (14.3)	3 (3.6)	44 (52.2)		1 (1.2)	6 (1.2)	7 (8.3)	5 (6.0)
DLE lesions										
P1	9		2		3		1		2	1
P2	11	4			3			1	2	1
P3	13		2	1	7			1		2
P4	7	2			2			2		1
P5	8	1			3	2		1	1	
P6	10	1	1		4			2	1	1
P7	9	3			5			1		
P8	9	2	3	1	1			1	1	
P9	10	1	2		5		1			1
Total	86 (100)	14 (16.3)	10 (11.6)	2 (2.3)	33 (38.4)	2 (2.3)	2 (2.3)	9 (10.5)	7 (8.1)	7 (8.1)

[15]. It is likely that the reduction of IFN-α subtype 5 and the increase of IFN-α subtype 1, 13 and 21 in DLE lesions may simply be the result of infiltration of PBMC in DLE lesion. Various biological responses to different IFN-α subtypes have been demonstrated. The IFN-α subtype 5 has the most active antiviral properties against influenza virus [14, 20]. Moreover, down-regulation of IFN-α subtype 5 has been observed in various virally infected tissues [15, 19]. It is also possible that cryptic viral infection in LE patients will be the cause of reduction of IFN-α subtype 5 in lesional DLE. It is probable that IFN-α subtype 5 might be the important protective subtypes in tissue and the decrease of IFN-α subtype 5 might result in immune imbalance leading to tissue inflammation.

Conclusion

The present study demonstrated up-regulation of IFN-α in cutaneous lesional and non-lesional DLE patients. Therefore, this is suggesting that IFN-α was an important cytokine in pathogenesis of DLE lesions. The reduction of IFN-α subtype 5 may also be crucial in maintaining DLE lesions. However, the functional consequence of different expression patterns of IFN-α subtype between normal skin and DLE lesion need further study.

Acknowledgement

This study was supported by The Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (HR1163A), Faculty of Medicine, Chulalongkorn University, Thailand. The authors have no conflict of interest to declare.

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