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

Gina Pistol, Cristiana Matache, Ana Calugaru, Crina Stavaru, Stefanita Tanaseanu, Ruxandra Ionescu, Sergiu Dumitrache, Maria Stefanescu

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+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 p56lck and p59fyn [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].
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 pathogenesis, 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, St. 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 x 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 x 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% CO₂. 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 CaCl₂) 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 x 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
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.
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, 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 T cells of both groups of subjects are presented. Paired Student’s t-test showed that T cell costimulation by CD3 × CD28 receptors induced for both groups of subjects a significant increase of phosphorylation level of some proteins in healthy donor T lymphocytes. In contrast, stimulation of T

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 in PBMCs was expressed as the ratio between CD147 and actin band intensities.

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.
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.

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 compared 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.

Acknowledgements

We thank Mr Razvan Stoian, Ms D. Preoteasa, and Ms D. Florescu for technical support. In addition, we thank all patients for their agreement and cooperation in this study. The Romanian Ministry of Education and Research grant no. 259/2003 - 2005 supported this work.

References

1. Kammer GM, Perl A, Richardson BC, Tsokos GC. Abnormal T cell signal transduction in systemic lupus erythematosus. Arthritis Rheum. 2002; 46: 1139–54.
2. Blasini AM, Brundula V, Paris M, Rivas L, Salazar S, Stekman IL, Rodriguez MA. Protein tyrosine kinase activity in T lymphocytes from patients with systemic lupus erythematosus. J Autoimmun. 1998; 11: 387–93.
3. Matache C, Stefanescu M, Onu A, Tanaseanu S, Matei I, Frade R, Szegli G. p56lck activity and expression in peripheral blood lymphocytes from patients with systemic lupus erythematosus. Autoimmunity. 1999; 29: 111–20.
4. Nambiar MP, Enyedy EJ, Fisher CU, Krishnan S, Warke VG, Gilliland WR, Oglesby RJ, Tsokos GC. Abnormal expression of various molecular forms and distribution of T cell receptor zeta chain in patients with systemic lupus erythematosus. Arthritis Rheum. 2002; 46: 163–74.
5. Nambiar MP, Krishnan S, Warke VG, Tsokos GC. TCR zeta-chain abnormalities in human systemic lupus erythematosus. Methods Mol Med. 2004; 102: 49–72.
6. Vassiliopoulos D, Kovacs B, Tsokos GC. TCR/CD3 complex–mediated signal transduction pathway in T cells and T cell lines from patients with systemic lupus erythematosus. J Immunol. 1995; 155: 2269–81.
7. Takeuchi T, Pang M, Amano K, Koide, Abe T. Reduced protein tyrosine phosphatase (PTPase) activity of CD45 on peripheral blood lymphocytes in
patients with systemic lupus erythematosus (SLE). J Clin Exp Immunol. 1997; 109: 20–6.

8. Theofilopoulos AN. Immune complexes in autoimmunity. In: Bona CA, Siminovitch KA, Zanetti M, Theofilopoulos AN, editors. The molecular pathology of autoimmune diseases. Switzerland: Harwood Academic Publishers; 1993. p. 229–44.

9. Matache C, Stefanescu M, Dragomir C, Tanaseanu S, Onu A, Ofiteru A, Szegei G. Matrix metalloproteinase-9 and its natural inhibitor TIMP-1 expressed or secreted by peripheral blood mononuclear cells from patients with systemic lupus erythematosus. J Autoimmun. 2003; 20: 323–31.

10. Kasinrerk W, Fiebigier E, Stefanova I, T Baumruker, Knapp W, Stockinger H. Human leukocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, mouse basigin, and chicken HT7 molecule. J Immunol. 1992; 149: 847–54.

11. Biswas C, Zhang Y, DeCastro R, Guo H, Nakamura T, Kataoka H, Nabeshima K. The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. Cancer Res. 1995; 55: 434–9.

12. Miyauchi T, Masuzawa Y, Muramatsu T. The basigin group of the immunoglobulin superfamily: complete conservation of a segment in and around transmembrane domains of human and mouse basigin and chicken HT7 antigen. J Biochem (Tokyo). 1991; 110: 770–4.

13. Fossum S, Mallett S, Barclay AN. The MRC OX-47 antigen is a member of the immunoglobulin superfamily with an unusual transmembrane sequence. Eur J Immunol. 1991; 21: 671–9.

14. DeCastro R, Zhang Y, Guo H, Kataoka H, Gordon MK, Toole B, Biswas G. Human keratinocytes express EMMPRIN, an extracellular matrix metalloproteinase inducer. J Invest Dermatol. 1996; 106: 1260–5.

15. Nehme CL, Fayos BE, Bartles JR. Distribution of the integral plasma membrane glycoprotein CE9 (MRC OX-47) among rat tissues and its induction by diverse stimuli of metabolic activation. Biochimie. 1995; 77: 693–8.

16. Koch C, Staffler G, Huttinge R, Hilgert I, Prager E, Cerny J, Steinlein P, Majdic O, Horejsi V, Stockinger H. T cell activation-associated epitopes of CD147 in regulation of the T cell response, and their definition by antibody affinity and antigen density. Int Immunol. 1999; 11: 777–86.

17. Renno T, Wilson A, Dunkel C, Coste I, Maisnier-Patin K, Benoit de Coignac A, Aubry JP, Lees RK, Bonnefoy JY, MacDonald HR, Gauchat JF. A role for CD147 in thymic development. J Immunol. 2002; 168: 4946–50.

18. Stonehouse TJ, Woodhead VE, Herridge PS, Ashrafian H, George M, Chain BM, Katz DR. Molecular characterization of U937-dependent T-cell co-stimulation. Immunology. 1999; 96: 35–47.

19. Woodhead VE, Stonehouse TJ, Binks MH, Speidel K, Fox DA, Gaya A, Hardie D, Henniker AJ, Horejsi V, Sagawa K, Skubitz KM, Taskov H, Todd RF 3rd, Van Agthoven A, Katz DR, Chain BM. Novel molecular mechanisms of dendritic cell-induced T cell activation. Int Immunol. 2000; 12: 1051–61.

20. Zhou J, Zhu P, Jiang J, Jiang Q, Wu ZB, Yao XY, Thang H, Lu N, Yang Y, Chen ZN. Involvement of CD147 in overexpression of MMP-2 and MMP-9 and enhancement of invasive potential of PMA-differentiated THP-1. Cell Biology. 2005; 6: 1–10.

21. Stockinger H, Ebel T, Hansmann C, Koch C, Majdic O, Prager E, Patel DD, Fox DA, Horejsi V, Sagawa K, Shen D-C. CD147 (neurothelin/basigin) Workshop Panel Report. In: Kishimoto T, Kikutani H, von dem Borne AEGK, Goyert SM, Mason DY, Miyasaka A, Morella T, Okumura K, Shaw S, Springer TA, Sugamura K, Zola H, editors. Leucocyte Typing VI, New York: Garland Publishing, Inc; 1997. p. 760–5.

22. Gabison EE, Hoang-Xuan T, Mavriel A, Menashi S. EMMPRIN/CD147, an MMP modulator in cancer, development and tissue repair. Biochimie. 2005; 87: 361–8.

23. Zucker S, Hymowitz M, Rollo EE, Mann R, Conner CE, Cao J, Foda HD, Tompkins DC, Toole BP. Tumorigenic potential of extracellular matrix metalloproteinase inducer. Am J Pathol. 2001; 158: 1921–8.

24. Caudroy S, Polette M, Nawrock-Raby B, Cao J, Toole BP, Zucker S, Birembaut P. EMMPRIN mediates MMP regulation in tumor and endothelial cells. Clin Exp Metastasis. 2002; 19: 697–702.

25. Yurchenko V, Zybarth G, O’Connor M, Dai WW, Franchin G, Hao T, Guo H, Hung HC, Toole B, Gallay P, Sherry B, Bukrinsky M. Active site residues of cyclophilin A are crucial for its signaling activity via CD147. J Biol Chem. 2002; 277: 22959–65.

26. Allain F, Vanpouille C, Carpentier M, Slomianny MC, Durieux S, Spik G. Interaction with glycosaminoglycans is required for cyclophilin B to trigger integrin-mediated adhesion of peripheral blood T lymphocytes to extracellular matrix. Proc Natl Acad Sci USA. 2002; 99: 2714–9.

27. Pushkarsky T, Yurchenko V, Vanpouille C, Brichacek B, Vaisman I, Hatakeyama S, Nakayama KI, Sherry B, Bukrinsky M. Cell surface expression of CD147/emmprin is regulated by cyclophilin 60. J Biol Chem. 2005; 280: 27866–71.

28. Zhou S, Zhou H, Walian PJ. CD147 is a regulatory subunit of the gamma-secretase complex in Alzheimer’s disease amyloid beta-peptide production. Proc Natl Acad Sci USA. 2005; 102: 7499–504.

29. Zhu P, Ding J, Zhou J, Dong WJ, Fan CM, Chen ZN. Expression of CD147 on monocytes/macrophages in
rheumatoid arthritis: its potential role in monocyte accumulation and matrix metalloproteinase production. *Arthritis Res Ther.* 2005; 7: 1023–33.

30. **Zhu P, Lu N, Shi ZG, Zhou J, Wu ZB, Yang Y, Ding J, Chen ZN.** CD147 overexpression on synoviocytes in rheumatoid arthritis enhances matrix metalloproteinase production and invasiveness of synoviocytes. *Arthritis Res Ther.* 2006; 8: 1–12.

31. **Yang JM, O’Neill P, Jin W, Foty R, Medina DJ, Xu Z, Lomas M, Arndt GM, Tang Y, Nakada M, Yan L, Hait WN.** Extracellular matrix metalloproteinase inducer (CD147) confers resistance of breast cancer cells to anoikis through inhibition of Bim. *J Biol Chem.* 2006; 281: 9719–27.

32. **Hochberg MC.** Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997; 40: 1725–35.

33. **Staffler G, Szekeres A, Schutz GJ, Saemann MD, Prager E, Zeyda M, Drbal K, Zlabinger GJ, Stulnig TM, Stockinger H.** Selective inhibition of T cell activation via CD147 through novel modulation of lipid rafts. *J Immunol.* 2003; 171: 1707–14.

34. **Igakura T, Kadomatsu K, Taguchi O, Muramatsu H, Kaname T, Miyauchi T, Yamamura K, Arimura K, Muramatsu T.** Roles of basigin, a member of the immunoglobulin superfamily, in behavior as to an irritating odor, lymphocyte response, and blood-brain barrier. *Biochem Biophys Res Commun.* 1996; 224: 33–6.

35. **Nabeshima K, Suzumiya J, Nagano M, Ohshima K, Toole BP, Tamura K, Iwasaki H, Kikuchi M.** Emmprin, a cell surface inducer of matrix metalloproteinases (MMPs), is expressed in T-cell lymphomas. *J Pathol.* 2004; 202: 341–51.

36. **Konttinen YT, Li TF, Mandelin J, Liljestrom M, Sorsa T, Santavirta S, Virtanen I.** Increased expression of extracellular matrix metalloproteinase inducer in rheumatoid synovium. *Arthritis Rheum.* 2000; 43: 275–80.

37. **Schmidt R, Bultmann A, Ungerer M, Joghetaei N, Bulbul O, Thieme S, Chavakis T, Toole BP, Gawaz M, Schomig A, May AE.** Extracellular matrix metalloproteinase inducer regulates matrix metalloproteinase activity in cardiovascular cells: implications in acute myocardial infarction. *Circulation.* 2006; 113: 834–41.

38. **Abe N, Osanai T, Fujiwara T, Kameda K, Matsunaga T, Okumura K.** C-reactive protein-induced upregulation of extracellular matrix metalloproteinase inducer in macrophages: inhibitory effect of fluvastatin. *Life Sci.* 2006; 78: 1021–8.

39. **Chiampanichayakul S, Peng-in P, Khunkaewla P, Stockinger H, Kasinrerk W.** CD147 contains different bioactive epitopes involving the regulation of cell adhesion and lymphocyte activation. *Immunobiology.* 2006; 211: 167–78.

40. **Jury EC, Kabouridis PS, Flores-Borja F, Mageed RA, Isenberg DA.** Altered lipid raft–associated signaling and ganglioside expression in T lymphocytes from patients with systemic lupus erythematosus. *J Clin Invest.* 2004; 113: 1176–87.