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Activity and expression of E-NTPDase is altered in peripheral lymphocytes of systemic lupus erythematosus patients

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disease, where there is irreversible breakdown of immunological self-tolerance. Extracellular adenosine triphosphate (ATP) and adenosine are signaling molecules that play an important part in the immune response. During inflammation and the immune response, a group of enzymes control these molecules, including ectonucleoside triphosphate diphosphohydrolase (E-NTPDase), E-5′-nucleotidase, and ecto-adenosine deaminase (E-ADA). We determined the activity and expression of E-NTPDase, the expression of E-5′-nucleotidase, the activity of E-ADA in lymphocytes and serum of SLE patients.
Methods: This study involved 35 patients with SLE and 30 healthy subjects as a control group. E-NTPDase activity and expression were increased in lymphocytes from SLE patients (31% and 37% for activity and expression, respectively) compared with the control group.
Results: An approximately 42% increase in E-ADA activity in lymphocytes was observed in SLE patients compared with the control group, in serum the ADA activity was decreased by 57% in SLE patients. Expression of E-5′-nucleotidase was not changed in SLE patients.
Conclusions: E-NTPDase and E-ADA perform key functions in the modulation of the immune and inflammatory response in SLE.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease; its course is unpredictable and progresses to acute flares (relapses) and periods of remission. The pathogenesis of SLE is characterized by impaired immune tolerance and autoantibody production [1]. Autoantibodies contribute to SLE through the formation of immune complexes and by interference with intracellular functions [2]. It is accepted that tissue injury results from the production of autoantibodies that combine with self-antigens to form immune complexes that drive inflammation and cellular damage [3]. B lymphocytes participate in the immune deregulation of SLE through the production of divers autoantibodies against soluble and cellular constituents, most commonly against intranuclear antigens [4]. T lymphocytes regulate B lymphocyte function and the production of pathogenic autoantibodies. T and B lymphocyte abnormalities have been described in SLE and their interaction are important in disease progression [1].

Purinergic signaling is involved in regulation of the inflammatory and immune responses. Under conditions of stress or injury, extracellular nucleotides such as ATP work as endogenous signaling molecules and parenchymal or immune cells release large amounts of ATP together with other nucleotides [5]. The role of ATP in the immune system is complex; however, it is known that in high concentrations, ATP is involved in the pro-inflammatory response via stimulation of

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lymphocyte proliferation and the release of pro-inflammatory cytokines [6]. Adenosine safeguards the maintenance of tissue integrity, facilitating Th2 pathways and inhibiting Th1 cell function [7].

The effects of nucleotides and their nucleoside derivative, adenosine, are mediated via a series of purinergic receptors which are expressed on immune cells surfaces, the P1 and P2 receptors, which are activated by adenosine and ATP, respectively, and intermediate the immunomodulatory effects of purines [8].

Extracellular adenine nucleotides and the nucleoside adenosine are controlled during inflammation and the immune response by a group of ectoenzymes expressed in immune cells [5]. Ectonucleoside triphosphate diphosphohydrolases (E-NTPDases; EC 3.6.1.5) are a group of enzymes capable of hydrolyzing nucleotide tri- and/or diphosphates to adenosine monophosphate (AMP). Eight subtypes of E-NTPDase are known, and E-NTPDase-1, which is known as CD39, plays a critical role in modulating the effect of ATP in inflammation. E-5′-nucleotidase (EC 3.1.3.5), also known as CD73, hydrolyzes AMP to adenosine and is involved in immunomodulation and inflammatory processes [9]. Ecto-adenosine deaminase (E-ADA; EC 3.5.4.4), which irreversibly catalyzes the deamination of adenosine and 2′-deoxyadenosine to inosine and deoxyinosine, is considered a key enzyme in the purine metabolism [10].

Given that SLE is an inflammatory and autoimmune disorder and that the purinergic system modulates the immune system and regulates pro- and anti-inflammatory events, this study determined the activity and expression of E-NTPDase, the expression of E-5′-nucleotidase, the activity of E-ADA in lymphocytes as well as the activity of ADA in serum of SLE patients.

2. Patients and methods

2.1. Chemicals

The reagents ATP, ADP, AMP, adenosine, Trizma base, sodium azide, and Coomassie Brilliant Blue G was bought from Sigma Chemical Co. The other reagents used in this experiment were of the highest purity.

2.2. Patients and samples

The population consisted of 35 SLE patients (SLE group), selected from the University Hospital of Santa Maria (HUSM), and 30 healthy subjects as a control group. The diagnosis of SLE was based on the Systemic Lupus Collaborating Clinics classification criteria (SLICC) [11]. For some statistical analysis, the SLE group was subdivided into remission (patients with resolution of clinical symptoms, normalization of laboratory results, and minimal maintenance of therapy) and relapse (patients with an increase in disease activity, requiring intensification of therapy). Subjects with hypertension, diabetes mellitus, alcoholism, cigarette smoking, and other autoimmune diseases were excluded from the work. Blood samples were collected in vacutainer tubes with EDTA as anticoagulant (for lymphocyte analyzes) and tubes without anticoagulant (for serum analysis). Ten milliliters of blood was collected from each subject and used for peripheral lymphocyte separation and other analyses. The same process was carried out for the control group. The study was approved by the Human Ethics Committee of the Health Science Center from the Federal University of Santa Maria, Brazil. All subjects provided written informed consent to participate in the study.

2.3. Isolation of lymphocytes-rich mononuclear cells from human blood and protein determination

Lymphocytes-rich peripheral blood mononuclear cells (PBMC) were isolated from peripheral human blood by centrifugation on Ficoll-Histopaque density gradients as described by Böyum [12]. The whole blood was diluted with 0.9% NaCl solution and added over 3 ml of Ficoll-histopaque and then centrifuged at 1800 rpm for 30 min at room temperature. After centrifugation, the cells were gently collected from the interface formed above the Ficoll-Hypaque into another conical tube. Ten ml of 0.9% NaCl solution were then added to the samples and centrifuged for a further 10 min at 1500 rpm. Then the supernatant was discarded and 5 ml of hemolytic buffer EDTA-ammonium chloride was added to the pellet and centrifuged at 1000 rpm for 10 min. Again the supernatant was discarded and then 0.9% NaCl solution added and centrifuged at 1200 rpm for 10 min. After removal of the supernatant, 1 ml of 0.9% NaCl solution was added and centrifuged for another 5 min at 1000 rpm. The pellet was homogenized with 0.9% NaCl solution to the required concentration of cells. The percentage of lymphocytes was > 93% as previously determined [13]. It was used serum albumin as standard to the measurement of proteins by the Coomassie Blue method [14].

2.4. E-NTPDase activity determination

After lymphocytes isolation, E-NTPDase activity was determined as previously described [16] with a reaction medium composed of 0.5 mmol/l $CaCl_2$, 120 mmol/l NaCl, 5 mmol/l KCl, 60 mmol/l glucose and 50 mmol/lTris- HCl buffer at pH 8.0 until reaching a volume of 200 µl. Twenty µl of the PBMCs were resuspended in a solution of 0.9% of NaCl and added to the reaction (2–4 µg of protein) and pre-incubated at 37 °C for 10 min, and incubation continued for 70 min. Substrate ATP or ADP at a final concentration of 2.0 mmol/l was added to start the reaction and 200 µl of 10% trichloracetic acid (TCA) to stop the reaction. The produced inorganic phosphate (Pi) was measured by a method previously described [17] using malachite green and KH_2PO_4 (as standard). The enzyme preparation was only added to the controls after the addition of TCA for correction of non-enzymatic nucleotide hydrolysis. Samples were taken in triplicate with enzyme activity reported in nmol of Pi released/min/mg protein.

2.5. E-ADA activity determination

The analysis of ADA activity, both in serum and lymphocytes, was performed using Guisti et al. method [18], which is based on measurement of the formation of ammonia released when ADA deaminates adenosine. The reaction used 25 µl of lymphocyte suspension or 50 µl of serum, was mixed to 21 mmol/l of adenosine, pH 6.5, and incubated at 37 °C for 1 h. Phenol, 106.2 mmol/l and 167.8 nmol/l sodium nitroprussiate and hypochlorite solution was added to stop the reaction. For the standard was used ammonium sulfate of 75 µmol/l. All samples were run in triplicate and ADA activity was shown in nmol NH_3/min/mg protein for lymphocytes and U/l for serum.

2.6. CD39 and CD73 expression on lymphocytes

E-NTPDase (CD39) and E-5′-Nucleotidase (CD73) expressions were assessed by flow cytometry. The cells were then incubated with anti-CD39, anti-CD73 and anti-CD45 and were analyzed by flow cytometry using the BD Accuri C6 flow cytometer. The antibodies used were: CD45 (Fluorophore- PerCP; Ab-clone- MEM-28; Company- Exbio), CD73 (Fluorophore- FITC; Ab-clone- AD2; Company- BD Pharmingen), CD39 (Fluorophore- PE; Ab-clone- TU66; Company- BD Pharmingen). Percentage of positive cells represented the results.

2.7. Analysis of purine levels in serum

The quantification of serum purine levels was performed by high pressure liquid chromatography (HPLC). The serum proteins were denaturated by 0.6 mol/l perchloric acid. All samples were then centrifuged (16,000 x *g* for 10 min at 4 °C), supernatants were neutralized with 4.0 N KOH, after a second centrifugation was performed (16,000 x *g* for 30 min at 4 °C). The supernatants were collected and centrifuged

once more (16,000 x *g* for 30 min at 4 °C). Aliquots of 20 µl were introduced to a reversed-phase HPLC (LC-20AT model, Shimadzu) using a C_{18} column (Ultra C18, 25 cm × 4.6 mm × 5 µm, Restek) at 260 nm with a mobile phase containing 60 mmol/l KH_2PO_4, 5 mmol/l tetrabutylammonium chloride in 30% methanol according to Voelter et al. [15]. The peaks were identified by their retention times and quantification by comparison with standards. Results are expressed as nmol compound/ml serum.

2.8. In vitro effects of drugs used in the treatment of patients with SLE on activity of enzymes E-NTPDase and E-ADA

The *in vitro* effects of prednisone, methotrexate, hidroxychloroquine and azathioprine on E-NTPDase and E-ADA activities were evaluated. Isolated lymphocytes from 3 healthy donors per treatment were incubated (for 80 min for NTPDase activity and 90 min for E-ADA activity) with different concentrations of these drugs in the medium reaction. The concentrations of drugs used *in vitro* represent the mean plasma values of the medication [16].

2.9. Statistical analysis

Normality of data was evaluated using the Kolmogorov–Smirnov test. The data were analyzed using unpaired Student's *t*-test or Mann-Whitney test (when data were not normally distributed) for comparisons between 2 groups and 1-way analysis of variance (for comparisons among three or more groups) followed by Dunnett's multiple comparisons test. The results were given as mean ± standard error of the mean (mean ± SEM) or median ± interquartile range and values of $P < 0.05$ were considered significant. Pearson's test correlation analysis was used to evaluate the correlation.

3. Results

3.1. General characteristics of the patients

The samples consisted of 35 SLE patients of both sexes (2 men, 33 women; 38.88 ± 12.64 y) and the group control consisted of 30 healthy subjects (4 men, 26 women; 27.34 ± 5.90 y). Table 1 shows the general characteristics and pharmacological treatments in patients with SLE and controls. The characteristics of the 2 groups of patients SLE in remission and relapse are shown in Table 2.

Table 1
General characteristics and pharmacological treatments in patients with systemic lupus erythematosus (SLE) and Controls.

	SLE Patients (n = 35)	Controls (n = 30)
Age (y)[a]	38.88 ± 12.64	27.34 ± 5.90
Gender (Female/male)	33/2	26/4
Current Therapy		
PRED	1(2.85%)	–
PRED + MTX	2(5.71%)	–
PRED + HCQ	8(22.85%)	–
PRED + AZA	1(2.85%)	–
PRED + AZA + HCQ	7(20%)	–
PRED + HCQ + MTX	7(20%)	–
HCQ	3(8.57%)	–
HCQ + MTX	3(8.57%)	–
HCQ + AZA	1(2.85%)	–
MTX	1(2.85%)	–
NONE	1 (2.85%)	30(100%)
Phase[b]		
Remission	20	-
Relapse	15	-
ANA + [b]	34(97.14%)	–

[a] Continuous variables are presents as means ± SD, PRED- Prednisone, MTX- Methotrexate, HCQ- Hidroxychloroquine, AZA- Azathioprine, NONE- no treatment.
[b] Number SLE patients recruited.

Table 2
General characteristics and pharmacological treatments in patients SLE remission and SLE relapse.

	SLE Remission n = 20	SLE Relapse n = 20
Age (y)[a]	40 ± 13.43	37.4 ± 1.13
Gender (Female/male)[b]	19/1	14/1
Current Therapy[b]		
PRED	–	1
PRED + MTX	1	1
PRED + HCQ	4	4
PRED + AZA	–	1
PRED + AZA + HCQ	3	4
PRED + HCQ + MTX	6	1
HCQ	3	–
HCQ + MTX	1	2
HCQ + AZA	1	–
MTX	–	1
NONE	1	–
Immunological Criteria		
Anti-dsDNA[b]		
Positive	2	5
Negative	5	2
No data	13	8
Anti-sm[b]		
Positive	1	6
Negative	7	2
No data	12	7

PRED- Prednisone, MTX- Methotrexate, HCQ- Hidroxychloroquine, AZA- Azathioprine, NONE- no treatment.
[a] Continuous variables are presents as means ± SD.
[b] Number SLE patients recruited.

3.2. E-NTPDase activity

The results obtained for ATP and ADP hydrolysis are shown in Fig. 1, demonstrating that E-NTPDase activity in lymphocytes was altered in patients with SLE. ATP hydrolysis was increased in the SLE patient group compared with the control group (40.30 ± 3.25 vs. 30.85 ± 2.4 nmol Pi released/min/mg of protein, respectively) ($P < 0.05$) (Fig. 1A). The same behavior was observed with ADP hydrolysis (42.94 ± 4.56 and 30.90 ± 1.95 nmol Pi released/min/mg of protein for patients and controls, respectively) ($P < 0.05$) (Fig. 1B).

Fig. 1C–D shows ATP and ADP hydrolysis in lymphocytes of SLE patients in remission and in relapse, respectively. Significant differences in the E-NTPDase activity (with ATP as a substrate) were not observed in the SLE remission group compared with the relapse group (41.31 ± 4.06 vs. 38.95 ± 5.47 nmol Pi released/min/mg of protein, respectively) (Fig. 1C). In addition, results obtained for lymphocyte E-NTPDase activity with ADP as a substrate are shown in Fig. 1D, where ADP hydrolysis did not differ in the SLE remission group (44.20 ± 6.32 nmol Pi released/min/mg of protein) compared with the SLE relapse group (41.26 ± 6.75 nmol Pi released/min/mg of protein).

3.3. CD39 and CD73 expression in lymphocytes

NTPDase1/CD39 expression was increased in SLE patients (15.20 ± 1.38% of $CD39^+$ lymphocytes) compared with the control group (11.09 ± 0.79% of $CD39^+$ lymphocytes) (Figs. 2A and 3; $P < 0.05$). Significant differences in CD73 expression were not found in the SLE group (38.44 ± 3.91% of $CD73^+$ lymphocytes) compared with the control group (37.31 ± 2.58% of $CD73^+$ lymphocytes) (Fig. 2B).

Fig. 2C shows NTPDase/CD39 expression in lymphocytes of patients

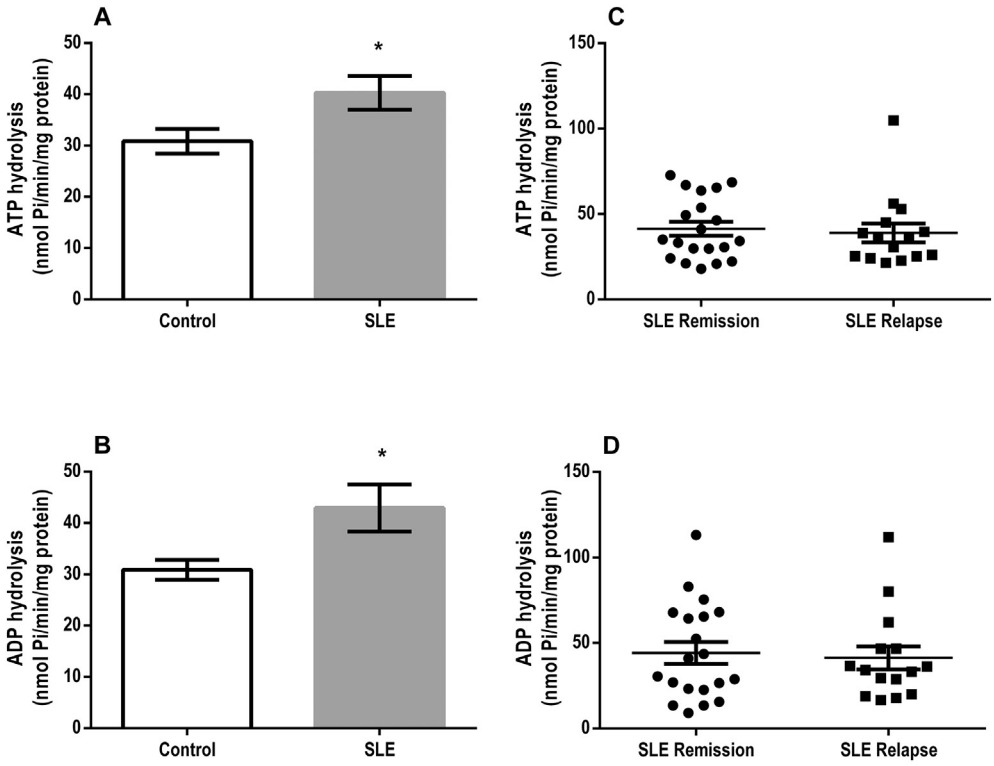

Fig. 1. Ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) activity [hydrolysis of ATP (A) and ADP (B)] in lymphocytes of systemic lupus erythematosus (SLE) patients and the control group. *$P < 0.05$, n = 30–35. E-NTPDase activity [hydrolysis of ATP (C) and ADP (D)] in lymphocytes of SLE patients during 2 different phases of the disease (remission and relapse). n = 15–20. Specific enzyme activities were reported as nmol of Pi released/min/mg of protein. Variables were expressed as mean ± SEM. (A, B, C and D) Student's *t*-test for independent samples was used for the analyses.

with SLE in remission and in relapse. Significant differences in CD39 expression were not found in the SLE remission group (12.7 ± 0.94% of $CD39^+$ lymphocytes) compared with the SLE relapse group (16.45 ± 1.86% of $CD39^+$ lymphocytes). Fig. 4A-B shows a statistically positive correlation between CD39 expression and the E-NTPDase activity (both ATP e ADP hydrolysis), $P < 0.01$.

3.4. E-ADA activity lymphocytes

Fig. 5A shows the E-ADA activity in lymphocytes of SLE patients. Adenosine deamination was higher in SLE patients compared with the control group (36.80 ± 4.15 vs. 25.98 ± 2.73 nmol NH_3/min/mg protein, respectively, $P < 0.05$). Statistically significant differences in E-ADA activity were not found in the SLE remission group compared

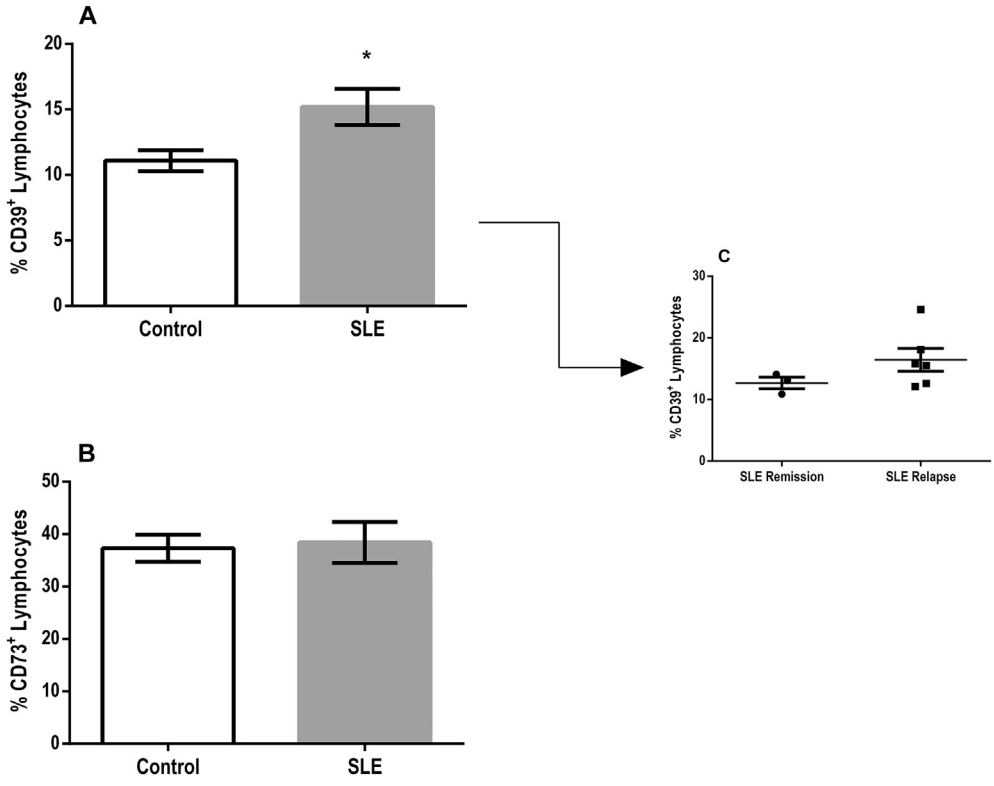

Fig. 2. Expression of CD39 (A) and CD73 (B) in lymphocytes from SLE and control subjects. *$P < 0.05$, n = 9. (C) Expression of CD39 in lymphocytes of SLE patients in remission and relapse. n = 3–6. Expression was reported as a percentage of lymphocytes expressing the surface marker (CD39 or CD73). Variables were expressed as mean ± SEM. Student's *t*-test for independent samples was used for all analyses.

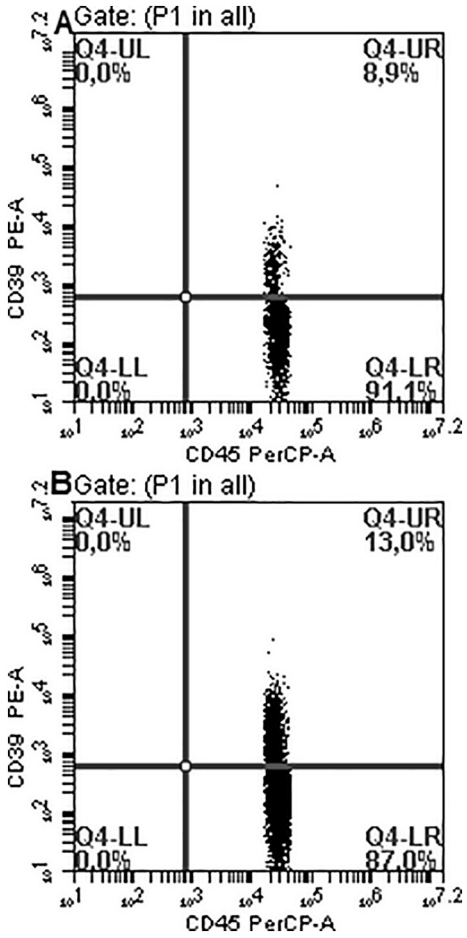

Fig. 3. CD39 expression was analyzed in gated $CD39^+$ $CD45^+$ cells. The percentage of $CD39^+$ cells in the $CD45^+$ cell population is indicated for healthy control (A) and SLE subjects (B). Cells were analyzed by flow cytometry.

with the SLE relapse group (37.91 ± 4.68 vs. 37.27 ± 8.33 nmol NH_3/min/mg protein, respectively) (Fig. 5B).

3.5. ADA activity serum

Adenosine deamination in serum was reduced in SLE patients compared with the control group (54.77 ± 25.04 vs. 103.9 ± 58.18 U/l, respectively, $P < 0.001$) (Fig. 5C). Statistically significant differences in ADA activity were not found in the SLE remission group compared with the SLE relapse group in serum (55.29 ± 20.1 vs. 52.55 ± 38.93 U/l, respectively) (Fig. 5D).

3.6. Purine levels measurement

The purine levels in the serum are shown in the Table 3. The concentration of ATP was increased in SLE patients compared with the control (2.35 ± 0.42 vs. 1.15 ± 0.25 nmol/ml, respectively, $P < 0.05$). ADP levels are also increased in the SLE group in relation to the control group (0.45 ± 0.22 vs. 0.00 ± 0.00 nmol/ml, respectively, $P < 0.05$). The concentration of the adenosine in serum was lower in the SLE group compared to the control (0.77 ± 0.22 vs. 2.39 ± 0.69 nmol/ml, respectively, $P < 0.05$). Significant differences in inosine, hypoxanthine, xanthine and uric acid were not found in the SLE group when compared with the control group.

3.7. Effects of drugs used in the treatment of SLE on E-NTPDase and E-ADA activities

The drugs were tested combined in the same way as the patients used, and each medication was tested in three different concentrations, which were: methotrexate (0, 0.0001, 0.001, and 0.01 mM), prednisone (0, 0.0002, 0.002, and 0.02 mM), hydroxychloroquine (0, 0.000014, 0.00014, and 0.0014 mM), and azathioprine (0, 0.0003, 0.003, and 0.03 mM). The results demonstrate that E-NTPDase and E-ADA activities were not altered by the presence of the medications at the above concentrations (data not shown).

4. Discussion

SLE is characterized by hyperactive and hyperresponsive B and T lymphocytes, which initiate and propagate the disease. Both types of immune cells act together to produce pathogenic autoantibodies. The disease is more frequent in women and most commonly described in the third and fourth decades of life [17], which is consistent with our results in terms of gender and age.

Autoantibodies form immune complexes that drive organ inflammation. The elevation in serum antinuclear antibody (ANA) levels is crucial in the process of SLE development and can be identified months to years preceding the onset of the disease [18]. According to the literature, 97.14% of patients with SLE present as ANA-positive [19].

Extracellular ATP is an important immune modulator that can be promptly released with other cellular components following stress or injury. In the immune system, ATP functions as an indicator of tissue destruction [20]. Extracellular ATP acts on inflammatory responses by binding to purinergic P2 receptors, which are expressed by almost all cell types, including cells of the immune system [21]. ATP levels can be regulated during inflammatory and immune responses by ectoenzymes [5].

In this context, we evaluated the role in lymphocytes of the enzymes E-NTPDase and E-ADA of SLE patients to improve our understanding of this disease. The results of this work showed that the E-NTPDase activity (using both ATP and ADP as substrates) and expression were increased in lymphocytes from patients with SLE and found a positive correlation between the activity of NTPDase (ATP and ADP hydrolysis) and the expression of CD39. Several studies have shown that E-NTPDase plays a role in the immune response and alterations in its activity have been reported in some diseases of autoimmune origin such as multiple sclerosis [22], diabetes [23], and rheumatoid arthritis (RA) [24].

The anti-inflammatory effects of E-NTPDase occur through 2 different mechanisms: either by removing the proinflammatory ATP or concomitantly generating immunosuppressive adenosine. Jaques et al. [24] demonstrated that E-NTPDase activity (using both ATP and ADP as substrates) was increased in lymphocytes from patients with RA, consistent with our results.

CD39 (E-NTPDase) expression is upregulated in activated T lymphocytes, indicating that it plays an important role in immune modulation and participates in the inflammatory response. This also suggests that CD39 could help reduce the immune response [25]. Based on our results, we suggest that there is an increase in ATP in the extracellular medium (Table 3), and the increased E-NTPDase activity and expression represent a compensatory mechanism to help control inflammation in SLE patients, attenuating the inflammatory effects and restoring balance. In the study of Moncrieffe et al. [26], the expression of CD39 was increased in inflammatory synovial T cells in human juvenile idiopathic arthritis, indicating an immunomodulatory mechanism dependent on ATP hydrolysis. Similarly, Herrath et al. [27] found that CD39 expression was increased in both $CD4^+$ T cell and $FOXP3^+$ regulatory T (Treg) cells in patients with RA, indicating that inflammation influenced CD39 expression. However, Loza et al. [28]

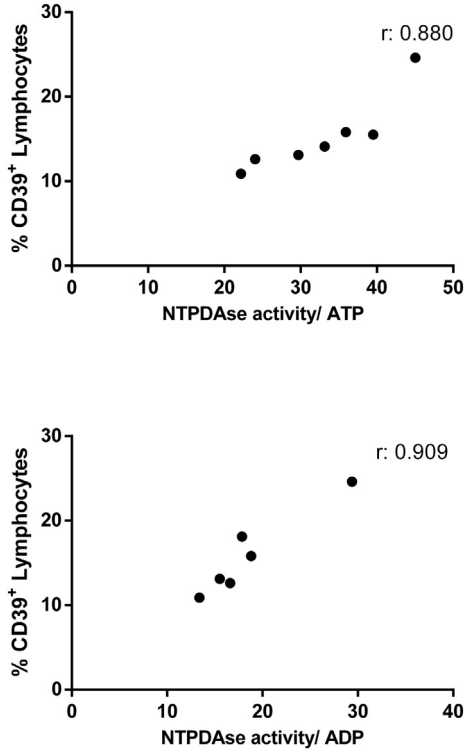

Fig. 4. Pearson's correlation analysis between % of CD 39 positive cells and E-NTPDase activity (ATP (A) and ADP (B) hydrolysis). $P < 0.05$.

showed decreased CD39 expression in Treg cells from SLE patients, demonstrating a defect restricted to this subset of cells. In relation to the nucleotide dosage, our results showed that the SLE group had an increase in the ATP level in relation to the control group, which may be due to the increase of the ATP release by the cells during inflammation. Although serum ADA is decreased, the reduced level of adenosine could be due to increased E-ADA activity in lymphocytes seen in this work. In addition, levels of AMP, inosine, hypoxanthine, xanthine and uric acid did not show differences between the SLE and control groups.

Table 3
Purine level measurement.

	Controls (nmol/ml)	SLE Patients (nmol/ml)
ATP	1.15 ± 0.25	2.35 ± 0.42*
ADP	0.00 ± 0.00	0.45 ± 0.22*
AMP	12.77 ± 1.3	9.45 ± 0.61
Adenosine	2.39 ± 0.69	0.77 ± 0.22*
Inosine	1.19 ± 0.46	0.74 ± 0.47
Hypoxanthine	8.36 ± 0.77	6.94 ± 1.10
Xanthine	4.34 ± 0.48	3.75 ± 0.62
Uric acid	109.5 ± 14.96	78.27 ± 14.15

Results are expressed as mean ± SEM, n = 4–10. Student's *t*-test for independent samples was used for all analyses, $*P < 0.05$.

We also evaluated E-NTPDase activity and expression in SLE patients during 2 phases of the disease: remission and relapse. In the current study, no significant differences were detected between the different subgroups of SLE in terms of E-NTPDase activity and expression. While there is still a lack of consensus on the criteria for defining SLE remission [29], a widely used definition is that patients in remission present resolution of clinical symptoms, normalization of laboratory results, and minimal maintenance of therapy. Yurasov et al. [30] concluded that patients in remission still exhibited increased numbers of autoantibodies and persistent abnormalities in tolerance, demonstrating that this imbalance is, in part, independent of the stage of disease. Therefore, both groups still exhibit autoantibodies and defects in tolerance which may explain the lack of differences between SLE in remission and in relapse.

We did not evaluate the activity of E-5′-nucleotidase in this study because the sensitivity of the colorimetric method used does not allow determination of enzyme activity. However, we evaluated the expression of CD73 in SLE patients and surprisingly, statistically significant differences were not found between SLE patients and the control group. In a study conducted by Dong-mei et al. [31], 5′-nucleotidase expression was decreased in Treg cells in SLE patients; however, subsets of lymphocytes were not separated in the current study. Stolk et al. [32]

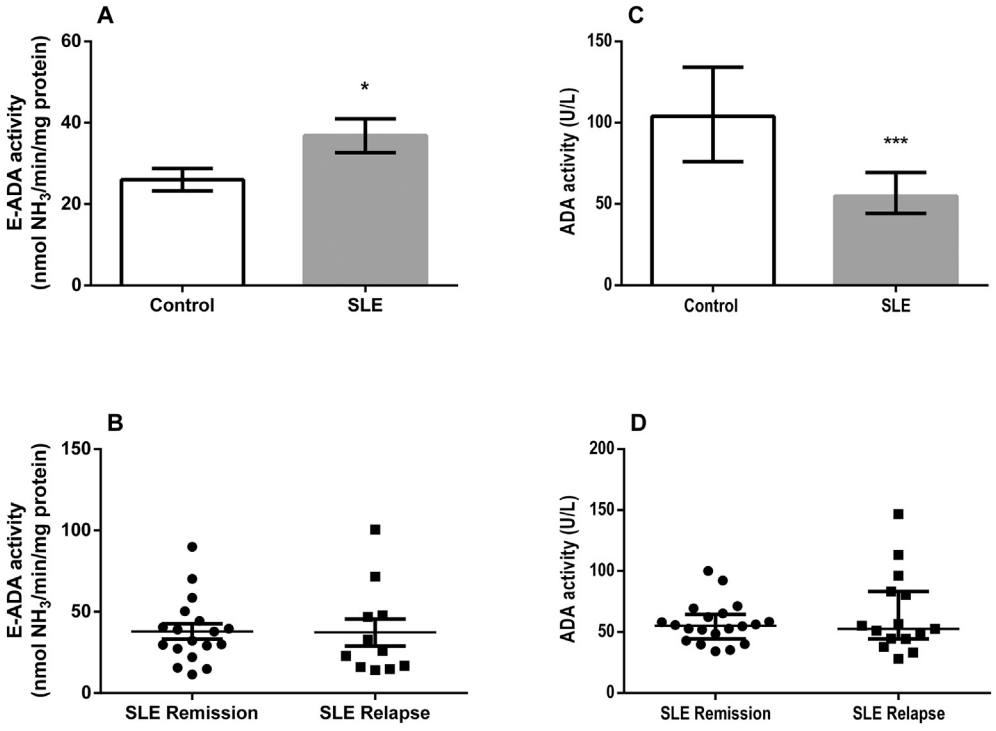

Fig. 5. Ecto-adenosine deamination in lymphocytes of control group and patients with SLE (A). $*P < 0.05$, n = 28–30 (SLE). (B) E-ADA activity in lymphocytes of SLE patients during 2 different phases of the disease (remission and relapse). n = 11–18. Variables were expressed as mean ± SEM. Student's *t*-test for independent samples. (C) ADA activity in serum of control group and patients with SLE. $***P < 0.001$, n = 30–35. (D) ADA activity in serum of SLE patients during 2 different phases of the disease (remission and relapse). n = 15–20. Variables were expressed as median ± interquartile range, Mann-Whitney test. Enzyme activities were reported as nmol NH_3/min/mg of protein in lymphocytes and U/l in serum.

demonstrated that the 5′-nucleotidase activity of lymphocytes was decreased in SLE patients, suggesting that purine metabolism in SLE might be disturbed.

Adenosine represents a powerful mechanism that orchestrates the inflammatory responses and ensures protection against tissue damage [33]. Upon its release, adenosine acts as a natural brake on immune cell function, limiting the inflammatory response, via activation of adenosine receptors (P1) [10]. Adenosine plays a central role in modulation of the lymphocyte response, decreasing interleukin (IL)-2, IL-4, and interferon-γ secretion through A_{2A} receptor activation [34], which contributes to the resolution of inflammation by facilitating Th2 pathways and inhibiting Th1 cell function [7]. The extracellular concentration of adenosine may be regulated by E-ADA, which converts adenosine to inosine [35]. In this context, E-ADA represents an important checkpoint to downregulate extracellular adenosine levels and serves to attenuate its immunosuppressive signaling [36]. The present study demonstrated that E-ADA activity was higher in lymphocytes from SLE patients. Alterations in E-ADA activity in lymphocytes of patients with multiple sclerosis [22], RA [24], and sickle cell anemia [37] have been previously demonstrated.

Hitoglou et al. [38] showed increased E-ADA activity in lymphocytes from SLE patients in the active phase, showing that E-ADA can be used as another biochemical approach for the pathophysiology evaluation of the disease. Downregulation of E-ADA may be associated with a greater anti-inflammatory Th2-like response, as upregulation may favor the Th1 immune response [39]. The ratios of Th1/Th2 cytokines are elevated in SLE [40]. Based on our results, we suggest that increased E-ADA activity may contribute to the inflammatory state present in SLE patients. We evaluated E-ADA activity in SLE patients during 2 different phases of the disease: remission and relapse. No significant differences were detected between the different subgroups of SLE.

ADA has a wide distribution in human tissues which could indicate its involvement in different diseases, many immune imbalances can be associated with altered ADA activity in the serum [41]. So in the present study we determined ADA activity in serum in SLE patients and control group. The results found revealed a decreased ADA activity in serum from SLE patients. Our data contrast with the results of the work Stancikova et al. [42] and Saghiri et al. [41] who found that ADA activity was higher in serum from SLE patients. The principal isoenzyme in serum is ADA-2, and the major source this isoenzyme are macrophages [43], which are able to release the enzyme into the surrounding environment [44]. Macrophages from patients with SLE show a defective phagocytic activity [45], we could suggest that reduced serum ADA activity in SLE patients would be altered due to defects in macrophages activity since these are the major source of serum ADA.

To exclude the effect of drugs commonly in the treatment of SLE, we investigated the influence of methotrexate, hydroxychloroquine, prednisone, and azathioprine in nucleotide hydrolysis and E-ADA activity*in vitro*. Our results (data not shown) did not show any significant alteration in E-NTPDase (hydrolysis of ATP and ADP) and E-ADA activities when methotrexate, hydroxychloroquine, prednisone, and azathioprine were used. We suggest that the observed increase in nucleotide hydrolysis was not caused by the drugs used to treat SLE.

5. Conclusion

We demonstrated that E-NTPDase activity and expression are altered in lymphocytes from SLE patients, suggesting a compensatory mechanism to help control inflammation. Likewise, E-ADA activity is altered in lymphocytes, suggesting that this enzyme may favor the Th1 immune response in SLE patients. Although increased NTPDase activity helps to control inflammation, this is not sufficient for its complete balance, since the increased ADA activity limits the anti-inflammatory effects of adenosine. Therefore, E-NTPDase and E-ADA have important functions in the modulation of the immune and inflammatory responses in SLE.

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