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ORIGINAL ARTICLE

Differential expression of the inflammasome complex genes in systemic lupus erythematosus

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Abstract

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder involving heterogeneous clinical manifestations and numerous susceptibility genes. Several findings evidence the critical role of inflammasomes in the predisposition to autoimmune diseases and in SLE. We investigated whether inflammasome polymorphins could affect susceptibility to develop and/or severity SLE. Moreover, differences in inflammasome activation in peripheral blood were also evaluated in SLE patients and controls. The distribution of 13 SNPs in eight inflammasome genes was evaluated. To assess inflammasome priming in peripheral blood monocytes of SLE and controls, differential expression of selected inflammasome genes and IL-1ß production was analyzed in resting condition as well as after LPS and ATP stimulation. Results showed that the gain-of-function variant rs10754558 (*NLRP3)* was significantly more frequent in SLE patients with nephritis, reinforcing the concept of a key role of *NLRP3* inflammasome not only in SLE but also especially in kidney disease. SLE monocytes in resting condition showed a higher level of IL-1ß expression and produced higher levels of IL-1ß when stimulated with LPS+ATP comparing to controls. The stimulation induced a significant expression of *NLRP1*, *AIM2*, *CASP1*, and *IL1B* genes, suggesting that the *NLRP1* inflammasome is responsible for the IL-1ß production observed in monocytes. These data emphasized once more the important contribution of inflammasome in SLE-associated inflammation.

Keywords Systemic lupus erythematosus · Polymorphisms · Inflammasome · Gene expression · Nephritis

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that predominantly affects women of childbearing age. SLE hallmark is the generation of autoantibodies that react with self-nuclear and cytoplasmic antigens, culminating in immunocomplex deposition in several organs, inducing cell death and organ failure (Davidson and Diamond 2001; Tsokos et al. 2007). Although under intense investigations, the genetic basis of human SLE is still not fully understood (Ghodke-Puranik and Niewold 2015; Tsao 2003; Croker and Kimberly 2005).

Several studies indicate that abnormal activation of genes related to the inflammatory response, resulting in an altered activation of IL-1ß and/or NF-kB, may contribute to the pathogenesis of autoimmune disorders with a strong inflammatory component, as observed in SLE (Shinkai and TH 2008; Shaw et al. 2011a; Aksentijevich et al. 2007; Magitta et al. 2009;

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Yang et al. 2015a; Kahlenberg and Kaplan 2014a). In the last few years, the innate immune signaling complex, called inflammasome, has garnered support for a role in triggering and maintaining SLE (Kahlenberg and Kaplan 2014a). Different innate immune cytoplasmic receptors, belonging to Nod-like Receptors/NLRs (i.e., NLRP1, NLRP3, NLRC4) or PYHIN (i.e., AIM2, IFI16) families, have been described as able to assemble an inflammasome in response to pathogen- or danger-associated molecular patterns (PAMPs or DAMPs) leading to caspase-1 activation and consequent cleavage and secretion of pro-inflammatory cytokines IL-1β and IL-18 (Shaw et al. 2011b; Man and Kanneganti 2015; Ito et al. 2014).

Several lupus-associated DAMPs (i.e., generation of reactive oxygen species due to inefficient clearance of cellular debris; impaired clearance of neutrophil extracellular trap (NET); accumulation of cytosolic self DNA) could be recognized by inflammasome receptors consequently inducing an inflammatory response (Yang and Chiang 2015).

NLRP3 inflammasome activation has been described as increase in lupus macrophages (Kahlenberg et al. 2013) and two recent studies suggested that this induction appeared to be, at least partially, caused by lupus-specific autoantibodies (Zhang et al. 2016; Shin et al. 2013a). NLRP3 inflammasome plays an important role also for the progression of SLE, contributing to the development of nephritis (Ka et al. 2015; Li et al. 2015).

Recently, it was demonstrated that the hyper-expression of NLRP3 in myeloid cells induces a severe disease in an experimental model of lupus (Lu et al. 2017). Even if NLRP3 remains the first candidate DAMPs' receptor involved in SLE pathogenesis, other inflammasome components have been pointed out as possible contributing factors. Our research group demonstrated that gain-of-function polymorphisms in the receptor *NLRP1* gene were associated to SLE and SLE-associated nephritis, rash, and arthritis (Pontillo et al. 2012). Accordingly, a deregulation not only of NLRP3 but also of NLRP1 inflammasomes has been reported in patients with SLE (Yang et al. 2014).

Accordingly, a deregulation not only of NLRP3 but also of NLRP1 inflammasomes has been reported in patients with SLE (Yang et al. 2014). Even though, the role of inflammasome in the pathogenesis of SLE should be more deeply elucidated (Yang et al. 2015b; Camargo et al. 2004; Wang et al. 2013; Wen et al. 2014). So, to better understand the possible impact of inflammasome gene disregulation in SLE development and its clinical phenotype, we analyzed a selected panel of single-nucleotide polymorphisms (SNPs) in *NLRP*1, *NLRP3*, *NLRC4*, *AIM2*, *CARD8*, *CASP1*, *IL1B*, and *IL18* genes; moreover, inflammasome activation was evaluated in monocytes from SLE patients to further characterize inflammasome profile in these individuals.

Materials and methods

Subject

We recruited 132 SLE patients (129 women/3 men, mean age 37.1 years ± 10.5) and 154 healthy controls (HC) (125 women/29 men, mean age 33.5 years ± 13.4) at the Clinical Hospital of Federal University of Pernambuco (HC-UFPE), from metropolitan region of Recife (Pernambuco, Brazil). Patients were classified according to the criteria of the American College of Rheumatology (ACR) (Hochberg 1997) and in the cumulative organic damage index (SLICC/ACR) or disease activity index (SLEDAI). The control group was composed of healthy volunteers without SLE or any other autoimmune diseases, or other problems that may impair the immune system. Subjects with diabetes mellitus, renal or hepatic dysfunction, acute or chronic inflammatory disease, cancer, and infection diseases were excluded from the study. Subjects were chosen randomly in the population, sex-, age-, and ethnicity-matched and from the same geographical area of the patients (metropolitan Recife, PE). The demographic, clinical, and laboratory profiles of patients and controls are reported in Table 1.

The following laboratory and clinical data regarding the SLE patients were collected: hematological alterations (hemolytic anemia, leucopenia, lymphopenia, thrombocytopenia), immunological alterations (Anticardiolipin, Anti-Sm, Anti-

Table 1 Demographic, clinical, and laboratory data of Brazilian case/control cohort of SLE. Data are expressed as number of individuals and percentage or means ± standard deviation. ANA: antinuclear antibody test

Characteristic	SLE ($n = 132$)	HC ($n = 154$)
Sex, male/female; *n* (%)	3 (2)/129 (98)	29 (19)/125 (81)
Age, years; mean ±SD	37.1 ± 10.5	33.5 ± 13.4
Lupus blood tests	*n* (%)	
Anti-dsDNA positiveness	37 (27%)	
ANA positiveness	114 (82%)	
Immunologic alterations[a]	44 (32%)	
Hematologic alterations[b]	82 (59%)	
Clinical manifestations	n (%)	
Cutaneous manifestations	89 (64%)	
Photosensitivity	85 (61%)	
Lupus arthritis	88 (63%)	
Oral ulcers	29 (20%)	
Nephritic disorders	58 (42%)	
Neuropsychiatric disorders[c]	12 (8%)	

[a] Anticardiolipin, anti-Sm, anti-RNP, anti-Ro/SSA, anti-La/SSB

[b] Hemolytic anemia, leucopenia, lymphopenia, thrombocytopenia

[c] Seizures, headache, psychosis

RNP, Anti-Ro/SSA, Anti-La/SSB), presence of antinuclear antibodies (ANA), presence of anti-double-strand DNA antibody (anti-ds-DNA), antiphospholipid syndrome (APS), photosensitivity, serositis (pleuritis, pericarditis), Lupus arthritis, cutaneous manifestations (malar or discoid rashes), oral ulcers, neuropsychiatric disorder (seizures, headache, psychosis), Raynaud phenomenon, and nephritic disorder. Patients were classified as having nephritic disease based on laboratory parameters, specifically changes in urine summary and 24-h proteinuria, as follows: persistent proteinuria (> 0.5 g/day or 3+) or abnormal cylindrury. All the participants provided a written informed consent approved by the local Research Ethics Committee (CAAE 03065312.3.0000.5208).

DNA isolation and genotyping

Genomic DNA from SLE patients and controls was extracted from peripheral blood using the DNA Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA).

Thirteen SNPs were selected within *NLRP1*, *NLRP3*, *NLRC4*, *AIM2*, *CARD8*, *CASP1*, *IL1B*, and *IL18* genes according to previously reported association studies (Pontillo et al. 2010; Pontillo et al. 2011) and/or their functional effect on protein activity or mRNA stability (Roberts et al. 2010; Hitomi et al. 2009), and minor allele frequency (MAF; > 5%) (Supplementary File 1).

SNPs were genotyped using allele-specific TaqMan assays (ThermoFisher Scientific, California, USA) and qPCR in a ABI7500 Real-Time PCR equipment (Thermo Fisher Scientific, California, USA). SDS software v2.3 (Thermo Fisher Scientific) was used for allelic discrimination.

Statistical analysis

SNPs distribution in case/control cohort as well as in SLE groups (stratified according to clinical presentation) was analyzed by multivariate association based on general linear model (GLM) adjusted for confounders variables (age, sex, race) using statistical program R package "SNP-association" version 1.5–2. Genetic analyses were performed taking into account co-dominant, dominant, recessive, and overdominant models for all SNPs. The Haploview software was used to investigate the linkage disequilibrium (LD) and to derive the haplotypes. A significant threshold of $p = 0.004$ was assumed after Bonferroni correction for multiple SNPs analysis ($p = 0.05/n$; $n = 13$ SNPs).

Inflammasome gene expression assays

Subjects

We investigated the relative mRNA expression of *IL-1β*, *NLRP1*, *NLRP3*, *NLRC4*, *AIM2*, *ASC/PYCARD*, and *CASP1* genes in peripheral blood–derived monocytes among subjects within the case/control study (10 SLE patients and 10 healthy individuals) and if their expression differed between the basal condition and after LPS+ATP stimulation.

All the patients selected for gene expression study had a remission for SLE. The healthy individuals were sex-, age-, and ethnicity-matched according to patients and did not present recent illness (autoimmune diseases, diabetes mellitus, renal or hepatic dysfunction, acute or chronic inflammatory disease, cancer, infection diseases) or any pharmacological treatment before blood collection.

Peripheral blood monocytes culture

Heparinized whole blood samples were obtained from ten post-menopausal SLE female patients (mean age 42.6 ± 12 years) and ten healthy post-menopausal female controls (HC) (mean age 57.5 ± 8.14 years). To establish a condition where both patients and controls ex vivo cell cultures would clear up from no reported inflammatory responses (in HC), cells were cultured overnight and after this period medium was changed. This procedure allowed cells to metabolize any ex vivo conditions before initiate inflammasome stimuli. To stimulate, we exposed 0.5×10^6 peripheral blood monocytes with 1 μg/ml lipopolysaccharide (LPS; Sigma-Aldrich) for 4 h and then with 1 mM adenosine triphosphate (ATP; Sigma-Aldrich) for 15′ in RPMI-1640 + 10% fetal bovine serum/FBS (ThermoFisher Scientific). Inflammasome genes modulation was evaluated in monocytes by real-time quantitative PCR and gene expression specific Taqman assays (Thermo Fisher Scientific).

IL-1β measurement

The secreted IL-1β was measured with ELISA (IL-1β assays, R&D systems, USA). Results were expressed in picograms per milliliter. Secretion differences between patients and controls were tested with Mann-Whitney U test with SPSS 15.0 (SPSS, Inc., Chicago, IL, USA).

Relative gene expression analysis

Total RNA was isolated using the RNAqueous micro kit (Ambion, Thermofisher Scientific, USA). RNA integrity was assessed by gel electrophoresis and quantification by Nanodrop 2000 (ThermoScientific). After retro-transcription of 0.5 μg total RNA (Super Script™ III Reverse Transcriptase (Invitrogen, Thermoscientific)), *IL1B* and selected inflammasome genes, namely *NLRP1*, *NLRP3*, *NLRC4*, *AIM2*, *ASC/PYCARD*, and *CASP1* were amplified with TaqMan® gene-specific assays and ABI Prism 7500 Real-Time PCR equipment. Gene modulation in SLE monocytes compared to HC and stimulated (LPS) versus unstimulated

resting (R) monocytes were calculated as 2exp-ΔCt ± standard deviation (fold change - FC). The SDS 2.3 software was used to obtain cycle quantification (Cq) values for relative gene expression analysis according to FC method (Schmittgen and Livak 2008). *GAPDH* was the reference gene used for normalization (ΔCt).

Statistical analysis

The comparison among expression levels of studied genes and patients and healthy control group were calculated using Student's *t* or Mann-Whitney *U* tests as appropriate. GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA) was used for statistical analyses and differences were accepted as significant for p values < 0.05.

Results

Genotyping study

We genotyped 13 SNPs located at eight inflammasome genes in 132 SLE unrelated patients and 154 healthy controls. Genotypes distribution was in Hardy-Weinberg equilibrium ($p > 0.05$). None of the studied polymorphisms resulted differently distributed in cases and controls (Table 2).

Linkage disequilibrium analysis revealed that *IL1B* SNPs rs1143643 and rs1143634 were in strong LD (D' = 100), whereas *NLRP1* rs12150220 and rs2670660, as well as *NLRP3* rs10754558 and rs35829419 in moderate LD (D′ = 78 and D' = 0.86, respectively). The distribution of *IL1B*, *NLRP1*, and *NLRP3* haplotypes was not significantly different between cases and controls (Supplementary File 2).

Then we analyzed SNPs distribution according to SLE clinical presentation: none of the studied SNPs resulted significantly associated to clinical features or laboratory parameters after Bonferroni correction with the exception of *NLRP3* rs10754558 which correlated with lupus nephritis (Table 3).

SLE individuals carrying rs10754558 minor G allele were significantly more frequent ($p = 0.0004$) in patients with nephritis (0.68) than in patients without kidney involvement (0.36), according to a dominant model of inheritance (C/G + G/G; OR = 3.88; 95%CI = 1.80–8.40). This result was poorly affected by confounders variables: age, sex, and race, ($p_{adj} = 0.0005$; $OR_{adj} = 4.0$; 95%CI = 1.79–8.92).

Of note, some SNPs resulted differently distributed according to clinical or laboratory data ($p < 0.05$), however, the analysis did not reach the statistical significance after Bonferroni correction (Table 3). *NLRP1* rs2670660 was less frequent in SLE patients positive for anti-DNA antibodies (0.08 versus 0.29) according to a recessive model of inheritance (G/G; $p_{adj} = 0.007$; $OR_{adj} = 0.22$). *CARD8* rs2043211 resulted more frequent in SLE patients with cutaneous manifestations (0.15 versus 0.02) according to a recessive model of inheritance (T/T; $p_{adj} = 0.022$; $OR_{adj} = 7.34$), and less frequent in SLE with hematologic involvement (0.27 versus 0.49) according to an overdominant model of inheritance (A/T; $p_{adj} = 0.024$; $OR_{adj} = 0.37$). *IL1B* rs1143643 was more frequent in SLE patients with photosensitivity (0.67 versus 0.44) according to a dominant model of inheritance (C/T + T/T; $p_{adj} = 0.009$; $OR_{adj} = 2.76$). *NLRC4* rs455060 was more

Table 2 Association results for case/control analysis. Inflammasome SNPs genotypes distribution in SLE patients (SLE) and healthy controls (HC) was analyzed by general linear model (GLM). Genotypes distribution in patients, p value and p value adjusted for sex, age, and race are reported. p value < 0.05 are underlined

Gene	SNP ID	Genotypes	SLE ($n = 132$)	HC ($n = 154$)	p	p_{adj}
NLRP1	rs2670660	A/A-A/G-G/G	43-62-19	47-66-22	0.985	0.940
NLRP1	rs12150220	A/A-A/T-T/T	68-47-9	75-55-16	0.607	0.860
NLRP3	rs35829419	C/C-C/A-A/A	126-6-0	143-9-2	0.609	0.633
NLRP3	rs10754558	C/C-C/G-G/G	61/60/11	60/64/30	0.012	0.074
NLRC4	rs455060	A/A-A/G-G/G	58-51-16	59-69-21	0.526	
AIM2	rs2276405	C/C-C/T-TT	112-5-0	117-6-0	1.0	
AIM2	rs35130877	T/T-G/T-T/T	126-0-0	152-0-0	1.0	
CARD8	rs2043211	A/A-A/T-T/T	58-51-6	84-59-7	0.681	
CASP1	rs572687	G/G-A/G-A/A	89-32-5	95-44-3	0.439	
IL1B	rs1143643	C/C-C/T-T/T	60-58-8	67-58-14	0.496	
IL1B	rs1143634	G/G-A/G-A/A	79-28-7	87-41-6	0.555	
IL18	rs1946519	C/C-A/C-A/A	41-53-20	46-60-27	0.876	

Table 3 Association results stratified for clinical and laboratory data. Inflammasome SNPs genotypes distribution in SLE patients was analyzed according to clinical and laboratory variables by general linear model (GLM). *p* value and *p* value adjusted for sex, age, and race are reported. *p* value < 0.05 are underlined; *p* value < 0.004 are indicated in bold characters. Adjusted p values are reported within brackets

Gene	SNP ID	Clinical/laboratory data	*p* value
NLRP1	rs2670660	Anti-DNA antibodies	0.008 (0.007)
NLRP3	rs10754558	Nephritis	0.0004 (0.0005)
NLRC4	rs455060	Neurological presentation	0.122 (0.027)
CARD8	rs2043211	Cutaneous manifestations	0.022 (0.022)
CARD8	rs2043211	Hematological involvement	0.036 (0.024)
IL1B	rs1143643	Photosensitivity	0.009 (0.008)

frequent in SLE women with neurologic presentation (0.56 versus 0.20) according to a dominant model of inheritance (A/G + G/G; $p_{adj} = 0.027$; $OR_{adj} = 5.16$).

No significant association with antinuclear antibodies (ANA) was evidenced, however, a different distribution of *NLRP1* rs2670220, *CARD8* rs2043211 was observed. *NLRP1* rs2670220 was less frequent between patients with immunological alterations (0.08 versus 0.26; $p_{adj} = 0.011$; $OR_{adj} = 0.25$). Similarly, *CARD8* rs2043211 was less frequent between patients with immunological alterations (0.01 versus 0.13; $p_{adj} = 0.013$; $OR_{adj} = 0.10$) (data not shown).

Inflammasome expression analysis

Then, we questioned whether an inflammasome signature characterizes SLE patients, as reported for other autoimmune and chronic inflammatory diseases (Shin et al. 2012a). So, we first evaluated the modulation of inflammasome genes expression in peripheral blood–derived monocytes of SLE and HC individuals, and then the production of IL-1β in these cells.

To investigate the hypothesis that the threshold of NLRP inflammasome responsiveness could be affected in SLE individuals, we evaluated the inflammasome genes modulation in monocytes from SLE versus HC. In un-stimulated resting monocytes we observed a higher expression of *IL1B* (fold change: 50.79; $p = 0.0212$; $t = 2.525$; df = 18) in SLE patients compared to HC (Fig. 1); however, we did not observe any significant modulation in *NLRP3*, *NLCR4*, *ASC/PYCARD*, *AIM2*, and *CASP1* expression ($p > 0.05$).

Within each group, LPS+ATP stimulation induced a significant increased expression of *NLRP1* (fold change 5.89; $p = 0.0009$, $t = 4.205$, df = 14), *CASP1* (fold change 8.68; $p = 0.0028$, $t = 3.427$, df = 19), *IL1B* (fold change 6.37; $p = 0.0051$, $t = 3.162$, df = 19), and *AIM2* (fold change 14.57; $p = 0.0009$, $t = 4.207$, df = 14 (Fig. 2). These data are

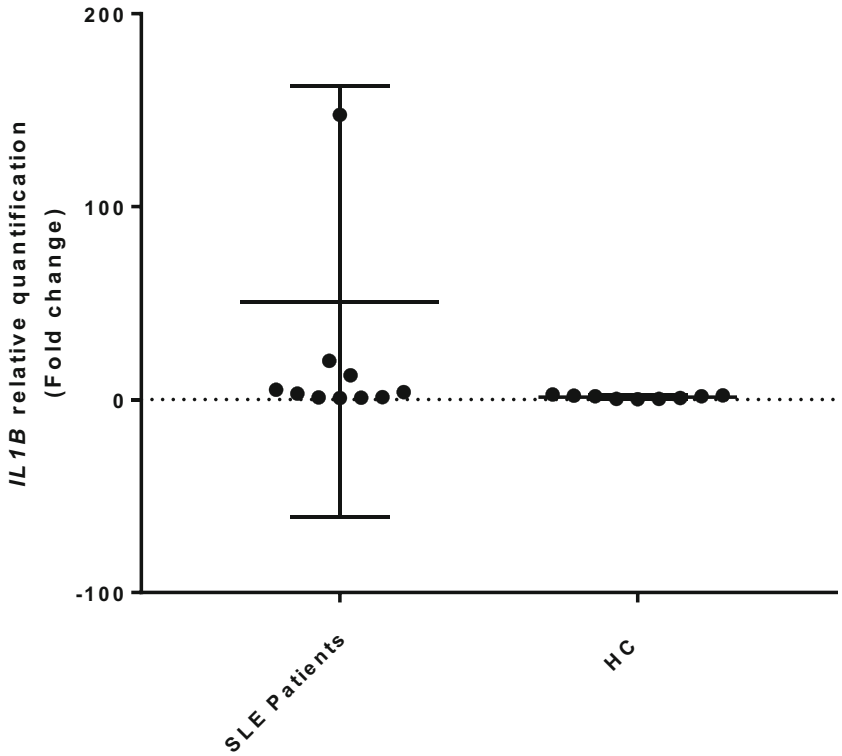

Fig. 1 Modulation of IL1B gene in monocytes isolated from SLE and HC individuals in a resting condition. A total of three out of 10 SLE individuals displayed a higher steady-state expression of *IL1B* transcripts in relation to SLE patients and HC individuals. *IL1B* relative expression (2exp-ΔCt ± standard deviation) between SLE individuals ($n = 10$) and HC (n = 10) showed statistically significant difference ($p = 0.0212$; $t = 2.525$). SLE: Systemic Lupus Erythematosus; HC: Healthy Controls

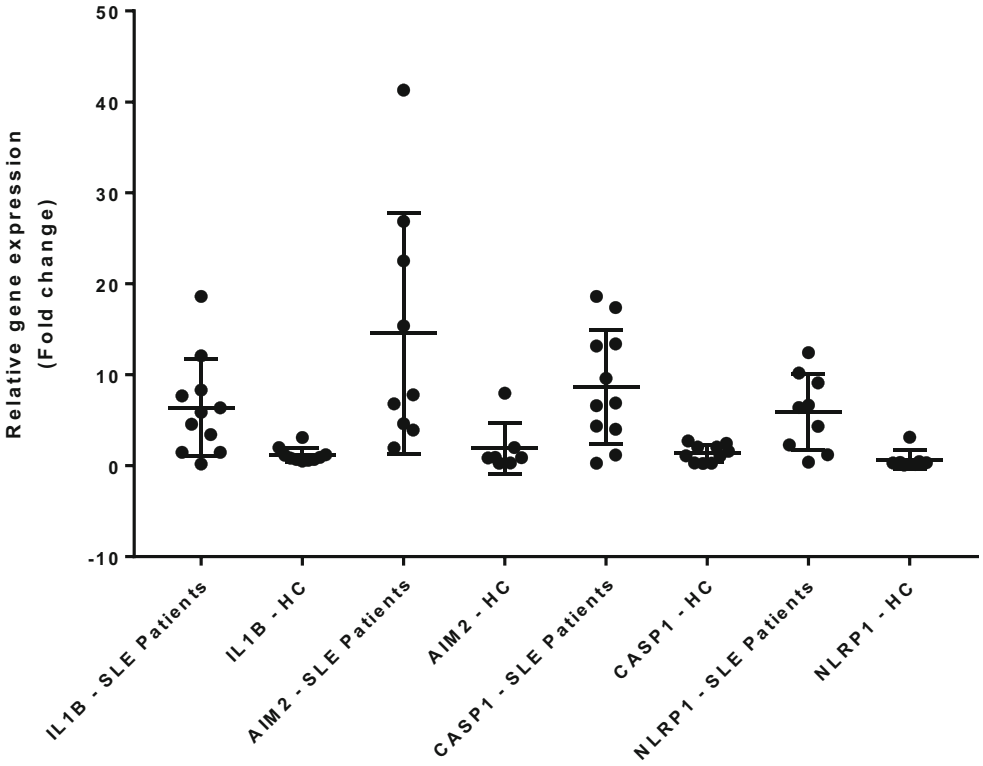

Fig. 2 Modulation of *IL1B* and inflammasome genes in monocytes isolated from SLE individuals and HC individuals with LPS+ATP stimulation. Relative expression (2exp-ΔCt ± standard deviation) between SLE individuals ($n = 10$) and HC ($n = 10$) showed statistically significant for *IL1B* ($p = 0.0051$; $t = 3.162$), *AIM2* ($p = 0.0009$; $t = 4.207$), *CASP1* ($p = 0.0028$; $t = 3.427$), and *NLRP1* ($p = 0.0009$; $t = 4.205$). SLE: Systemic Lupus Erythematosus; HC: Healthy Controls

compatible with the NLRP1 inflammasome being responsible for the IL-1ß production observed in monocytes.

As expected, monocytes from SLE patients produced higher levels of IL-1ß comparing to HC in LPS (lipopolysaccharide) stimulated monocytes as well as in LPS+ATP stimulated cells, however, only the last condition was statistically different ($p = 5.6$ exp-4) (Fig. 3). Intriguingly, this effect appeared to be emphasized in the presence of LPS+ATP, suggesting that the inflammasomes respond actively to LPS and ATP associated.

Discussion

Recent studies have reported the association of *NLRP1*, *NLRP3*, and *IL1B* genes with SLE in terms of susceptibility factors and/or disease severity modulation (Magitta et al. 2009; Pontillo et al. 2012; Wang et al. 2013; Wen et al. 2014). Studies concerning specific polymorphisms in inflammasome-related genes and the relationships with SLE susceptibility are necessary to better understand the involvement of these molecules in SLE pathology. Here, we

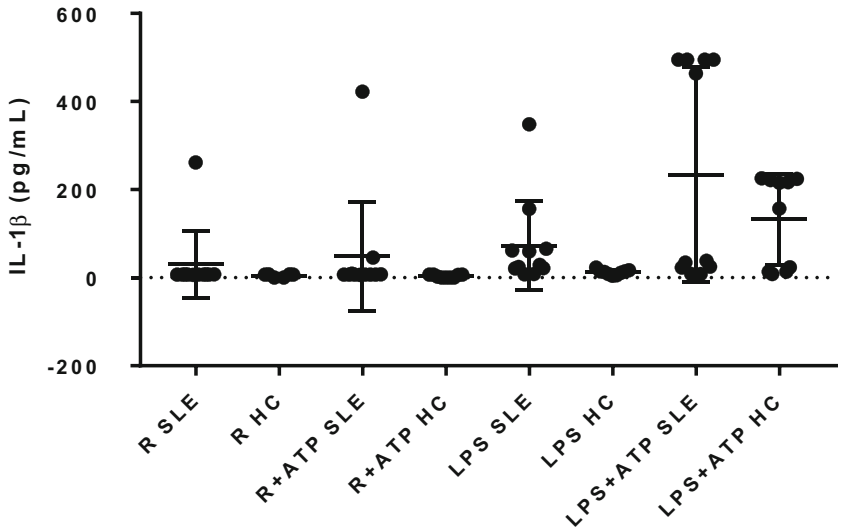

Fig. 3 Production of IL-1ß in monocytes isolated from SLE and HC individuals. Concentration of IL-1ß (pg/mL) in supernatants of monocytes from SLE individuals ($n = 10$) and HC ($n = 10$) in unstimulated/resting condition or stimulated with 1 μg/mL LPS for 4 h and 1 mM ATP for 15 min. Only LPS+ATP condition showed statistically significant difference between groups ($p = 5.6$ exp-4). SLE: Systemic Lupus Erythematosus; HC: Healthy Controls; R: Resting condition; LPS: stimulated with LPS; LPS+ATP: stimulated with LPS and ATP

demonstrated the relation between inflammasome SNPs and SLE clinical manifestation as well as a chronic expression of some inflammasome components in monocytes from SLE patients.

The previously observed association between *NLRP1* polymorphisms and SLE (Li et al. 2015) was not replicated in our study performed on a different Brazilian group of patients and controls, even if the frequency of the SNPs in the populations was similarly distributed in the Southeast of Brazil when comparing to Northeast population in Brazil. On the other hand, the *NLRP3* rs10754558 gain-of-function variant associated with augmented risk to development of lupus nephritis, which apparently is sustained by NLRP3 inflammasome expression findings in experimental model using LPS stimulated monocytes from SLE patients and HC (Shin et al. 2012a; Tsai et al. 2011; Zhao et al. 2013; Zhao et al. 2015; Kahlenberg et al. 2011; Huang et al. 2017; Fu et al. 2017).

The gene expression profile of SLE patients showed an upregulation for *IL1B* gene in resting condition and for *IL1B*, *AIM2*, *CASP1*, and *NLRP1* genes in LPS+ATP-stimulated monocytes when comparing to healthy individuals, suggesting that cells are dramatically sensitized to ligands and respond quickly for signs of stimulation.

Immune complexes formed secondary to antibody recognition of DNA or RNA antigens have been shown to stimulate inflammasome activation through upregulation of TLR-dependent activation of NF-kB and subsequent activation of the NLRP1 and NLRP3 inflammasomes, producing high amounts of IL-1β (Shin et al. 2012b, 2013b; Levandowski et al. 2013). Thereby, the upregulation of inflammasome components is expected since in autoimmune diseases there are abundant releases of DAMPs upon tissue damage, which may activate the inflammasome (Shin et al. 2013b; Kahlenberg and Kaplan 2014b). Therefore, besides the excessive IL-1β secretion, the deregulated activation of these complexes may exacerbate the cell death, contributing to the inflammatory process and its maintenance in SLE disease. Our findings suggest that SLE monocytes may be dramatically sensitized to ligands and respond faster for signs of stimulation, contributing to the establishment of the exacerbated inflammation observed in the disease.

These differences in inflammasome genes expression between patients and healthy controls are underlined by the results observed analyzing IL-1β secretion in monocytes supernatants. In all studied conditions, the SLE monocytes secreted higher amounts of IL-1β. The exact mechanisms responsible for the production and secretion of IL-1β remain unclear, but two signals are traditionally required. The first signal, in our case LPS, induces the transcription of pro-IL-1β and inflammasome subunits (Shin et al. 2012b, 2013b). One-second signal is provided by reduction of intracellular K+ generated by ATP promoting a rapid activation of caspase-1 and then enhancing secretion of mature IL-1β (Perregaux and Gabel 1998; Perregaux and Gabel 1994).

In conclusion, our results indicate that the inflammasome is an important player in lupus pathogenesis. SNPs in genes of inflammasome components are involved in the disease and a chronic expression of some of them was observed, indicating a dysfunction of this protein complex in SLE disease.

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Compliance with ethical standards

Conflict of interest statement The authors declare that they have no conflict of interest.

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