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Levels of STING in rheumatoid arthritis

Inflammation in RA

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Abstract
Aim: STING (stimulator of Type-I Interferon genes) is a transmembrane carrier protein that is responsible for the transduction of (deoxyribonucleic acid) DNA-triggered signals. In this study, we aimed to investigate the serum level of STING and its prognostic value related to the disease activity in patients with rheumatoid arthritis (RA).
Material and Methods: We enrolled 80 patients with RA, and age-, gender- and body mass index (BMI)- matched 80 healthy individuals. Serum levels of STING were investigated using enzyme-linked immunosorbent assay kits. The SPSS (Statistical Package for the Social Sciences) program (IBM, Armonk, NY, USA) was used for statistical analysis of the data. Descriptive statistics were expressed as mean ± standard deviation or median (minimum-maximum) for discrete and continuous numerical variables, and the number of cases and (%) for categorical variables. Cross-table statistics were used to compare categorical variables (Chi-square, Fisher). Comparisons between multiple groups were made with Post Hoc Tukey analysis. Results were defined as $p<0.05$ statistical significance.
Results: Neutrophilia, higher values of erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) and lower hemoglobin values were detected in the RA group (Neutrophil: 4.54 ± 1.71x109/L, CRP: 1.03 ± 1.30 mg/dL, ESR: 30.27 ± 18.1 mm/h, and Hgb: 12.88 ± 1.50 g/dL; p values: 0.013, 0.001, 0.001 and 0.029, respectively). The mean level of serum STING was 3422.75 ± 398,92 pg/mL in the RA group, whereas 3548.70 ± 126,03 pg/mL in the control group. Male patients and patients with higher ESR and CRP values had higher STING levels compared with female patients, and patients with lower ESR and CRP values (p-values: 0.044, 0.006 and 0.046, respectively).
Discussion: Serum STING levels were similar in RA patients and healthy controls. Additional knowledge related to the STING pathway will be beneficial for the development of new immunotherapeutic strategies in many inflammatory diseases such as RA.

Keywords
STING, Rheumatoid Arthritis, Inflammation, Autoimmunity, Type-I Interferon

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Introduction

STING (stimulator of Type-I Interferon genes) is a transmembrane carrier protein encoded by Transmembrane protein 173 (TMEM173) that plays a role in innate immunity. Human STING is 379 amino acids in length. STING is localized in the endoplasmic reticulum (ER) of numerous cell types such as macrophages and dendritic cells, as well as endothelial and epithelial cells. This ER-related signaling molecule is involved in the control of transcription of many host defense genes such as interferon (IFN) and pro-inflammatory cytokines, following the detection and recognition of cyclic dinucleotides (CDNs) or aberrant (deoxyribonucleic acid) DNA molecules in the cell cytoplasm [1]. STING has three functional domains: the cytoplasmic C-terminal tail (342-379 amino acids), the central globular domain (155-341 amino acids), and four N-terminal transmembrane motifs (1-154 amino acids) that present STING to the ER and receive signals. STING is responsible for the transduction of DNA-triggered signals.

Examples of the sources of DNA that induce the formation of CDNs include the genomes of pathogens such as Herpes simplex virus type 1 (HSV-1). In addition, some bacteria can secrete CDNs when they infect the host. Recent studies have shown that self-DNA that comes out of the nucleus of the host cell during cell division and/or as a result of DNA damage is also the potent activator of the STING pathway. It has been shown that such DNA molecules may be responsible for autoinflammatory diseases such as systemic lupus erythematosus (SLE) or Aicardi–Goiteres syndrome (AGS), or inflammation-associated cancer [2]. Many DNA viruses such as adenovirus, vaccinia virus, papillomavirus, and/or bacterial infections (such as listeria monocytogenes or mycobacterium tuberculosis) and/or parasites (malaria) have been reported to trigger STING-dependent processes. Type-I interferon (Type-I IFN) activation occurs as a result of STING overexpression and induces nuclear factor-kappa B (NF-kB) and Type-I IFN regulatory factor 3 (IRF3) [3]. In addition, there are opposite mechanisms in the body to prevent self-DNA from initiating host defense through DNA sensors. TREX1 likely inhibits the activation of STING signals by intrinsic self-DNA. DNase II has the function of preventing STING in phagocytes. These DNases are indirect negative regulators of the STING pathway, and when these DNases are defective, basal and continuous STING activity and then inflammatory diseases occur [4].

Rheumatoid arthritis is one of the autoimmune rheumatic diseases. Therefore, examining the effects of the STING pathway in autoimmune diseases may contribute to the elucidation of the etiopathogenesis of RA. In this study, we aimed to investigate serum levels of STING, and its prognostic value related to the disease activity in RA patients.

Material and Methods

Study design and Data collection

The study was designed by following local ethical rules (date: 27/12/2016 and no: 23) and the Helsinki Declarations. Written- and informed- consent form was obtained from all participants. We enrolled 80 patients with RA who fulfilled the American College of Rheumatology (ACR) classification criteria [5], and age-, gender- and body mass index (BMI)- matched 80 healthy individuals. All patients were diagnosed by the same rheumatology team, between June 2017 and November 2017. Disease activity score 28-ESR (DAS28-ESR) was calculated as: (0.56 x √number of tender joints) + (0.28 x √number of swollen joints) + (0.70 x ESR) + (0.014 x visual pain scale) [6]. Demographic characteristics and laboratory parameters at diagnosis were noted for each participant. Venous blood samples were collected at 8.00-10.00 am after 12 hours of fasting, and all samples were centrifuged and stored at -80 degrees. Serum levels of STING were investigated using enzyme-linked immunosorbent assay (ELISA) kits.

Inclusion and Exclusion criteria

Inclusion criteria were defined as: having RA and/ or being a volunteer for the control group.

Exclusion criteria were defined as: smoking, having at least one of the following diseases such as hypertension, diabetes mellitus, coronary arterial disease, cardiac valve disease, and/ or having abnormal thyroid functions, abnormal renal and/or hepatic function tests, recent infectious disease in the last three months, having active solid organ and/or hematologic malignancies.

Statistical analysis

The Statistical Package for the Social Sciences (version 21.0 of the SPSS, IBM, Armonk, NY, USA) program was used for statistical analysis of the data in the study. Descriptive statistics were expressed as mean ± standard deviation or median (minimum-maximum) for discrete and continuous numerical variables, and number (n) of cases and (%) for categorical variables. Cross-table statistics were used to compare categorical variables (Chi-square, Fisher). Normally distributed parametric data were compared with Student's t-test and ANOVA, and non-parametric data that did not conform to normal distribution were compared with the Mann-Whitney U and the Kruskal-Wallis tests. Comparisons between multiple groups were made with Post Hoc Tukey analysis. Results were defined as $p<0.05$ statistical significance.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

The mean age was 46.43 ± 12.8 years for the whole study group (distribution range: 18-67 years), and one hundred, twenty-one (n=121, 75.6%) patients were female. The vast majority of individuals in both groups were female (n=61, 76.3% in the RA group; n=60, 75% in the control group). Both groups were similar in terms of age, gender and body mass index (BMI) ($p>0.05$). The mean value of DAS28-ESR was 3.29 ± 0.68 among RA patients (distribution range: 2.4-4.8). Laboratory parameters at diagnosis including complete blood count, acute phase reactants and serum uric acid are shown in Table 1. As expected, neutrophilia, higher values of ESR and c-reactive protein (CRP), and lower hemoglobin values were detected in the RA group (Neutrophil: 4.54 ± 1.71x109/L, CRP: 1.03 ± 1.30 mg/dL, ESR: 30.27 ± 18.1 mm/h, and Hgb: 12.88 ± 1.50 g/dL; p values: 0.013, 0.001, 0.001 and 0.029 respectively). Twenty-six (n=26, 32.5%) RA patients had positive anti-nuclear antibody (ANA), whereas 60 (75%) RA patients with positive rheumatoid factor (RF), and 51 (63.7%) RA patients with

Table 1. Demographic data and the baseline laboratory parameters of the whole study group.

Variables	RA (n=80) (mean ± SD)	Healthy Controls (n=80) (mean ± SD)	p value
Age	52.5±10.3	40.2±12.2	P > 0.2*
BMI (kg/m2)	26.8±5.0	26.0±4.7	P > 0.2*
Leukocyte (x109/L)	7.20±2.23	6.63±1.87	0.080
Neutrophil (x109/L)	4.54±1.71	3.93±1.33	0.013*
Hemoglobin (g/dL)	12.88±1.50	13.45±1.73	0.029*
Platelet (x109/L)	290.6±849.9	282.0±747.9	0.668
Uric acid (mg/dL)	4.31±1.12	4.39±1.33	0.982
Sedimentation (mm/h)	30.27±18.1	17.63±11.7	0.001*
C-reactive protein (mg/dL)	1.03±1.30	0.33±0.54	0.001*
STING (pg/mL)	3422.75±398.92	3548.70±126.03	0.420

RA: rheumatoid arthritis, BMI: body mass index, STING: stimulator of interferon genes.
*. This is a lower bound of the true significance

Table 2. Mean levels of STING in RA patients according to different variables.

Variables		RA (n=80) n (%)	STING levels (mean±SD)	p-value
Age	< 45	18 (22.5%)	3338.47±760.2	0.557
	> 45	62 (77.5%)	3447.21±205.7	
Gender	Female	61 (76.3%)	3391.25±447.6	0.044*
	Male	19 (23.8%)	3523.87±127.8	
ANA	(+)	26 (32.5%)	3293.03±640.1	0.070
	(-)	54 (67.5%)	3485.20±178.0	
RF	(+)	60 (75%)	3426.08±447.8	0.898
	(-)	20 (25%)	3412,75±197,1	
Anti-CCP	(+)	51 (63.7%)	3399.28±483.6	0.342
	(-)	29 (36.3%)	3464.01±169.1	
Leukocyte (109/L)	< 10.000	72 (90%)	3412.95±417.1	0.312
	> 10.000	8 (10%)	3510.93±144.8	
Sedimentation (mm/h)	< 30	45 (56.3%)	3356.69±502.0	0.006*
	> 30	35 (43.8%)	3507.68±174.3	
C-reactive protein (mg/dL)	< 0.50	45 (56.3%)	3351.86±511.7	0.046*
	> 0.50	35 (43.8%)	3513.89±124.9	

RA: rheumatoid arthritis, STING: stimulator of interferon genes, ANA: antinuclear antibody, RF: rheumatoid factor, anti-CCP: anti-citrullinated protein.

Table 3. A correlation analysis of STING and different clinical variables.

Variables	r	p-value
Age	-0,141	0,214
BMI	-0,042	0,714
DAS 28	0,158	0,16
Anti-CCP	0,109	0,336
RF	+0,294**	0,008*
Leukocyte	0,199	0,077
Neutrophil	0,213	0,058
Hemoglobin	0,07	0,537
Platelet	0,009	0,936
Uric acid	-0,008	0,947
C-reactive protein	0,17	0,131
Sedimentation	0,127	0,262

STING: stimulator of interferon genes, BMI: body mass index, DAS: disease activity score, RF: rheumatoid factor, anti-CCP: anti-citrullinated protein, *: p<0.05 is statistically significant, **: p=0.01 is statistically significant.

positive anti-citrullinated protein (anti-CCP). A vast majority of patients were on treatment with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs). The three most commonly used drugs were methotrexate, glucocorticoids and antimalarials, respectively.

The mean level of serum STING was 3422.75 ± 398,92 pg/mL in the RA group, whereas 3548.70 ± 126,03 pg/mL in the control group. There was no statistical difference between the groups. Male patients and patients with higher ESR and CRP values had higher STING levels compared with female patients, and patients with lower ESR and CRP values (p-values: 0.044, 0.006, and 0.046, respectively) (Table 2). In addition, there was a positive correlation between RF positivity and serum STING levels in RA patients (r=294, p=0.008) (Table 3).

Discussion

We conducted the study to reveal the possible role of the STING pathway in the pathogenesis of RA. RA has multiple complex immunogenetic mechanisms in the underlying pathogenesis. Our data demonstrate similar levels of serum STING both of the RA patients and healthy controls. However, this does not mean that STING is not involved in the pathogenesis of RA. Recently, the main cytokine tumor necrosis factor-alpha (TNF-α) has been shown to trigger the cyclic guanosine monophosphate-AMP synthase (cGAS) related- STING pathway in RA [7]. Triptolide has also been shown as a new immunosuppressant and anti-inflammatory agent in RA. It plays a role in the down-regulation of the cGAS and STING protein and reduces the levels of proinflammatory cytokines such as interleukin-1beta (IL-1β), interleukin 6 (IL-6), and TNF-α [8].

Recent studies have shown that overactivation of the STING pathway as a result of overexpression of self-DNA molecules originating from possible necrotic or inappropriately apoptotic cells plays an important role in the pathogenesis of autoimmunity, not only in RA [9]. Many variable mutations in the innate immune signaling are also culprit factors in inflammatory vasculopathy and a variety of interferonopathies [10]. As an example, TREX1 acts as a negative regulator for STING, and TREX1 mutations have been reported to occur in inflammatory diseases such as SLE [11].

SLE is one of the most investigated autoimmune diseases in association with STING. The role of the STING pathway has been discussed by many researchers. Kato Y et al. observed a high type- I IFN activity induced by the cytosolic DNA-sensitive STING pathway in SLE patients [12]. Similarly, Wang J et al reported the hyperactive cGAS related-STING pathway in SLE patients, and they showed that type- I IFN overproduction occurs in connection with the contribution of the Interferon-induced protein with tetratricopeptide repeats 3 (IFIT3) gene in this overactivation [13]. Konig N et al. found traces of STING mutation in 5 patients with chilblain lupus erythematosus, a rare inflammatory form of chronic cutaneous lupus erythematosus with uncertain pathogenesis, and they blamed the functional anomaly of the STING pathway in the pathogenesis of the disease [14].

The effect of the STING-dependent pathway has also been proven using specific bifunctional autoantibodies. It has been shown not only in SLE, but also in the development of ANA-

mediated inflammatory arthritis. Pawaria S et al. noticed that autoreactive B cell activation could not be achieved in double-knockout (DNase II−/−, Ifnar1−/−) mice due to deficiency of Toll-like receptor 9 (TLR9) activation [15]. Similarly, Baum R et al. investigated inflammation in another study in triple knockout mice with STING or melanoma 2 (AIM2) defects. Mice with AIM2 defect induced a limited inflammatory response against accumulated endosomal self-DNA, while mice with STING defect showed regression of inflammation in the joints. Thus, the researchers showed that the cytosolic and endosomal nucleic acid-sensitive pathways are TLR7/9-dependent, and autoantibody production is disrupted in any defect, thus they play an active role in the formation of the disease [16].

In fact, the effect of the STING signaling pathway has been demonstrated not only in inflammatory processes. Baum R et al. also proved the necessity of the intact STING pathway in bone formation. Thus, researchers have demonstrated the pathogenic role of disruptions in the STING pathway in bone anomalies observed in autoimmune diseases [17]. Besides, Hartlova et al. worked with patients diagnosed with ataxia-telangiectasia (AT). AT protein kinase gene (ATM gene) is involved in the DNA repair mechanism. In the presence of a genetic defect, unrepaired DNA residues trigger type- I IFN and cause a serious increase in viral and bacterial responses, and at the same time, the DNA fragments accumulated in the cytoplasm activate the DNA-sensitive STING pathway, thereby activating the TLR-1 [18]. Liu Y et al. detected an autosomal dominant mutation in the TMEM173 gene in 6 children with infant-onset systemic and/or organ-specific inflammation and vasculopathy. Also, ESR and CRP which are indices of systemic inflammation, have also been reported to be elevated in these children who were defined as STING-associated vasculopathy with onset in infancy (SAVI) [19]. Similarly, although there was no statistically significant difference in serum STING levels between the RA group and the control group, high sedimentation rate (> 30 mm/s) and high CRP (> 0.50 mg) /dl) values were related to higher serum STING levels in our study. A statistically significant positive correlation was also found between mean serum STING levels and serum RF values in patients with RA (p=0.008, r=294). This situation may be explained by the relationship between hyper inflammation- and autoimmunity-related mechanisms and serum STING levels, as expected.

There are few limitations in thisstudy. First, the number of study groups is relatively small for cytokine and/or gene trials. Secondly, concurrent evaluation of serum STING levels and related-gene polymorphisms can provide more accurate knowledge. Also, further studies could compare more homogeneous patient groups with only differences in disease activity scores (mild or severe disease).

Conclusion

Serum STING levels were similar in RA patients and in healthy controls. Additional knowledge related to STING pathway will be beneficial for the development of new immunotherapeutic targets in many inflammatory diseases such as RA. It could be useful to compare the results of our study with the findings of future studies with larger sample groups.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

1. Barber GN. Innate immune DNA sensing pathways: STING, AIMIII and the regulation of interferon production and inflammatory responses. Curr Opin Immunol. 2011;23(1):10-20.

2. Barber GN. STING: infection, inflammation and cancer. Nat Rev Immunol. 2015;15(12):760-70.

3. Liu X, Wang C. The emerging roles of the STING adaptor protein in immunity and diseases. Immunology. 2016;147(3):285-91.

4. Smith S, Jefferies C. Role of DNA/RNA sensors and contribution to autoimmunity. Cytokine Growth Factor Rev. 2014;25(6):745-57.

5. Aletaha D, Neogi T, Silman AJ, Furovits J, Felson DT, Bingham CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.

6. Inoue E, Yamanaka H, Hara M, Tomatsu T, Kamatani N. Comparison of Disease Activity Score (DAS)28- erythrocyte sedimentation rate and DAS28- C-reactive protein threshold values. Ann Rheum Dis. 2007;66(3):407-9.

7. Willemsen J, Neuhoff MT, Hoyler T, Noir E, Tessier C, Sarret S, et al. TNF leads to mtDNA release and cGAS/STING-dependent interferon responses that support inflammatory arthritis. Cell Rep. 2021;37(6):109977.

8. Xu A, Yang R, Zhang M, Wang X, Di Y, Jiang B, et al. Macrophage targeted triptolide micelles capable of cGAS-STING pathway inhibition for rheumatoid arthritis treatment. J Drug Target. 2022;30(9):961-2.

9. Burdette DL, Monroe KM, Sotelo-Troha K, Iwig JS, Eckert B, Hyodo M, et al. STING is a direct innate immune sensor of cyclic di-GMP. Nature. 2011;478(7370):515-8.

10. Yan N. Immune Diseases Associated with TREX1 and STING Dysfunction. J Interferon Cytokine Res. 2017;37(5):198-206.

11. Ahn J, Ruiz P, Barber GN. Intrinsic self-DNA triggers inflammatory disease dependent on STING. J Immunol. 2014;193(9):4634-42.

12. Akazawa S, Mackin L, Jhala G, Fynch S, Catterall T, Selck C, et al. Deficiency of the innate immune adaptor STING promotes autoreactive T cell expansion in NOD mice. Diabetologia. 2021;64(4):878-89.

13. Motwani M, McGowan J, Antonovitch J, Gao KM, Jiang Z, Sharma S, et al. CGAS-STING Pathway Does Not Promote Autoimmunity in Murine Models of SLE. Front Immunol. 2021;12:605930.

14. Konig N, Fiehn C, Wolf C, Schuster M, Costa EC, Tungler V, et al. Familial chilblain lupus due to a gain-of-function mutation in STING. Ann Rheum Dis. 2017;76(2):468-72.

15. Pawaria S, Moody K, Busto P, Nundel K, Choi CH, Ghayur T, et al. Cutting Edge: DNase II deficiency prevents activation of autoreactive B cells by double-stranded DNA endogenous ligands. J Immunol. 2015;194(4):1403-7.

16. Baum R, Sharma S, Carpenter S, Li QZ, Busto P, Fitzgerald KA, et al. Cutting edge: AIM2 and endosomal TLRs differentially regulate arthritis and autoantibody production in DNase II-deficient mice. J Immunol. 2015;194(3):873-7.

17. Baum R, Sharma S, Organ JM, Jakobs C, Hornung V, Burr DB, et al. STING Contributes to Abnormal Bone Formation Induced by Deficiency of DNase II in Mice. Arthritis Rheumatol. 2017;69(2):460-71.

18. Hartlova A, Erttmann SF, Raffi FA, Schmalz AM, Resch U, Anugula S, et al. DNA damage primes the type I interferon system via the cytosolic DNA sensor STING to promote anti-microbial innate immunity. Immunity. 2015(17);42(2):332-43.

19. Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Sanchez GAM, et al. Activated Stıng in a vascular and pulmonary syndrome. N Engl J Med. 2014;371(6):507-18.

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