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Genetically Determined TNF- α Secretion Influences the Development of Dermatomyositis and Systemic Lupus Erythematosus

Lyubomir A. Dourmishev¹, Maria H. Hristova², Zornitsa G. Kamenarska³, Alexey S. Savov⁴, Anton I. Vinkov⁵, Radka Kaneva⁶, Vanjo Mitev⁶

¹ Department of Dermatology and Venereology, Medical University of Sofia, Sofia, Bulgaria

² Department of Nephrology, Faculty of Medicine, Medical University of Sofia, Sofia, Bulgaria

³ Molecular Medicine Center, Medical University of Sofia, Sofia, Bulgaria

⁴ National Genetic Laboratory, Maichin Dom Hospital, Bulgaria

⁵ Department of Neurology, Hôpitaux Drôme Nord, Romans-sur-Isère, France

⁶ Department of Medical Chemistry and Biochemistry, Molecular Medicine Center, Medical University of Sofia, Sofia, Bulgaria

Correspondence:

Lyubomir A. Dourmishev, Department of Dermatology and Venereology, Medical University of Sofia, 1 St Georgi Sofijski Str., 1431 Sofia, Bulgaria
E-mail: l_dourmishev@mail.bg
Tel: +359 2 9230 438

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Background: Abnormal secretion of TNF- α is known to play a role in the pathogenesis of dermatomyositis and systemic lupus erythematosus.

Materials and methods: In the present study we have analyzed the concentrations of TNF- α in the sera of 30 patients with systemic lupus erythematosus (SLE), 28 with dermatomyositis (DM) and 30 healthy controls by standard ELISA tests.

Results: We have found that -308A allele increases TNF- α secretion, while -1031C and -863A alleles decrease it. The -857C/T and 489G/A polymorphisms appeared in strong linkage disequilibrium ($D'=0.93$) but they did not seem to affect TNF- α secretion.

Conclusion: TNF- α polymorphisms play a significant role in its secretion and influence the development of DM and SLE.

BACKGROUND

Dermatomyositis and systemic lupus erythematosus are autoimmune diseases of unknown origin but inherited genes, viruses, ultraviolet light and certain medication may play role in their etiopathogenesis.

Cytokine imbalances are known to play a key role in the pathogenesis of both DM and SLE. One of the intensely investigated pro-inflammatory proteins is the tumor necrosis factor alpha (TNF- α) which plays an important role in the inflammatory and immune responses. DM and SLE patients are characterized by disturbances in their TNF- α levels compared to healthy controls.¹⁻³ The serum con-

centration of TNF- α depends on many factors but the ones that are most important are the genetic polymorphisms. In previous studies, we have found that the TNF- α -1031C/-863C/-857C/-308G/+489G haplotypes are associated with both DM and SLE, especially in women.^{4,5} Although there are plenty of studies, the data concerning the role of TNF- α polymorphisms in its secretion vary a lot. In an in vitro study, the TNF- α level and the transcriptional promoter activity produced by concanavalin A-activated peripheral blood mononuclear cells were higher for the -857T or -1031C alleles than for the -857C or -1031T alleles, respectively.⁶ However,

another research group has reported that subjects with -863A tightly linked with -1031C have a significantly lower serum TNF- α level.⁷ According to some authors, individuals homozygous for TNF- α -308A have higher serum TNF- α level than the TNF- α -308G homozygotes^{8,9}, however, a recent study has demonstrated that the -308GG, -1031TT and the -857TT+CT genotypes are associated with higher TNF- α level than the -308GA+AA, -1031TC+CC and -857CC genotypes¹⁰.

The aim of the present study was to analyze the TNF- α serum concentrations in patients with DM and SLE and search for correlations on terms of the TNF- α polymorphisms we have studied earlier.^{4,5}

MATERIALS AND METHODS

Twenty-eight patients with dermatomyositis meeting the criteria of Bohan and Peter¹¹ and Targoff et al.¹² and 30 with systemic lupus erythematosus meeting the American College of Rheumatology (ACR) criteria were included in this study. Only patients with definitive or probable disease were included. In the DM group, 22 patients were female and 6 male with mean age of 53 years (range 28 - 82 yrs). In the SLE group, 23 were female and 7 male with mean age of 44 years (range of 25-78 yrs). The patients were followed up for a mean of 10 years at the Department of Dermatology and Venereology, and the Department of Nephrology at the Medical University, Sofia, and at the Department of Nephrology in the Ministry of Interior Hospital, Sofia. The control group consisted of 30 age-, gender- and ethnicity-matched healthy volunteers randomly selected from the Biobank of the Molecular Medicine Center.

The serum TNF- α levels were measured using a standard ELISA test (Gen-Probe Diaclone SAS, France) according to the protocols provided by the manufacturing company.

The procedures described herein were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The study was approved by the local ethics committee at the Medical University, Sofia. Mann-Whitney U test was used to determine the statistical significance.

RESULTS AND DISCUSSION

According to the Centers for Disease Control and Prevention (CDC) the TNF- α normal reference range is lower than 10 pg/ml. The concentration of TNF- α was below the limit of detection in the majority of

the individuals studied. Only in several cases did we observe the TNF- α serum levels to increase above 10 pg/ml.

We found that the -308GA genotype is associated with a higher secretion of TNF- α compared to the -308GG ($p=0.008$). These results are consistent with the results reported by other authors and confirm the functional significance of the -308G/A polymorphism.^{9,13-15}

A difference was found in the secretion of TNF- α in patients with SLE carrying the -1031TT, -1031TC, -863CC, and -863CA genotypes ($p=0.009$ and $p=0.018$, respectively). The -1031TT and -863CC genotypes lead to a higher secretion of TNF- α compared to the -1031TC and -863CA genotypes.⁶ Our results are fully consistent with the data obtained by other authors who reported significantly lower levels of TNF- α in carriers of the -1031C and -863A alleles.^{7,16} As in previous studies, we also found a strong linkage disequilibrium between the -863 and -1031C alleles ($D'=0.88$). Thus, the obtained results support the hypothesis that the -863C/A polymorphism might affect the binding of the transcription factor NF κ B which leads to differences in the gene expression levels. However, the leading role of the -1031T/C cannot be excluded.

The -857C/T and 489G/A polymorphisms appeared in strong linkage disequilibrium ($D'=0.93$) but they do not seem to have any functional significance concerning the TNF- α secretion. Unlike van Heel et al., who have found an allele dependent expression of TNF- α based on the fact that only the TNF- α -857T but not -857C allele binds to the octamer binding transcription factor OCT1 (or POU2F1)¹⁷, we have found no differences in the expressions neither between the patients nor between the controls. A possible explanation for the observed differences is that in the current work we have not carried out an ex vivo stimulation with lipopolysaccharides. Unlike the 'housekeeping' genes, the gene expression of TNF- α is not constant. It largely depends on external factors and the single nucleotide polymorphisms only provide the potential for high or low expression of the protein in relation to the transcription factors which can join the corresponding domain of the promoter region.

Due to the low frequency of the -238GA and -238AA genotypes, their impact could not be assessed.

This study is the first to examine the functional role of the haplotype combinations formed by the six SNPs. Their frequency among the entire

population is given in **Table 1**. The role of the haplotype combinations was confirmed by the statistically significant difference in the extinction between the genotypes TCCGGG/TCCAGG and CACGGG/CCCGGG.

These results are in line with those of other authors who have found that individuals with the haplotype -1031/-863/-857/-308 >TCCG have a significantly higher secretion of TNF- α than those with the CACG haplotype.¹⁶ A partial explanation

Furthermore, their approach to compare the average concentration of each haplotype with that of all other diminish the differential potential of the study.

Of note, no statistically significant difference in the secretion was found in the present study for the haplotypes TCCAGG, TACGGG, TCTGGA, TCCGGG and CACGGG but this may be due to the masking effect which the TCCGGG haplotype exhibits within the common genotype.

Table 1. Haplotype frequency

<i>TNFA</i> -1031/-863/-857/-308/-238/489	Frequency	Mean concentration (pg/ml) $\pm$ SD
1031 (TT); 863 (CC); 857 (CT); 308 (GG); 238 (GG); 489 (GA)	22.7	9.1 $\pm$ 6.3
1031 (TT); 863 (CC); 857 (CC); 308 (GG); 238 (GG); 489 (GG)	21.6	10.6 $\pm$ 16.9
1031 (TC); 863 (CA); 857 (CC); 308 (GG); 238 (GG); 489 (GG)	15.9	7.2 $\pm$ 1.9
1031 (TT); 863 (CC); 857 (CC); 308 (GA); 238 (GG); 489 (GG)	9.1	11.7 $\pm$ 9.3
1031 (TT); 863 (CC); 857 (TT); 308 (GG); 238 (GG); 489 (AA)	7.6	26.8 $\pm$ 48.8
1031 (CC); 863 (CA); 857 (CC); 308 (GG); 238 (GG); 489 (GG)	3.4	6.2 $\pm$ 1
1031 (TT); 863 (CC); 857 (CT); 308 (GA); 238 (GG); 489 (GA)	3.4	8.5 $\pm$ 0.4
1031 (TT); 863 (CC); 857 (CC); 308 (GA); 238 (GG); 489 (GA)	2.3	12.5 $\pm$ 2.6
1031 (TT); 863 (CA); 857 (CC); 308 (GG); 238 (GG); 489 (GG)	2.3	9.5 $\pm$ 3.5
1031 (TC); 863 (CA); 857 (CC); 308 (GA); 238 (GG); 489 (GG)	2.3	6.4 $\pm$ 0.3
1031 (TC); 863 (CA); 857 (CT); 308 (GG); 238 (GG); 489 (GA)	2.3	6.6 $\pm$ 0.1
1031 (TC); 863 (AA); 857 (CT); 308 (GG); 238 (GG); 489 (GA)	2.3	5.9 $\pm$ 1.8
1031 (TT); 863 (CC); 857 (CT); 308 (GA); 238 (GG); 489 (GG)	1.2	10
1031 (TC); 863 (CC); 857 (CT); 308 (GG); 238 (GG); 489 (GA)	1.2	6.3
1031 (TC); 863 (CC); 857 (CT); 308 (GG); 238 (GA); 489 (GA)	1.2	6.6
1031 (TT); 863 (CA); 857 (CC); 308 (GA); 238 (GG); 489 (GG)	1.2	7.2

of the observed results is given in the study of van Heel et al. according to which the haplotype combination -863C -857C is a prerequisite for binding to NFkB, but not to OST1, thereby avoiding the inhibitory effect of NFkB on OST1 transcriptional activity.¹⁷ Thus this haplotype is associated with a higher expression of TNF- α .

In another study, Mekinian et al. investigated the functional role of -857/-308/-238 haplotypes in healthy subjects and found no statistically significant differences in the TNF- α concentrations.¹⁸ However, they have not taken into account the influence of the polymorphisms -1031T/C and -863C/A which obviously play a key role in the TNF- α expres-

In conclusion, the TNF- α polymorphisms play a significant role in its secretion and influence the development of DM and SLE. Although our understanding of the TNF- α role in DM and SLE has increased considerably over the past years, a wide-scale multicenter studies of all cytokine gene polymorphisms are still of importance in future.

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Генетически обусловленная секреция TNF- α влияет на развитие дерматомиозита и системной красной волчанки

Любомир А. Дурмишев¹, Мария Х. Христова², Зорница Г. Каменарска³, Алексей С. Савов⁴,
Антон И. Винков⁵, Радка Канева⁶, Ваньо Митев⁷

¹ Кафедра дерматологии и венерологии, Медицинский университет - София, София, Болгария

² Кафедра нефрологии, Факультет медицины, Медицинский университет - София, София, Болгария

³ Кафедра медицинской химии и биохимии, Центр молекулярной медицины, Медицинский университет - София, София, Болгария

⁴ Национальная генетическая лаборатория, СБАЛАГ „Майчин дом“, Болгария

⁵ Кафедра неврологии, Больница Дром Норд, Роман-сюр-Изер, Франция

⁶ Кафедра медицинской химии и биохимии, Центр молекулярной медицины, Медицинский университет - София, София, Болгария

⁷ Кафедра медицинской химии и биохимии, Медицинский университет София, София, Болгария

Адрес для корреспонденции:

Любомир А. Дурмишев, Кафедра дерматологии и венерологии, Медицинский университет - София, ул. „Георги Странски“ №1, 1431 София, Болгария

E-mail: l_dourmishev@mail.bg

Tel: +359 2 9230 438

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Введение: Известно, что аномальная секреция TNF- α играет определённую роль в патогенезе дерматомиозита и системной красной волчанки.

Материалы и методы: В настоящем исследовании мы проанализировали концентрации TNF- α в сыворотках 30 больных с системной красной волчанкой (СКВ), 28 с дерматомиозитом (ДМ) и 30 здоровых представителей контрольной группы с помощью стандартных тестов ELISA.

Результаты: Мы обнаружили, что аллель -308A увеличивает секрецию TNF- α , тогда как аллели -1031C и -863A уменьшают её. Полиморфизмы -857C / T и 489G / A проявились в сильном неравновесном сцеплении ($D' = 0,93$), но они, по-видимому, не влияют на секрецию TNF- α .

Заключение: Полиморфизмы TNF- α играют значительную роль в её секреции и влияют на развитие ДМ и СКВ.