AB0169

SNP (1513A>C AND 489C>T) OF P2X7 RECEPTOR IN SYSTEMIC LUPUS ERYTHEMATOSUS WITH SEROSITIS

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Background: our preliminary data demonstrated that expression and activity of P2X7R was impaired in Systemic Lupus Erythematosus (SLE) and associated with a reduced production of IL-1 β . Serositis is a typical manifestation of SLE characterized by a marked inflammation, which has been suggested to be an "inflammasome driven" manifestation.

Objectives: to investigate the role of 1513A>C (rs3751143) and 489C>T (rs208294) Single Nucleotide Polymorphisms (SNPs) which are differently associated with loss or gain of function, respectively, of P2X7R, a potent activator of the NLRP3 inflammasome and IL-1 β release, in patients with SLE and with a history of serositis (SLE-S).

Methods: DNA was extracted from whole blood and used for evaluation of 2 P2X7R SNPs (1513A>C and 489C>T). Considering the combined action of these two SNPs, the overall activity of P2X7R was divided, into three groups: GOF (gain of function), normal function (NF), and LOF (loss of function). In addition, peripheral blood mononuclear cells (PBMCs) were isolated from venous blood and employed to evaluate P2X7R and NLRP3 expression by RT-PCR, assess P2X7R activity as Benzoyl ATP (BzATP)-induced intracellular Calcium ([Ca²⁺]_i) increments and evaluate in vitro IL-1 β following stimulation with lipopolysaccharide (LPS) and BzATP, either separately or in combination.

Results: 33 SLE patients (pts), 11 with (SLE-S) and 22 without serositis (SLE-NS) were enrolled. Mean age was 40.9 $\pm$ 10.9 years and disease duration was 135.3 $\pm$ 108.6 months. No significant difference in disease activity and clinical characteristic was found between the two groups (table 1). Evaluating 1513A>C SNP, 20 pts were positive for A/A and 13 for A/C phenotype respectively, while in case of 489C>T SNP, 7 pts presented C/C, 12 C/T and 14 T/T phenotype with a comparable distribution between SLE-NS and SLE-S (table 1). After combination of different phenotypes, 9 pts presented normal function (NF), 22 gain of function (GOF) and 2 loss of function (LOF) with no significant difference between SLE-S and SLE-NS (table 1). P2X7R activity, (evaluated as IL-1 β production and [Ca²⁺]_i increments,) and expression (evaluated with RT-PCR) were comparable between SLE-S and SLE-NS. No significant difference was found between expression and activity of P2X7R and NLRP3 and the two SNPs evaluated (table 2).

Table 1 Comparison between patients with a positive history of serositis (SLE-S) vs patients without history of serositis (SLE-NS)

	SLE-NS 22 (66.7%)	SLE-S 11 (33.3%)	P (SLE-NS vs SLE-S)
Demographic parameters			
No. female/male	21/1	9/2	ns
Age, mean $\pm$ SE years	40.8 $\pm$ 8.4	44.2 $\pm$ 13.1	0.36
Disease duration (months)	134.7 $\pm$ 98.3	122.5 $\pm$ 96.2	0.28
Ongoing treatment			
Hydroxychloroquine (200 mg/day)	18 (81.8%)	8 (72.7%)	0.36
Corticosteroids (2.5 up to 12.5 mg/day)	18 (81.2%)	9 (81.2%)	1
Major immunosuppressive treatment (Mycophenolate mofetil, Cyclosporine A, Azathioprine, MTX, PEX, RTX)	10 (45.5%)	4 (36.6%)	0.25
Ongoing dosage of steroids mg mean $\pm$ SE	3.7 $\pm$ 0.59	5.1 $\pm$ 1.6	0.34
Cumulative dosage of steroids gr mean $\pm$ SE	19.8 $\pm$ 3.6	19.5 $\pm$ 6.7	0.95
Current clinimetric parameters			
SLEDAI-2K, mean $\pm$ SE	3.3 $\pm$ 0.7	3.9 $\pm$ 0.8	0.61
SUCC, mean $\pm$ SE	0.33 $\pm$ 0.7	0.45 $\pm$ 0.2	0.55
SNP 1513A>C= A/C	9 (40.9%)	4 (36.4%)	0.55
SNP 1513A>C= AA	13 (59.9%)	7 (63.6%)	0.54
SNP 489C>T = CC	5 (22.7%)	2 (18.2%)	0.57
SNP 489C>T = CT	7 (31.8%)	5 (45.5%)	0.35
SNP 489C>T = TT	10 (45.5%)	4 (36.4%)	0.45
LOF	7 (31.8%)	4 (36.4%)	0.54
GOF	15 (68.2%)	7 (63.4%)	0.55

Conclusion: our data indicate that the 1513A>C and 489C>T SNPs do not seem linked with reduced activity and expression of P2X7R that we previously observed in our cohort of lupus patients. Furthermore, no significant association was found between expression of these SNPs and development of serositis.

REFERENCES

- [1] P2X7 receptor in systemic lupus erythematosus (SLE). Exploring a novel pathogenetic pathway in lupus related serositis. F. Furini. Ann Rheum Dis, volume 77, supplement Suppl, year 2018, page A871

Table 2: Activity and expression of P2X7R-NLRP3 inflammasome axis: influence of combined effect of two SNP

	GOF	LOF	p (GOF vs LOF)
Δ Ca ²⁺ (Fura2) nM $\pm$ SE	54.8 $\pm$ 40.0	74.2 $\pm$ 14.5	0.42
IL-1β levels in monocytes supernatants			
pg/ml; mean $\pm$ SE			
RPMI	81.3 $\pm$ 29.9	48.2 $\pm$ 22.0	0.49
LPS	130.7 $\pm$ 26.9	231.1 $\pm$ 70.1	0.71
LPS+BzATP	2159.1 $\pm$ 203.2	2392.7 $\pm$ 166.9	0.46
BzATP	110.0 $\pm$ 31.6	153.9 $\pm$ 55.1	0.46
RT-PCR P2X7R mean ($\pm$ SE)	1.1 $\pm$ 0.13	0.97 $\pm$ 0.1	0.63
RT-PCR NLRP3 mean ($\pm$ SE)	4.7 $\pm$ 0.3	3.7 $\pm$ 0.7	0.47

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AB0170

TUBERCULOSIS IN PATIENTS WITH SYSTEMIC RHEUMATIC DISEASES : THE TUNISIAN EXPERIENCE

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Background: The incidence of tuberculosis among patients with systemic rheumatic diseases is much higher than in the general population. The clinical manifestations of both systemic rheumatic disease activity and tuberculosis, (i.e. fever, weight loss, asthenia) may overlap or lead to confusion. The immunosuppression of systemic diseases makes the management of patients with tuberculosis more complicated.

Objectives: The purpose of our study was to describe the clinical characteristics of patients with systemic rheumatic diseases and tuberculosis.

Methods: A retrospective study, from 1998 to 2018, in the internal medicine service in Fattouma Bourguiba hospital, Tunisia, of 59 patients suffering from connective tissues disease, treated by corticosteroids linked in one or several treatments to immunosuppressants, who subsequently developed tuberculosis.

Results: Fifty nine patients were included (46 women and 13 man) with a mean age of 47 years (range from 18-83 years). Systemic illnesses were: systemic lupus erythematosus (13.6%, n = 8), Gougerot-Sjögren syndrome (secondary or primary) (18.6%, n = 11), systemic sclerosis (5.1%, n = 3), rheumatoid arthritis (n = 1), Takayasu arteritis (n=1), Horton disease (n=2), periarteritis nodosa (n=1), Wegener's granulomatosis (n= 2) and Behçet's disease (n=1). 45 patients (76.6%) were treated with corticosteroids and/or immunosuppressants in 3 cases (methotrexate in one case, cyclophosphamide in one case and azathioprine in one case) before the tuberculosis was diagnosed. The clinical manifestations most commonly observed were : weight loss in 55.9%, fatigue in 50.8% and fever in 24.7%. The tuberculin skin tests was positive in 45.8%. Quantiferon-TB-Gold was positive in eleven cases. Twelve patients had an abnormal chest X-ray. The location of the tuberculosis was pulmonary (32.3%, n = 19), ganglionic (33.9%, n = 16), urogenital (20.3%, n = 12), lymphatic (n = 5), abdominal (n=4), cerebral (n=2), ocular (n=2), osteoarticular (n= 2) and more than one location in 23.7% of the cases. The diagnosis of tuberculosis was confirmed by bacteriology in 13.6% (n = 8) and in thirteen cases, histologically (22.03%). The systemic rheumatic disease was clinically active at the time of the diagnosis of tuberculosis in 8 patients. The diagnosis of **systemic rheumatic diseases** was made before that of tuberculosis in 13 patients and concomitantly in 5. Under tuberculosis treatment by four drugs then by two drugs, the evolution of tuberculosis was favourable in most of our patients. Three of the patients developed an allergy in isoniazid. Nine patients have developed hepatotoxicity with pyrazinamide. Retrobulbar neuritis was observed in 3 cases treated with ethambutol.

Conclusion: This study confirms the often extra-pulmonary character of tuberculosis in patients with systemic disease as well as the difficulty of diagnosis and problems multiplied by this association. The screening strategies for tuberculosis should probably be extended in all patients with systemic rheumatic diseases receiving glucocorticoids and/or immunosuppressive therapy.

REFERENCES

- [1] SEGAL B, SNELLER M: Infectious complications of immunosuppressive therapy in patients with rheumatic diseases. *Rheum Dis Clin North Am* 1997; 23: 219-37.
- [2] Hernández-Cruz B1 et al, Mycobacterium tuberculosis infection in patients with systemic rheumatic diseases. A case-series. *Clin Exp Rheumatol*. 1999 May-Jun;17(3):289-96.

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AB0171

THE REGULATION AND PHARMACOLOGICAL MODULATION OF IMMUNE COMPLEX INDUCED PRODUCTION OF TYPE III IFN BY PLASMACYTOID DENDRITIC CELLS

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Background: The type I Interferons (IFNs) are the most important drivers of the IFN gene signature in Systemic Lupus Erythematosus (SLE). However, both type II and type III IFNs (IFN- λ 1-3) can be measured in a proportion of patients with SLE and contribute to the IFN signature. The exact role of type III IFNs in SLE is not completely clear, but serum levels of type III IFN correlate with disease activity and specific organ manifestations, such as arthritis, nephritis and anti-dsDNA antibodies. Type III IFN can be induced in pDCs by TLR9 agonist Oligodinucleotide (ODN) 2216 and many viruses. Whether type III IFN can also be induced in pDCs by nucleic acid containing immune complexes (IC), has, to our knowledge, not been investigated before.

Objectives: We asked if RNA containing immune complexes (RNA-IC), which trigger the synthesis of large amounts of IFN- α by plasmacytoid dendritic cells (pDCs), can act as stimuli for type III IFN production, and how this production is regulated by Natural Killer (NK) cells and different cytokines. We also investigated if the type III IFN production could be blocked by hydroxychloroquine (HCQ) and an interleukin receptor 1 associated kinase 4 inhibitor (IRAK4i).

Methods: Peripheral blood mononuclear cells (PBMCs) from SLE patients or healthy individuals were used to isolate pDCs and natural killer (NK) cells, or were depleted of monocytes. Cells were stimulated with RNA-IC, and cytokines were measured by immunoassays. mRNA expression in RNA-IC stimulated pDCs and NK cells was analyzed with a microarray. The effect of HCQ and IRAK4i on the IFN- λ 1/3 production was investigated in pDCs and NK cells from healthy individuals.

Results: Type III IFN mRNA expression was strongly upregulated in co-cultures of pDC-NK cells stimulated with RNA-IC. High levels of IFN- λ 1/3 and IFN- λ 2 (medians 2000 pg/ml and 100 pg/ml) were detected in supernatants from RNA-IC stimulated pDC-NK cell co-cultures. IFN- λ 2 enhanced IFN- λ 1/3 and IFN- α production by purified pDCs. Interleukin (IL) -3, IL-6, and GM-CSF significantly enhanced IFN- λ 1/3 production (4-5 fold) by RNA-IC stimulated pDCs. Monocyte depleted PBMCs and pDC-NK cell co-cultures from 15% and 9% of SLE patients produced IFN- λ 1/3 in response to RNA-IC stimulation. Exogenous IFN- α 2b and GM-CSF in pDC-NK cell co-cultures increased the proportion of patients responding to RNA-IC stimulation from 9 to 36%. IFN- λ 1/3 production by RNA-IC-stimulated pDCs and pDC-NK cells was significantly inhibited by HCQ (by 99% and 93% respectively) and an IRAK4i (by 98% and 96% respectively).

Conclusion: pDCs produce both type I and type III IFN in response to RNA containing immune complexes. This is promoted by activated NK cells as well as a number of pro-inflammatory cytokines, including IFN type I and type III, considered important in SLE. Consequently, in order to achieve a proper control the IFN driven autoimmune process in SLE, both type I and type III IFN need to be targeted. In this system of stimulated, co-cultivated pDCs and NK cells, HCQ and an IRAK4 inhibitor blocked the type III IFN production.

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AB0172

EXPRESSION OF SLAMF6 AND ITS FUNCTIONAL SIGNIFICANCE IN PODOCYTES OF LUPUS NEPHRITIS

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Background: Systemic lupus erythematosus (SLE) is multisystem disorder that is caused by tissue damage resulting from antibody and complement-fixing immune complex deposition. Lupus nephritis (LN) is frequent complication and one of the most serious manifestations of SLE. The expression of nephrin, as podocyte marker, is reduced in various renal diseases that develop into nephrotic syndrome¹. The alteration of the structural protein in podocytes is known as a mechanism of proteinuria in LN.

Objectives: The signaling lymphocyte activation molecule family (the SLAM family) of type I transmembrane receptors consists of nine related members of the immunoglobulin superfamily and has been reported to mediate important regulatory signals between immune cells through their homophilic and heterophilic interactions². The 1q23 region (*Sle1b* region in mouse) on human chromosome 1 including the SLAMF cluster of genes, containing SLAMF6 has been identified as a lupus susceptibility locus³. It has been shown that the expression of signaling lymphocyte activation molecule family 6 (SLAMF6) is enhanced in CD4+T cells of SLE patients and is involved in IL-17 production⁴. We sought to examine the functional role of SLAMF6 in lupus podocytes.

Methods: We evaluated the co-expression of nephrin, a podocyte marker and SLAMF6 in kidney of normal controls and LN patients, also in B6 and MRL/lpr mice at the age of 8wk and 16wk by immunofluorescence analysis. We also examined nephrin positive SLAMF6 expression in isolated podocytes from B6 and MRL/lpr kidneys. Then, we analyzed the expression of SLAMF6 in CD4+T cells of isolated kidney and spleen in B6 and MRL/lpr mice. We treated human podocytes with IgG from healthy individuals and LN patients for 24 h and 48h and analyzed the expression of SLAMF6 by real-time PCR.

Results: In the histopathology, the expression of SLAMF6 was increased in nephrin positive area in LN patients and MRL/lpr mice compared to each control groups. Although the expression of nephrin in MRL/lpr mice kidney at 16 wk old decreased compared to B6 mice at same age, the expression of SLAMF6 in podocytes increased in diseased MRL/lpr mice compared to B6 mice. Similarly, the expression of SLAMF6 in CD4+ T cells increased in diseased MRL/lpr mice kidney and spleen compared to B6 mice. The level of SLAMF6 mRNA elevated in human podocytes exposed to LN-derived IgG compared to healthy individuals-derived IgG.

Conclusion: The expression of SLAMF6 is enhanced in LN podocytes, suggesting that the possibility of cooperating with CD4+T cells contributing to its dysfunction. Further examination is needed to investigate in detail how SLAMF6 is involved in the development of LN in the future.

REFERENCES

- [1] *J Am Soc Nephrol*. 2003 Aug;14(8):2063-71.,
- [2] *Nat Rev Immunol*. 2003 Oct;3(10):813-21.,
- [3] *Nat Rev Rheumatol*. 2010 Jun;6(6):348-57.,
- [4] *J Immunol* 2012; 188:1206-1212.

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