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Roles of CD147 on T lymphocytes activation and MMP-9 secretion in Systemic Lupus Erythematosus

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Abstract

The cellular and molecular mechanisms involved in many abnormalities described in Systemic Lupus Erythematosus (SLE) are still unclear. Some of these abnormalities referred to the hyperactivation of T lymphocytes and the enhanced secretion of MMP-9 by peripheral blood mononuclear cells (PBMCs). Therefore, in this paper we investigated the potential role of CD147 molecule in these abnormalities. Our results demonstrated that CD147 molecule is overexpressed on $CD3^+$T lymphocytes from SLE patients when compared with $CD3^+$T lymphocytes from healthy donors. Monoclonal anti-CD147 antibodies, MEM-M6/1 clone, were able to inhibit protein tyrosine phosphorylation only in CD3 $\times$ CD28 costimulated T lymphocytes from SLE patients. However, this monoclonal antibody was unable to inhibit the enhanced activity of MMP-9 secreted by SLE PBMCs.

Keywords: systemic lupus erythematosus • CD147 positive T lymphocytes • tyrosine phosphorylation • MMP-9

Introduction

Systemic lupus erythematosus (SLE) is considered the prototype of systemic autoimmune diseases, characterized by T and B lymphocytes dysfunctions and many abnormalities of intracellular signaling pathways. Thus, among other defects, peripheral T lymphocytes of SLE patients display abnormal signal transduction mediated by TCR/CD3 [1]. This comprises aberrant regulation of protein tyrosine kinases $p56^{lck}$ and $p59^{fyn}$ [2, 3], decreased expression of ξ chain of TCR/CD3 [4, 5], increased intracellular calcium mobilization mediated by CD3 [6], reduction of protein tyrosine phosphatase CD45 expression and function [7]. All these defects suggest that peripheral SLE T lymphocytes have an activated phenotype facilitating the cooperation with B lymphocytes, finally leading to high-affinity autoantibodies secretion and to immune complex-mediated tissue damages [8].

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Besides immunologic abnormalities, SLE was characterized by high levels of some molecules involved in mediation of inflammatory processes such as matrix metalloproteinases (MMPs). Previously, we demonstrated that PBMCs freshly isolated from SLE patients express a significantly higher activity and spontaneously release higher levels of MMP-9, as compared to healthy donor PBMCs [9].

CD147, also known as M6 antigen [10], EMMPRIN [11] or human basigin [12] is a 50–60 kD transmembrane glycoprotein [10], broadly expressed on the surface of both haematopoietic and non-haematopoietic cells [13–15]. CD147 is expressed on the surface of all immune cells [16–20]. Resting T lymphocytes have a weak expression of CD147, but the expression of this molecule is rapidly increased after cellular activation [16, 21].

Multiple biological activities of CD147 have been demonstrated, this molecule playing roles in: (a) tumor invasion, being a potent inducer of MMPs synthesis and secretion from stromal and tumor cells [22–24]; (b) mediation of inflammatory processes, as a type I receptor for Cyclophilins A and B [25–27]; (c) amyloid plaques formation in Alzheimer's disease patients, as member of the gamma-secretase complex [28]; (d) monocyte accumulation and MMPs production in patients with rheumatoid arthritis [29, 30]; (e) anoikis resistance of breast carcinoma cells [31], and others.

Based on previously reported data, we hypothesized that CD147 could play a role in SLE pathology, altering signal transduction mediated by TCR/CD3 and costimulatory molecules, and also MMPs expression and secretion. In this paper, we demonstrated that SLE PBMCs presented a significantly increased number of $CD3^+CD147^+$ T lymphocytes and an augmented density of CD147 molecules on $CD3^+$T lymphocytes than healthy donor cells. The triggering of CD147 molecules concomitantly with TCR/CD3 and CD28 costimulation on SLE T lymphocytes induced an important reduction of total tyrosine phosphorylation level of cellular proteins. In addition, the activity of secreted MMP-9 by SLE PBMCs directly correlated with the percentage of SLE $CD147^+$ T lymphocytes.

Materials and methods

Subjects

Peripheral blood samples were obtained from 40 SLE patients, all females (19 active SLE and 21 inactive SLE) fulfilling the criteria of the American Rheumatism Association [32]. Patients hospitalized at Colentina Clinical Hospital, Sf. Maria Clinical Hospital and Carol Davila Clinical Hospital of Nephrology, Bucharest, Romania, received treatment according to disease activity. Twenty-one healthy donors, matched for sex and age, were studied as controls. Due to the reduced number of peripheral T lymphocytes in SLE patients, the tests described below were not performed on all the subjects. For this study, ethical approval was granted and all subjects provided their informed consent.

Cell isolation

From heparinized peripheral blood of SLE patients and healthy donors, mononuclear cells were isolated by Ficoll-Paque (Amersham Pharmacia Biotech) gradient centrifugation, following manufacturer's protocol.

Flow cytometry analysis

To evaluate the percentage of $CD147^+$T lymphocytes, 1×10^6 PBMCs in RPMI 1640 medium (Sigma Aldrich) containing 2% Fetal Bovine Serum (FCS, Gibco BRL, Eggenstein, Germany) and 0.02% sodium azide were double stained with FITC conjugated anti-CD147 and PE conjugated anti-CD3 monoclonal antibodies (BD Pharmingen). Appropriate isotype controls (mouse IgG1 and mouse IgG2, respectively) were included in all FACS experiments. Cells were analyzed using FACS Calibur Flow Cytometer (Becton Dickinson) and data were evaluated using WinMDI 2.7 free software (Joseph Trotter, Scripps Research Institute, La Jolla, CA, USA; http://facs.scripps.edu). The results were expressed as the percentage of CD147 positive T lymphocytes from $CD3^+$T lymphocytes or as median of fluorescence intensity (MFI) of $CD147^+CD3^+$T lymphocytes.

Immunoblotting analysis of CD147 expression on PBMCs

PBMCs (1×10^6) freshly isolated from healthy donors and SLE patients were washed with cold PBS and lysed in buffer containing 20 mM Tris-HCl pH 7.5, 1% NP 40, 150 mM NaCl, 1 mM sodium vanadate, 20 mM NaF, 5 mM EDTA and protease inhibitor mixture. Lysates were centrifuged at 15,000 rpm for 15 min. Cleared lysates were resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes (Trans-Blot® Transfer Medium, Bio-Rad). Proteins were detected with a CD147 specific monoclonal antibody (1 μg/ml, MEM-M6/1 clone, Chemicon) or with actin specific polyclonal antibodies (1/400) (Sigma Aldrich). Blots were developed with peroxidase–-conjugated secondary antibodies (Amersham Pharmacia Biotech), and ECL (Amersham Pharmacia Biotech). Immunoblotting images were scanned and the expression levels of CD147 and actin in PBMCs were evaluated using Total Lab v1.11

software (Phoretix, Newcastle, UK). The expression level of CD147 on PBMCs was evaluated like the ratio value between the intensity bands of CD147 and actin.

Cell stimulation

Freshly isolated PBMCs (1×10^6 cells in 100 µl RPMI-1640 medium containing 4 mM L-glutamine, 100 units/ml penicillin G, 0.1 mg/ml streptomycin and 10% FCS) were experimentally stimulated for 5 min, as follows: (1) with 1 µg/ml monoclonal anti-CD147 antibodies (MEM-M6/1 clone, Chemicon); (2) with 10 µg/ml monoclonal anti-CD3 antibodies (UCHT1 clone, BD Pharmingen) plus 2 µg/ml monoclonal anti-CD28 antibodies (CD28.2 clone, BD Pharmingen) in absence or presence of 1 µg/ml anti-CD147 antibodies, as described Staffler *et al.* [33]; (3) rested unstimulated cells as control. The reaction was stopped by adding cold buffer (20 mM Tris-HCl pH 7.5 containing 150 mM NaCl and 5 mM EDTA). Subsequently, the cells were lysed in lysis buffer and centrifuged for 15 min at 15,000 rpm. The supernatants were collected and analyzed for tyrosine phosphorylation level by immunoblotting.

Immunoblotting analysis of tyrosine phosphorylation level

Cellular proteins were resolved by SDS-PAGE and subsequently transferred on nitrocellulose membrane. Tyrosine phosphorylation level of proteins was detected using monoclonal anti-phosphotyrosine antibodies (1µg/ml, Santa Cruz), peroxidase–-conjugated anti-mouse IgG antibodies (Amersham Pharmacia Biotech), and ECL system. Immunoblotting images were scanned and the level of tyrosine phosphorylation of each protein was estimated using Total Lab v1.11 software. The results were expressed as Arbitrary Units (AU).

Zymographic analysis of secreted MMP-9

On 24 wells culture plate, freshly isolated PBMCs (2×10^6/ml) were incubated for 24 hrs in conditioned RPMI-1640 medium containing 4 mM glutamine, 10% serum replacement (Sigma Aldrich) and antibiotics, at 37°C and 5% CO_2. The supernatants were collected and analyzed for the activity of secreted MMP-9 by gelatin zymography on 8% SDS-PAGE containing 0.1% gelatin. After electrophoresis, the gels were incubated in 2.5% Triton X-100 for 30 min and then in substrate buffer (50 mM Tris-HCl, pH 7.6 containing 0.2 M NaCl and 5 mM $CaCl_2$) for 18 hrs at 37°C. The lytic activity of gelatinases was identified by Coomassie Brilliant Blue R staining as a clear band on a blue background. The zymograms were scanned and analyzed by densitometry using TotalLab v1.11 software. Molecular weight markers were used to identify the gelatinase type.

In order to inhibit the activity of secreted MMP-9, freshly isolated PBMCs (1×10^6/ml) were incubated for 24 hrs with different concentrations (1, 2.5, 5 µg/ml) of soluble or immobilized monoclonal anti-CD147 antibodies. To immobilize anti-CD147 antibodies, culture plates were precoated with 8 µg/well rabbit anti-mouse IgG (Chemicon). MMP-9 activity in collected culture media was analyzed by gelatin zymography.

Statistical analysis

The significance of the differences between SLE patients and healthy donors were established using unpaired or paired Student's t-test, $p < 0.05$ being considered as significant. Spearman's coefficients were calculated to establish the correlation between the experimentally determined parameters. A correlation coefficient $|r| < 0.5$ with a probability $p < 0.05$ was considered as significant.

Results

$CD3^+CD147^+$T lymphocytes in SLE and healthy donor PBMCs

Peripheral T lymphocytes freshly isolated from eighteen SLE patients (nine with active SLE and nine with inactive SLE) and 11 healthy donors were evaluated for the expression of CD147 molecule by FACS. CD3 positive cells were gated using FL1-H/FL2-H density plot and then analyzed for CD147 positive T lymphocytes using the quadrants analysis. Figure 1 presents a FACS analysis using WinMDi 2.7 software. As can be seen, only few $CD3^+$T lymphocytes are CD147 positive (2.5%). However, when FACS images were analyzed for all studied subjects the results showed that SLE patients have a significantly increased percentage of $CD147^+$T lymphocytes (3.05 ± 2.01) in $CD3^+$T population than healthy donors (1.26 ± 0.96), the difference between the groups being significant ($p = 0.003$, Student's t-test). Additionally, we analyzed the percentages of $CD3^+CD147^+$T lymphocytes in SLE patients in accordance with disease activity (Fig. 2). The highest percentage of $CD3^+$T lymphocytes expressing CD147 molecules was found in peripheral blood of patients with active SLE. Indeed, the statistical analysis by Student's t-test showed significant difference between active SLE patients and healthy donors ($p = 0.018$) and a limited difference between active and inactive SLE patients ($p = 0.069$).

To estimate the density of CD147 molecules on SLE and healthy donors T lymphocytes, MFI was

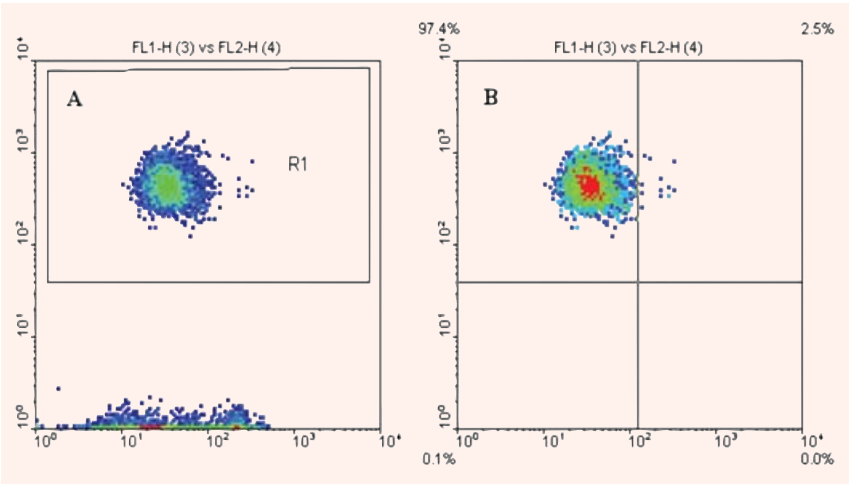


Fig. 1 FACS analysis of CD3+CD147+T lymphocytes. PBMCs freshly isolated from one subject were double stained using FITC conjugated monoclonal anti-CD147 antibodies (FL1-H) and PE conjugated monoclonal anti-CD3 antibodies (FL2-H). CD3$^+$T lymphocytes were gated (**A**, gate R1). The percentage of CD3$^+$CD147$^+$T lymphocytes from R1 is identified in upper right quadrant (**B**).

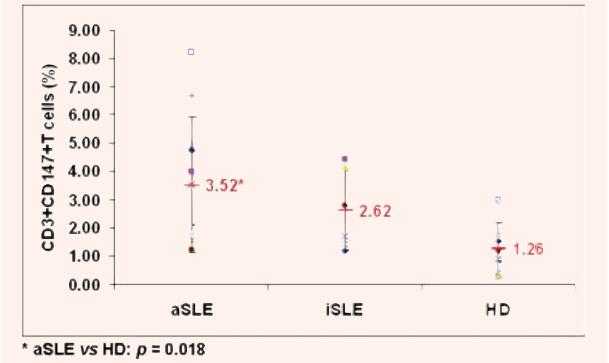


Fig. 2 The percentage of CD147 positive cells in CD3$^+$T lymphocytes from SLE patients and healthy donors. Points represent the percentages of CD3$^+$CD147$^+$T lymphocytes obtained for each active (aSLE) and inactive (iSLE) SLE patient and healthy donor (HD) as well as the mean values ± SD calculated for each group of subjects.

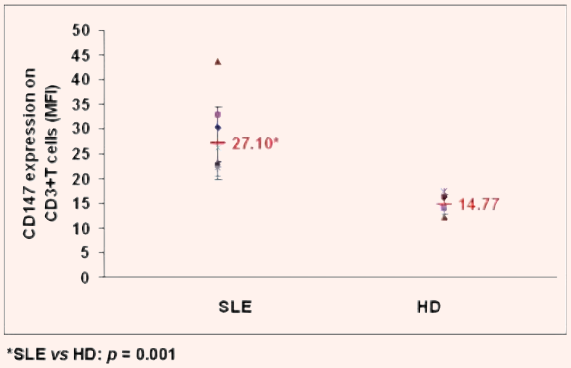
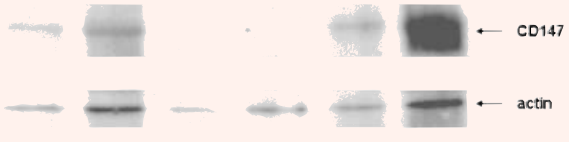


Fig. 3 FACS analysis for the density of CD147 molecules on CD3$^+$T lymphocytes from SLE patients and healthy donors. Median of fluorescence intensities (MFI) that corresponds to the density of CD147 molecules on CD3+T lymphocytes is presented as points for each studied subject. The mean values ± SD of MFI for each group of subjects are included in histogram.

evaluated by FACS. CD3$^+$T lymphocytes were gated and the histograms were used to quantify the MFI for each subject. All the data showed that the expression level of CD147 molecules on CD3$^+$T lymphocytes was also higher in SLE patients than in healthy donors (p = 0.001), as can be seen in Figure 3.

Subsequently, PBMCs isolated from three healthy donors and three SLE patients (two in inactive and one in active stage of disease) were analyzed by immunoblotting using specific anti-CD147 and anti-actin antibodies. As can be see in Figure 4, the immunoblotting results are in line with those obtained by FACS, generally showing that the expression level of CD147 is more increased in PBMCs from active SLE patients than in inactive SLE patients or from healthy donors PBMCs. However, FACS results display the expression level of CD147 molecules only on CD3$^+$T cells while immunoblotting reflects the expression level of CD147 on all cellular populations contained by PBMCs pool.



	HD1	HD2	HD3	iSLE	iSLE	aSLE
	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6
CD147	26,059	41,819	0	4,838	33,748	474,675
actin	26,665	88,776	13,941	38,402	27,483	109,032
CD147/actin	0.98	0.47	0.00	0.13	1.23	4.35

Fig. 4 Immunoblotting analysis of CD147 expression on PBMCs. PBMCs isolated from three healthy donors and three SLE patients (two in inactive and one in active stage of disease) were analyzed by immunoblotting using specific anti-CD147 and anti-actin antibodies. Immunoblotting was analyzed by densitometry and the expression level of CD147 on PBMCs was expressed as the ratio between CD147 and actin band intensities.

Modulation of tyrosine phosphorylation level by triggering of CD147 molecules

PBMCs freshly isolated from SLE patients and healthy donors were experimentally stimulated using soluble monoclonal anti-CD3 and anti-CD28 antibodies, in the presence or absence of anti-CD147 antibodies, MEM-M6/1 clone. After 5 min of stimulation, the cells were lysed and analyzed for tyrosine phosphorylation level. As Koch *et al.* [16] have demonstrated, this antibody reacts with resting and activated T cells and possesses a relatively high affinity for the N terminal Ig domain of monomeric CD147. In addition, Staffler *et al.* [33] demonstrated that the optimal concentration between 1 μg/ml and 0.1 μg/ml of soluble MEM-M6/6 antibody is able to inhibit anti-CD3-induced T cell proliferation. Increasing or decreasing of MEM-M6/6 antibody concentration abrogated the inhibitory effect. In line with these data and with our experiments (data not shown) we analyzed the inhibitory potential of MEM-M6/1, using 1 μg/ml monoclonal antibody. A representative image of immunoblotting experiments performed on unstimulated or stimulated PBMCs isolated from one SLE patient and one healthy donor is shown in Figure 5. As can be seen, only CD147 stimulation did not significantly modify the tyrosine phosphorylation level in PBMCs of the two analyzed subjects. In contrast,

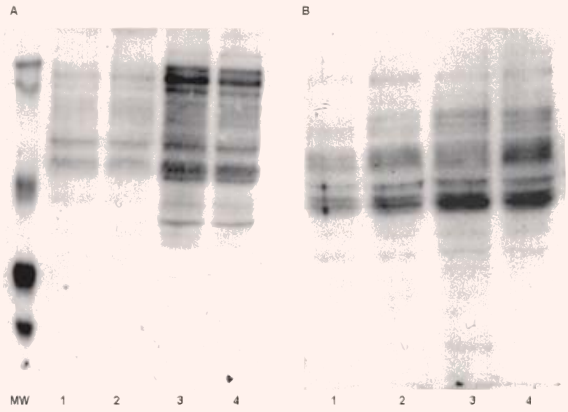


Fig. 5 Tyrosine phosphorylation pattern in unstimulated and stimulated T lymphocytes. PBMCs isolated from one SLE patient (**A**) and one healthy donor (**B**) were stimulated with different types of monoclonal antibodies, as follows: (1) unstimulated, (2) stimulated by anti-CD147 antibodies, (3) stimulated by anti-CD3 and anti-CD28 antibodies, (4) costimulated by antibodies directed to CD3, CD28 and CD147 receptors. After 5 min of stimulation, the cells were lysed and analyzed by immunoblotting with monoclonal anti-phosphotyrosine antibodies and ECL system. The standards of known molecular weight (MW) were included in each experiment.

when T lymphocytes were costimulated by CD3 and CD28 receptors, a significantly increase in tyrosine phosphorylation was observed, especially in SLE PBMCs. Stimulation of T lymphocytes by CD3 × CD28 × CD147, in comparison with CD3 × CD28 costimulation, induced different modulation of tyrosine phosphorylation level in SLE and healthy donor T lymphocytes. While anti-CD147 antibodies decreased the tyrosine phosphorylation level of CD3 × CD28 costimulated SLE T lymphocytes, the same antibodies induced a slight increase of phosphorylation level of some proteins in healthy donor T lymphocytes. In Figure 6, the mean values ± SD of total tyrosine phosphorylation level in unstimulated and stimulated PBMCs from nine SLE patients and nine healthy donors are presented. Paired Student's t-test showed that T cells costimulation by CD3 x CD28 receptors induced for both groups of subjects a significant increase of total tyrosine phosphorylation level when compared with unstimulated T cells (p = 0.003 for SLE patients and p = 0.003 for healthy donors, respectively). Concomitantly, stimulation of T

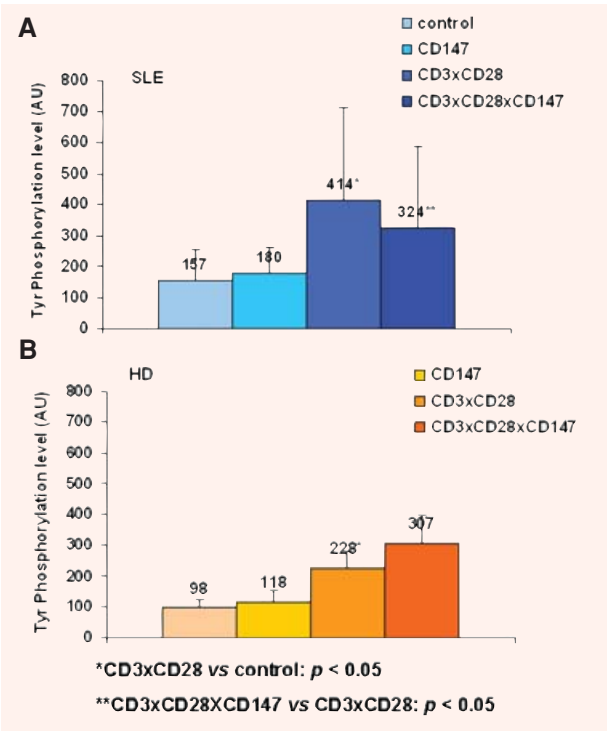


Fig. 6 Tyrosine phosphorylation level in unstimulated and stimulated T lymphocytes. PBMCs isolated from SLE patients and healthy donors were stimulated as in Figure 5 and analyzed by densitometry for total tyrosine phosphorylation level. For each type of stimulation and for SLE **(A)** and healthy donors **(B)** group the mean values ± SD was calculated and indicated on histograms.

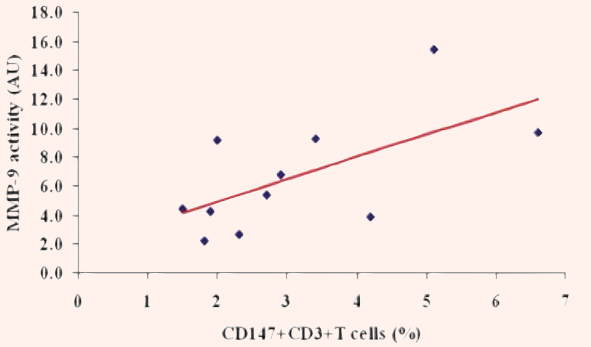


Fig. 7 Correlation between the percentage of $CD3^+CD147^+$T lymphocytes and the activity of secreted MMP-9. PBMCs freshly isolated from peripheral blood of SLE patients were analyzed by FACS in order to determine the percentage of $CD3^+CD147^+$T lymphocytes. In addition, PBMCs from the same patients were cultured for 24 hrs and the culture supernatants were analyzed by gelatin zymography. Zymograms were scanned and the intensity of bands was determined. Subsequently, these two parameters were correlated by Spearman's analysis.

lymphocytes by CD3 × CD28 and CD147 receptors confirmed the results presented in Figure 5. The total tyrosine phosphorylation level in SLE T lymphocytes activated by CD3 × CD28 receptors significantly decreased in the presence of anti-CD147 antibodies (p = 0.002, by paired Student's t-test). On contrary, CD147 triggering did not induce important changes in healthy donor T lymphocytes activated by CD3 × CD28 receptors (p = 0.195 by paired Student's t-test).

Subsequently it was verified if the modulation of tyrosine phosphorylation dependent on CD147 expression on T lymphocytes. For this purpose, PBMCs from three SLE patients were analyzed by FACS for CD147 expression (MFI) and the results were correlated with anti-CD147 antibodies capacity to modulate the tyrosine phosphorylation level in CD3 × CD28 costimulated T lymphocytes. No correlation was found by this analysis.

Correlation between the percentage of $CD3^+CD147^+$T lymphocytes and the activity of MMP-9 secreted by PBMCs

Literature data demonstrated the role of CD147 molecules in MMPs induction [22–24]. On the other hand, it is known that SLE PBMCs produce and secrete large amounts of MMP-9 as compared with healthy donor PBMCs [9]. To test if secreted MMP-9 activity is related to CD147 expression, we analyzed these two parameters for PBMCs isolated from ten SLE patients (Fig. 7). Spearman's analysis showed a significant direct correlation between the percentage of $CD3^+CD147^+$T lymphocytes and the activity of MMP-9 secreted in supernatants of cultured SLE PBMCs (r = 0.627, p = 0.038).

Modulation of MMPs secretion by monoclonal anti-CD147 antibodies

In order to verify if the blocking of CD147 molecules could inhibit the secretion of MMPs, SLE PBMCs were cultured for 24 hrs in the absence or presence of soluble or immobilized monoclonal anti-CD147

antibodies. The obtained results demonstrated that this anti-CD147 antibodies clone did not inhibit the activity of different types of MMPs, as it can be seen in Figure 8.

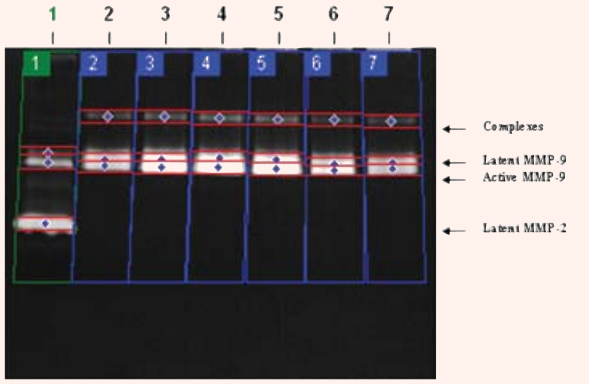


Fig. 8 Modulation of MMPs activity in PBMCs cultured in absence or presence of monoclonal anti-CD147 antibodies. PBMCs of one SLE patient were cultured in absence (Line 2) or presence of anti-CD147 antibodies immobilized by rabbit anti-mouse IgG antibodies (Lines 4, 5) or soluble (lines 6, 7). Two controls were included in all experiments: positive control (fetal calf serum as source of latent and active forms of MMP-9 and latent MMP-2) (Line 1) and negative control (supernatant of PBMCs cultured only in the presence of rabbit anti-mouse IgG antibodies) (Line 3). After 24 hours, supernatants were collected and analyzed by gelatin zymography.

Discussion

Previously, we demonstrated an increased expression and secretion of MMP-9 by PBMCs from SLE patients [9]. To explain these results, we proposed to study the occurrence of $CD147^+$T lymphocytes in PBMCs freshly isolated from SLE patients and healthy donors, taking into account that CD147 is overexpressed on activated T lymphocytes [34] and SLE T lymphocytes are in continuously activated state by autoantigens and, being known that CD147 is one of the MMPs inducers [35].

FACS analysis showed significantly increased percentage of $CD3^+CD147^+$T lymphocytes associated with higher CD147 expression level on $CD3^+$T lymphocytes in SLE patients when compare with healthy donors. Furthermore, when SLE patients were divided into active and inactive patients a higher percentage of $CD3^+CD147^+$T lymphocytes was found in active SLE PBMCs. These results additionally proved the activation state of T lymphocytes from active SLE patients. An increased expression of CD147, but on monocytes/macrophages, was reported in other pathologies with inflammatory components, like rheumatoid arthritis and acute myocardial infarction [29, 36, 37], some authors demonstrating the role of C Reactive Protein in the enhancement of CD147 expression on macrophages [38].

Based on the involvement of CD147 in cellular activation, several reports demonstrated that CD147 might be a potential therapeutic target. Therefore, different monoclonal antibodies were generated and different cellular responses were observed. Thus, an anti-CD147 antibody inhibits the activation of both CD45RA (naive) T cells and CD45RO (memory) T cells [19]. Another monoclonal antibody, MEM-M6/6, that recognizes membrane proximal domain 2 of CD147 but not MEM-M6/1, raised against membrane distal domain 1, prevents TCR stimulation-dependent reorganization of microdomains and inhibits TCR-mediated T cell proliferation [33]. However, MEM-M6/6 antibodies were unable to affect major protein tyrosine phosphorylation upon TCR/CD3 T cell stimulation. Recently, another group of researchers [39] generated other five monoclonal antibodies, two of them raised against membrane distal domain 1 of CD147, like MEM-M6/1. Using peripheral T lymphocytes isolated from healthy subjects and TCR/CD3 stimulation for a long time (30 min or 24 hrs), authors demonstrated that these two anti-CD147 antibodies strongly inhibited cell proliferation without significant modifications of tyrosine phosphorylation level.

Starting from this evidence and based on own results, we investigated the CD147 mediated signal transduction in SLE and healthy donors T lymphocytes. It is well known that intracellular level of protein tyrosine phosphorylation could be a measure of early cellular responses to extracellular stimulation. Therefore, SLE and healthy donor PBMCs were stimulated for short time (5 min) using commercial monoclonal anti-CD147 antibodies, a MEM-M6/1 clone. MEM-6/1 antibodies were unable to significantly modify the tyrosine phosphorylation level in SLE or healthy donor T lymphocytes. When healthy donor T lymphocytes were concomitantly stimulated using anti-CD3, anti-CD28 and anti-CD147 antibodies, we did not observe an important modification of phosphorylation level, in agreement with Staffler *et al.* [33]

results. Although MEM-M6/1 was considered as a non-functional antibody, in our experimental conditions (non-purified T lymphocytes and short time activation with soluble antibodies) this antibody was able to inhibit the tyrosine phosphorylation level induced by CD3 × CD28 costimulation of T lymphocytes from SLE patients. This effect could not be attributed to differences in CD147 expression on T cell surface because no correlation between the reduction of tyrosine phosphorylation level and the CD147 expression level was found. In SLE T lymphocytes, different to healthy donor cells, an increased lipid rafts formation upon CD3 × CD28 costimulation was described. On the other hand, the lipid rafts of T lymphocytes are more susceptible to be disorganized in SLE patients than in healthy donors [40]. It was demonstrated that in TCR/CD3 stimulated cells, only a small part of CD147 molecules were found in lipid raft domains. However, if TCR/CD3 stimulated T cells were incubated with MEM-M6/1 or MEM-M6/6 antibodies, CD147 molecules were concentrated in non lipid raft fractions [33]. Based on such observations, we proposed that MEM-M6/1 antibodies could be able to disturb preformed lipid rafts on SLE T lymphocytes and thus to inhibit signal transduction mediated by TCR/CD3 and CD28 receptors.

Since CD147 was considered one of the MMPs inducers [35], in a second set of experiments we analyzed the relationship between the activities of spontaneously secreted MMPs and the percentage of $CD147^+$T lymphocytes. Therefore, the MMP-9 activity in supernatants of cultured SLE PBMCs and the percentage of SLE $CD147^+$T lymphocytes were concomitantly analyzed. The results of these experiments showed a strong positive correlation between these two parameters, demonstrating the important role of $CD147^+$T lymphocytes in the enhancement of MMP-9 secretion by SLE PBMCs, as we previously reported [9].

These results encouraged us to use anti-CD147 antibodies in order to inhibit secretion of MMP-9 by SLE PBMCs. Different to the capacity of anti-CD147 antibodies (MEM-M6/1) to inhibit tyrosine phosphorylation in CD3 × CD28 costimulated SLE T lymphocytes, these antibodies were unable to inhibit the activity of secreted MMP-9 by SLE PBMCs. Our result was not surprising taking into account different bioactive functions of CD147 epitopes, responsible for different cellular activities. Thus, it was demonstrated that epitopes involved in cell aggregation are different from those that regulate lymphocyte activation [39]. Similarly, in our studies MEM-M6/1 anti-CD147 antibodies were able to downregulate early steps of SLE T cell activation but not to suppress MMP-9 secretion, a later cellular response.

Taken together, our results showed that manipulation of CD147 molecule inhibited early signaling events in continuously activated SLE T lymphocytes, and could re-establish some of abnormal cellular functions, suggesting that CD147 could be a potential therapeutic target in SLE.

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