Atorvastatin reduced soluble receptors of TNF-alpha in systemic lupus erythematosus

G.A. Ferreira[1,3], A.L. Teixeira[2], D.C. Calderaro[3], E.I. Sato[4]

[1]Department of Locomotor System and [2]Department of Internal Medicine, Medical School, Universidade Federal de Minas Gerais, [3]Rheumatology Service, Hospital das Clínicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; [4]Rheumatology Division, Universidade Federal de São Paulo/Escola Paulista de Medicina (UNIFESP/EPM), São Paulo, Brazil.

Abstract

Objective

The aim of this study was to evaluate the efficacy of atorvastatin to reduce the plasma levels of TNF system molecules (TNF-α, sTNFR1 and sTNFR2) and to assess their association with risk factors for accelerate atherosclerosis and clinical disease activity scores in SLE patients.

Methods

In a previous study, 64 female SLE patients received 20 mg/day of atorvastatin and 24 SLE patients (non-treated group) were followed for 8 weeks. Plasma levels of TNF-α, sTNFR 1 and sTNFR 2 were measured by ELISA, at baseline and at the end of the study.

Results

The plasma levels of sTNFR1 and sTNFR 2 showed a positive correlation with SLEDAI score. We also found a positive correlation between TNF-α and sTNFR 1 levels and SLICC score. Patients with current nephritis and patients with anti-ds-DNA antibodies presented higher sTNFR1 and sTNFR2 levels. Patients with abdominal obesity and arterial hypertension also had higher plasma levels of soluble receptors. At the end of 8 weeks, we observed a significant decrease in sTNFR1 plasma levels in patients receiving atorvastatin [median (percentile), 876.5 (717–1284 pg/ml) vs. 748 (629.6–917.3 pg/ml), p=0.03], without difference regarding TNF-α and sTNFR2 plasma levels. The SLEDAI and SLICC scores were independent determinants of the plasma levels of sRTNF1.

Conclusion

Atorvastatin reduced soluble receptors of TNF-α. The plasma levels of TNF-α, sTNFR1 and sTNFR2 may play a role in SLE activity and atherosclerosis, and might be evaluated as targets for new therapies.

Key words

systemic lupus erythematosus, atherosclerosis, receptors, tumour necrosis factor, atorvastatin

Gilda Aparecida Ferreira, MD, PhD
Antonio Lúcio Teixeira, MD, PhD
Debora Cerqueira Calderaro, MD
Emília Inoue Sato, MD, PhD

Please address correspondence to:
Dr Gilda Aparecida Ferreira,
Department of Locomotor System,
Medical School,
Universidade Federal de Minas Gerais,
Av. Alfredo Balena, 190,
CEP 30130-100, Belo Horizonte,
Minas Gerais, Brazil.
E-mail: gildaferreira9@gmail.com

Received on January 8, 2015; accepted in revised form on June 23, 2015.

Funding: this study was partly funded by CNPq and Fapemig.

Competing interests: none declared.

Introduction

An increased prevalence of atherosclerosis among systemic lupus erythematosus (SLE) patients has been largely reported (1, 2, 5-8). Its pathogenesis is incompletely understood and probably are multifactorial (1, 4, 7). Traditional risk factors for atherosclerosis (diabetes, hyperlipidaemia, hypertension, obesity and sedentary lifestyle), as well as metabolic syndrome are common among patients with SLE, however, they are not sufficient to explain the accelerated atherosclerosis in these patients (9, 10). Other aspects related to atherosclerosis in these patients include chronic inflammation, antiphospholipid antibodies, use of steroids, high levels of homocysteine and anti-oxidised low-density lipoprotein (LDL) antibodies (1, 7-9). Therefore, recent evidence suggests that the immune system, with immune cell activation, inflammation-driven plaque formation and subsequent rupture, are important in atherosclerosis pathogenesis, what is thought to be associated with accelerated atherosclerosis in SLE patients (1, 5). Traditional pro-inflammatory markers associated with SLE-accelerated cardiovascular disease (SLEACVD) include interferon-γ (IFN-γ), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), interleukin-10 (IL-10) and transforming growth factor β (TGF-β). T-cells and B-cells dysregulation in SLE also plays a role in the development of accelerated atherosclerosis (1, 11).

TNF-α is a cytokine that has been proposed as a major player in inflammatory cell activation and recruitment, and plays a central role in the development of many chronic inflammatory diseases. Depending on its receptors and their multiple signal transduction pathways, TNF-α has both immune-regulatory and pro-inflammatory effects. TNF-α mediates its biological effects through two membrane receptors, TNF-α receptor type I (TNFR1 or p55) and TNF-α receptor type II (TNFR2 or p75). TNFR2 is involved in the anti-apoptotic and inflammatory effects of TNF-α, whereas TNFR1 is involved in both apoptotic/anti-inflammatory and anti-apoptotic/inflammatory signalling (5).

Some authors have described that in many SLE patients serum TNF-α levels are increased and this increase correlates with disease activity, anti-double-stranded DNA (anti-dsDNA) antibodies and active nephritis (12, 13). For instance, Weckerle *et al*. (2011), in a large scale study including 653 SLE patients, described that serum TNF-α is significantly higher in SLE patients than non-autoimmune disease controls, although no association between TNF-α levels and autoantibodies, clinical criteria for the diagnosis of SLE or age at the time of sampling was observed (13). Levels of soluble TNFR1 and TNFR 2 are increased in SLE as well, and their increase correlates with disease activity (3). Therapeutic blocking of TNF-α in SLE has showed no benefits, while in other autoimmune diseases, like rheumatoid arthritis, rarely it has been linked to lupus-like syndromes (4). Thereby, the physiological role of TNF-α and its receptors in the pathogenesis of SLE remains uncertain (13).

TNF-α appears to play an important role in the initiation and perpetuation of atherosclerotic lesions in the general population. It increases adhesion molecules expression on the surface of vascular endothelium cells and promotes enhanced levels of chemotactic proteins, which allows the recruitment of monocytes and T cells into the vessel wall. In SLE, elevated levels of TNF-α have been linked to higher coronary calcium scores, cardiovascular disease, altered lipid profile and increased levels of soluble vascular adhesion molecule (VCAM-1), but its exact role on the development of vascular injury in SLE remains unclear (11). Data on the role of sTNFR1 and sTNFR2 in the pathogenesis of SLEACVD is lacking.

In the last few decades, statins have become one of the most effective strategies in reducing the risk of cardiovascular disease. They inhibit 3-hydroxi-3metil-glutaril-coenzime A (HMG-CoA) reductase, an enzyme that catalyses the conversion of HMG-CoA to mevalonate during cholesterol synthesis (14). Statins also play a role in the modulation of cytokines and cell adhesion molecules, reducing intercellular adhesion molecule-1 (ICAM-1), IL-6,

TNF-α, and IL-1 levels (15). Statins immune-modulatory effects, through the inhibition of transcription of various genes induced by NF-κB, and the inhibition of IFN-γ induced HLA-class II expression in endothelial cells, have also been reported (16). Ferreira *et al*. (2007) described an improvement of endothelium-dependent vasodilation in SLE patients after 8-week 20 mg/day atorvastatin therapy, independent of traditional risk factors for atherosclerosis (17). Ferreira *et al*. (2010) also demonstrated the decrease of CXCL9, an IFN-regulated cytokine associated with recruitment and retention of activated T-cells within vascular wall lesions during atherogenesis and possibly associated to the occurrence of lupus nephritis, which confirms the pleiotropic effects of atorvastatin and suggests a possible role for this medication in SLE treatment, beyond its lipid-lowering effects (18).

The aim of this study was to evaluate the efficacy of atorvastatin to reduce the plasma levels of TNF system molecules (TNF-α, TNFR1 and TNFR2) and to assess their association with risk factors for accelerated atherosclerosis and disease activity scores in SLE patients.

Methods

Patients and study design

In a previous study 88 SLE patients, with regular follow-up at the Rheumatology Outpatient Clinic of the Medical School Hospital of Federal University of Minas Gerais, Belo Horizonte, Brazil, were evaluated. The first 64 patients were allocated in the intervention group and received atorvastatin 20 mg/day. Other consecutive 24 SLE patients without specific intervention constituted the non-treated group. The patients were not randomised. Both groups were followed for 8 weeks (17, 18).

Inclusion criteria were: female sex, SLE according to the ACR revised classification criteria (19), disease diagnosis ≥1 year and age >18 years. The exclusion criteria were current or past use of lipid-lowering drugs in the last 6 months, serum creatinine >1.2 mg/dl, pregnancy, myopathy or elevated creatinine phosphokinase, liver disease, cyclosporine use and acute or chronic infectious conditions at the time of study visit.

SLE disease activity and damage were measured using SLEDAI (20) and SLICC/ACR score (21). Renal disease was defined as proteinuria >0.5 g/24 h or the presence of cell casts, or renal biopsy compatible with lupus nephritis. In the intervention group, 33 patients presented arterial hypertension, dyslipidemia and/or obesity and the remaining 31 had no traditional coronary heart disease (CHD) risk factors.

In order to reinforce and to check adherence to the protocol, phone calls or personal contacts were performed 30 days after the beginning of the study, and atorvastatin tablets were counted at the end of the study.

At baseline and after 8-week, all participants underwent complete clinical examination and blood sampling after 12-hours fasting for laboratory analysis. All plasma samples were kept at -80°C until laboratory tests. Medical records were reviewed in order to obtain information regarding all disease manifestations and medications used at the time of study. All participants signed the informed consent form approved by the institutional Ethics Committee.

Laboratory tests

The following laboratory tests were performed according to standard routine techniques: complete blood count, creatinine, fasting glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), very low density cholesterol (VLDL-c), triglycerides, creatine phosphokinase, complement (C3, C4, CH50) serum levels, liver function tests and urinalysis. Anti-dsDNA antibodies were detected by indirect immunofluorescence using *Crithidia luciliae* as substrate. Anticardiolipin antibodies were determined by in-house enzyme immunoassay and were considered positive when higher than 20 GPL units or 11 MPL units. Homocysteine serum levels were measured by liquid chromatography (reference range <15 mmlo/L).

The concentration of TNF-α (Quantikine, R&D Systems, Minneapolis, MN) and sTNFR1 and sTNFR2 (Duoset, R&D Systems, Minneapolis, MN) were measured with commercially available ELISA kits. All samples were assayed in duplicate, and intra- and inter-assay coefficients of variation were below 5 and 10%, respectively. The detection limits for TNF-α and sTNFRs assays were 0.018 and 5.0 pg/ml, respectively.

Statistical analysis

Statistical packages Minitab 13.2 and SPSS for windows 12.0 were used. Descriptive analyses were performed and data are presented as mean, median, standard-deviation (SD) and percentile. The normal distribution of the variables was tested by Kolmogorov-Smirnov test. Paired and independent Student *t*-test or Mann-Whitney tests were applied according to the normal or non-normal distribution. Spearman and Pearson's correlation analyses were used to investigate a potential association among variables. Multiple linear regression models were designed to determine the most significant associations. Significance level was set as $p<0.05$.

Results

Patients were classified as white (64.8%) and non-white (35.2%), with a mean (SD) age of 32 (8) years and a mean (SD) disease duration of 8.9 (4.8) years. SLEDAI and SLICC scores ranged from 0 to 18 (mean 4.16) and from 0 to 4 (mean 1.06), respectively. The clinical and laboratory abnormalities present at the time of the study were: serositis (7.9%), cutaneous vasculitis (6.8%), arthritis (9.2%), nephritis (18%), positive anti-dsDNA antibodies (16%) and positive anticardiolipin antibodies (11%), without significant difference between groups (data not showed). No patient had confirmed coronary or cerebrovascular event. Seventy-two patients (88%) were on regular use of prednisone, 54 (61%) on chloroquine diphosphate and 46 (52%) were receiving additional immunosuppressive agents (azathioprine (22.7%); cyclophosphamide (21.8%); and methotrexate (7.9%)). Eight patients were also on regular use of low-dose aspirin. The most prevalent CHD risk factors were: arterial hypertension (30%), dyslipidaemia (36%), obesity (16%) and abdominal obesity (32%). Ten patients

Table I. Demographic characteristics and baseline coronary heart disease risk factors in SLE patients divided in 64 treated with atorvastatin (intervention group) and in 24 patients not receiving atorvastatin (non-treated group).

	Intervention group (n=64)	Non-treated group (n=24)	*p*-value
Age (years)	32 ± 8	34 ± 7.5	NS
Disease duration (months)	8.5 ± 4.9	8.5 ± 4.7	NS
Height (cm)	158 ± 5.8	159 ± 4.9	NS
BMI (Kg/m^2)[#]	24.7 ± 4.2	26.9 ± 5.2	NS
Systolic blood pressure (mmHg)[$]	117 (110-127)	120 (110-130)	NS
Diastolic blood pressure (mmHg)[$]	74 (70-80)	80 (78-83)	**0.01**
Total cholesterol (mg/dL)	162.5 ± 36	162 ± 23	NS
LDL-cholesterol (mg/dL)	92 ± 30	95 ± 18	NS
HDL-cholesterol (mg/dL)	47 ± 12	41 ± 8	**0.049**
Triglycerides (mg/dL)	115 ± 55	136 ± 119	NS
SLEDAI score[*]	4.4 ± 5	3.3 ± 3.9	NS
SLICC score[**]	1.1 ± 1.2	0.79 ± 0.77	NS

[$]Median values (range), [#]BMI: body mass index. [*]SLEDAI: Systemic Lupus Erythematosus Disease Activity Index. [**]SLICC:The Systemic Lupus International Collaborating Clinics/American College of Rheumatology. Damage Index for Systemic Lupus Erythematosus

Table II. Correlations of variables with plasma levels of TNF system molecules (TNF-α, sTNFR 1, sTNFR 2) in 88 SLE patients at baseline.

Variable	TNF-α		sTNFR 1		sTNFR 2	
	r	*p*	r	*p*	r	*p*
Triglycerides (mg/dl)	0.267	**0.016**	0.398	**<0.001**	0.320	**0.003**
Total cholesterol (mg/dl)	0.031	0.781	0.254	**0.023**	0.444	**<0.001**
LDL- cholesterol (mg/dl)	0.032	0.776	0.161	0.156	0.468	**<0.001**
HDL- cholesterol (mg/dl)	-0.114	0.453	-0.24	0.061	-0.257	**0.018**
Homocysteine (µmol/l)	0.320	**0.005**	0.280	0.014	0.237	**0.033**
BMI(kg/m^2)[#]	0.149	0.185	0.191	0.089	0.291	**0.007**
SLEDAI (score)[*]	0.103	0.362	0.362	**0.003**	0.280	**0.009**
SLICC (score)[**]	0.340	**0.002**	0.383	**<0.001**	0.21	0.058
Current prednisone dosage (mg/day)	0.201	0.066	0.30	**0.007**	0.246	**0.024**
Cumulative prednisone dosage (mg)	0.010	0.947	0.380	**0.008**	0.185	0.193

[#]BMI: body mass index. [*]SLEDAI: Systemic Lupus Erythematosus Disease Activity Index. [**]SLICC:The Systemic Lupus International Collaborating Clinics/American College of Rheumatology. Damage Index for Systemic Lupus Erythematosus.

(12%) had homocysteine above the normal levels. Regarding demographic and CHD risk factors, we did not find a significant difference in most of the variables between groups (Table I), except HDL-c levels, that were lower, and mean diastolic blood pressure, that was higher, in the non-treated group in comparison with the intervention group.

The Table II shows the correlations of some variables with plasma levels of TNF-α, sTNFR1 and sTNFR2 at baseline in a univariate analysis. The plasma levels of sTNFR1(r=0.362; *p*=0.003) and sTNFR2 (r=0.280; *p*=0.009) showed a weak correlation with SLEDAI score. We also found a correlation between TNF-α (r=0.340; *p*=0.002) and sTNFR1 (r=0.383; *p*<0.001) levels and the SLICC score. The plasma levels of sTNFR1 [median (interquartile range) values, 1163.68 (884.32-1499.06) *vs*. 811.12 (629.65–1064.68), *p*=0.003] and sTNFR2 [3537.8±894.16 *vs*. 3037±733.32, *p*=0.029) were higher in patients with positive than negative anti-dsDNA antibodies.

The associations between plasma levels of the TNF-α and its soluble receptors (sTNFR1 and sTNFR 2) and clinical abnormalities present at baseline in 88 SLE patients are summarized in Table III. Patients with current nephritis presented higher plasma levels of sTNFR1 and sTNFR2, while patients with active serositis presented higher level of TNF-α and sTNFR2 and patients with active arthritis presented higher level of sTNFR1.

The associations between traditional CHD risk factors and plasma levels of TNF-α, sTNFR 1 and sTNFR2 are disclosed in Table IV. Dyslipidaemia, abdominal obesity and hypertension were associated with higher levels of sTNFR1 and sTNFR2.

At the end of 8 weeks, there was a significant decrease in the plasma levels of sTNFR1 [876.5 (717–1284) *vs*. 748 (629.6–913), *p*=0.032] in the intervention group. In contrast, atorvastatin did not change plasma levels of TNF-α (*p*=0.934) and sTNFR2 (*p*=0.746). No difference in plasma levels of TNF, sTNFR1 and sTNFR2 was observed between baseline and at the end of the study in non-treated group (Table V).

In a binary linear regression model, the score of SLEDAI and SLICC were independent determinants of the plasma levels of sTNFR1 (Table VI). In another binary linear regression analysis, the plasma levels of HDL, LDL and SLEDAI score were independently associated with high levels of sTNFR2 (Table VII).

Discussion

The significance of the TNF-α involvement in the pathogenesis of SLE remains controversial and there are few studies assessing the role of its soluble receptors (sTNFR1 and sTNFR2). From the genetic standpoint, a number of studies suggest that the TNF-α gene polymorphisms are involved in the susceptibility to SLE (22-24). There is a close association between TNF-α gene expression and clinical manifestations. In addition, the increased plasma levels of TNF-α are observed in SLE patients and associated with disease activity and certain systemic manifestations (25). In line with the study of Sabry *et al*. we found significant association between plasma levels of TNF-α and its soluble receptors (sTNFR 1 and sTNFR2) and clinical manifestations, particularly with nephritis and serositis (12).

Table III. Plasma levels of TNF system in 88 SLE patients with and without clinical abnormalities at baseline.

	Active Arthritis			Active Nephritis			Active Serositis		
	Present	Absent	*p*	Present	Absent	*p*	Present	Absent	*p*
TNF-α*	1.28 (0.57-3.8)	0.93 (0.47-3.09)	0.607	3.49 (0.82-7.83)	0.93 (0.52-3.07)	0.131	3.94 (3.90-12.45)	0.94 (0.52-3.13)	**0.037**
sTNFR1*	889.32 (711.49-1249.46)	728.78 (553.73-898.85)	**0.043**	1397.58 (832.83-1810.44)	783.91 (626.21-1052.20)	**0.001**	1810.44 (1163.79-1913.98)	828.89 (648.39-1103.50)	0.327
sTNFR2#	3147.97 (±740.43)	3039.14 (±901.21)	0.555	3602.01 (±1089.58)	3024.73 (±658.58)	**0.008**	4286.23 (±810.93)	3084.18 (±746.74)	**0.008**

*Data expressed in median (interquartile range). #Data expressed in mean±standard deviation. Mann-Whitney Test.

Table IV. Plasma levels of TNF system molecules in 88 SLE patients with and without traditional coronary heart disease risk factors at baseline.

	Dyslipidemia			Abdominal Obesity			Arterial Hypertension		
	Present	Absent	*p*	Present	Absent	*p*	Present	Absent	*p*
TNF-α*	1.70 (0.42-4.02)	0.87 (0.51-3.01)	0.351	1.63 (0.27-3.94)	0.91 (0.54-3.38)	0.985	1.80 (0.52-3.67)	0.91 (0.48-3.88)	0.685
sTNFR1*	986.98 (661.59-1427.88)	782.50 (619.30-920.36)	**0.027**	929.79 (816.56-1397.58)	756.42 (626.21-1039.95)	**0.013**	928.67 (767.07-1500.75)	810.22 (633.09-1044.17)	**0.014**
sTNFR 2#	3451.09 (±816.97)	2954.00 (±691.20)	**0.005**	3450.80 (±791.62)	2964.43 (±649.40)	**0.008**	3578.85 (±772.47)	2926.65 (±702.59)	**<0.001**

*Data expressed in median (interquartile range). #Data expressed in mean±standard deviation. Mann-Whitney Test.

SLE is an immune-mediated disease with a large amounts of immune complexes deposited in tissues, especially in the glomerulus. Immune complexes induce macrophages to produce high levels of TNF-α (26). TNF-α was found to be increased in glomerulus in all forms of lupus nephritis, and the level of TNF-α expression was correlated with renal inflammatory activity (27). Svenungsson *et al*. showed that high levels of TNF-α and soluble sTNFR were all consistently associated with previous arterial disease and renal disease in SLE (28). These findings may justify the higher plasma levels of sTNFR1 and sTNFR2 in the patients with current nephritis in the present study.

The knowledge of the cytokine profiles in SLE not only provides new insight into the disease pathogenesis but also presents potential clinical applications. Some cytokines, such as interleukin 6 (IL-6), interleukin 10 (IL-10), interferon alpha (INF-α), and TNF-α can serve as biomarkers to monitor disease activity and to predict disease severity (29, 12). In the current study and that of Gabay *et al*. (30), plasma levels of sTNFR1 and sTNFR 2 showed a positive correlation with SLEDAI score and were higher in patients with positive anti-dsDNA antibody. These results suggest perhaps the analysis of sTNFR is better than searching the cytokine TNF-α for monitoring disease activity in LES patients which goes in line with previous report in other inflammatory and/or infectious diseases (31, 32).

In addition, the manipulation of these cytokines may represent a potential therapeutic strategy for the treatment of SLE (33). However, the therapeutic blocking of TNF-α has showed no benefits in SLE patients and, in other autoimmune diseases, it has been linked to lupus-like syndromes. Studies indicated that increased availability of apoptotic antigens after anti-TNF-α treatment might play a role in the autoantibody formation and, as a consequence, the manifestation of the lupus-like syndrome (34). Exposure of apoptotic material to the immune system has been suggested as a possible trigger for the production of anti-dsDNA and other autoantibodies in SLE patients.

Table V. TNF-α, sTNFR-1 and sTNFR-2 plasma levels (pg/mL) at baseline and after 8 weeks of study.

	Intervention group n=64			Non-treated group n=24		
	Baseline	8 weeks	*p*	Baseline	8 weeks	*p*
TNF-α*	1.3 (0.639-3.997)	1.8995 (0.6275-4.604)	0.934	0.608 (0.282-2.21)	0.621 (0.27-2.249)	0.523
TNFR 1*	876.5 (717-1284)	748 (629.6-917.3)	0.032	742.566 (526.292-983.853)	732.554 (623.106-1091.904)	0.089
TNFR 2#	3120.26±818.15	3090.16±756.28	0.746	3190.76±689.64	3303.88±591.7	0.186

*Data expressed in median (interquartile range). #Data expressed in mean±standard deviation.

Table VI. Multiple linear regression analysis of 88 SLE patients considering the plasma levels of sTNFR1 as the dependent variable.

Independent Variable	Unstandardised Coefficient B	#CI 95% Coefficient B		Standard Error	t	*p*
Constant	6.504	6.382	6.625	0.061	106.205	0.000
SLEDAI(score)*	0.035	0.019	0.051	0.008	4.385	0.000
SLICC (score)**	0.121	0.055	0.188	0.033	3.626	0.000

R^2 = 0.31. #Confidence Interval. *SLEDAI: Systemic Lupus Erythematosus Disease Activity Index. **SLICC:The Systemic Lupus International Collaborating Clinics/American College of Rheumatology. Damage Index for Systemic Lupus Erythematosus.

Table VII. Multiple linear regression analysis of 88 SLE patients considering the plasma levels of sTNFR2 as the dependent variable.

Independent Variable	Unstandardised Coefficient B	#CI 95% Coefficient B		Standard Error	t	*p*
Constant	2704.384	1953.404	3455.363	377.365	7.166	0.000
HDL (mg/dl)	-17.165	-29.130	-5.200	6.012	-2.855	**0.005**
LDL (mg/dl)	10.938	5.866	16.011	2.549	4.291	**0.000**
SLEDAI(score)*	49.705	19.754	79.657	15.051	3.303	**0.001**

R^2 = 0.36. #Confidence Interval. *SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

In previous study, markers of apoptotic activity correlated with TNF-α activity, suggesting that TNF-α can function as an inductor of apoptosis (35). In this and Svenungsson *et al*. study, the levels of sTNFRs associated with higher levels of antibodies to dsDNA, suggesting that enhanced TNF-α--induced apoptosis could be one possible mechanism behind this association (28).

TNF-α is a pro-inflammatory cytokine whose expression and circulating levels are increased with obesity and decreased with weight loss. Lower levels of adiposity are associated with preponderance of anti-inflammatory cytokines and lower expression of pro-inflammatory cytokines (36). In this study the plasma levels of sTNFR1 and sTNFR2 were higher in patients with abdominal obesity and the BMI mean correlated with plasma levels of sRTNF2.

Inflammatory cytokines such as Il-6 and TNF-α stimulate lipolysis and increase free fatty acids (37). Elevated free fatty acids results in increased concentrations of soluble endothelial activation markers including E-selectin, VCAM-1 and ICAM-1. Furthermore, free fatty acids can decrease endothelial nitric oxide production and increase reactive oxygen species, predisposing to endothelial dysfunction, a potential mechanism underlying atherosclerosis and coronary artery disease (38). As reported here, atorvastatin effect in reducing sTNFR1 levels can contribute to the control of endothelial dysfunction and accelerated atherosclerosis in SLE patients.

Preliminary studies showed that many SLE patients present dyslipoproteinemia, characterised by high levels of triglycerides and low level of HDL, which is associated with disease activity (39,40).

Among its multiple effects, TNF-α has the capacity to induce dyslipoproteinemia, insulin resistance, and endothelial cell activation and it is present in atherosclerotic plaques (41). In a nested case-control study, the authors noticed an association between elevated triglycerides and enhanced TNF-α activity among patients with SLE and previous cardiovascular disease (42). In the Svenungsson *et al*. study, the serum levels of TNF-α, sTNFR1, and sTNFR2 correlated with increased level of TGs. The levels of TNF and sTNFR2 showed a significant correlation with low HDL levels, thus demonstrating an association with the "lupus pattern of dyslipoproteinemia (28)." We confirmed this previous report demonstrating a positive correlation between plasma levels of TNF-α and its sTNFRs and triglycerides. Moreover, we found a correlation between the total cholesterol level and the sTNFRs.

HDL-associated apolipoprotein A-I has been shown to decrease TNF-α production through its inhibition of contact-mediated activation of monocytes by binding to stimulated T cells (25). Thus, low HDL levels seem to be a consequence of active SLE, but may also indirectly contribute to enhanced inflammation in SLE. We also showed a negative association between plasma levels of HDL-cholesterol and sTNFR2. These findings suggest a possible role for these cytokines in traditional CHD factors and atherosclerotic disease in general population and SLE patients.

Accumulating evidence has suggested that statins exert immune-modulatory and anti-inflammatory functions independent of their lipid-lowering effects. By the virtue of pleiotropic immune-modulatory properties, statins might be applied for the treatment of both autoimmunity and atherosclerosis (43). Several studies evaluated the effects of statins on the disease activity and atherosclerosis in SLE patients. For instance, Lawman *et al*. demonstrated that the administration of atorvastatin to lupus-prone NZB/WF1 mice determined a significant reduction in anti-dsDNA antibodies and proteinuria (44). Additionally our group demonstrated, in the same sample of this study, a surprisingly reduction of SLE activity measured by SLEDAI, improving of endothelial-dependent vasodilatation and decreasing in plasma levels of CXCL9 (an IFN-γ regulated chemokine) after an 8-week controlled trial of atorvastatin (17, 18).

It has been recently demonstrated that atorvastatin directly activates Erk5, which results in a potent blockade of the TNF-α induced VCAM-1 and ICAM-1 expression through the inhibition of Rac-1 activity, ROS formation and NF-kB function in endothelial cells, turning down inflammation (45).

Therefore atorvastatin effect in reducing inflammation, herein evidenced by sTNFR1 plasma levels decrease, may have a role on control of disease activity and to reduce atherosclerosis in LES patients.

References

1. WADE SN, MAJOR AS: The problem of accelerated atherosclerosis in systemic lupus erythematosus: Insights into a complex comorbidity. *Thromb Haemost* 2011; 106: 849-57.
2. SCHUR PH, GLADMAN DD: Overview of the clinical manifestations of systemic lupus erythematosus in adults. *In* UpToDate online. Accessed on 03/05/12.
3. ZHU LJ, LANDOLT-MARTICORENA C, LI T *et al*.: Altered expression of TNF-α signaling pathway proteins in systemic lupus erythematosus. *J Rheumatol* 2010; 37: 1658-66.
4. ZHU LJ, YANG X, YU XQ: Anti-TNF-α in systemic lupus erythematosus. *J Biomed Biotechnol* 2010; 2010: 465898.
5. PARAMESWARAN N, PATIAL S: Tumor necrosis factor-α signaling in macrophages. *Crit Ver Eukaryot Gene Expr* 2010; 20: 87-103.
6. COSTENBADER KH, SHUR PH: Coronary heart disease in systemic lupus erythematosus. In UpTo date online. Accessed on 03/04/12.
7. MANZI S, MEILAHN EM, RAIRIE JE *et al*.: Age-specific incidence rates of myocardial infarction and angina in women with sustemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol* 1997; 145: 408-15.
8. HAK AE, KARLSON EW, FESKANICH D *et al*.: Systemic lupus erythematosus and the risk of cardiovascular disease: results form the nurses'health study. *Artrhitis Rheum* 2009; 61: 1396.
9. ESDAILE JM, ABRAHAMOWICZ M, GRODZICKY T *et al*.: Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum* 2001; 44: 2331-37.
10. CHIN CW, TAN RS, THUMBOO J: Endothelial function and arterial stiffness assessment as early surrogate markers of vascular risk in patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 2013; 31: 295-301.
11. KAHLENBERG JM, KAPLAN MJ: The interplay of inflammation and cardiovascular disease in systemic lupus erythematosus. *Arthritis Res Ther* 2011; 13: 203-10.
12. SABRY A, SHEASHAA H, EL-HUSSEINI A *et al*.: Proinflammatory cytokines (TNF-α and IL-6) in Egyptian patients with SLE: its correlation with disease activity. *Cytokine* 2006; 35: 148-53.
13. WECKERLE CE, MANGALE D, FRANEK BS *et al*.: Large-scale analysis of tumor necrosis fator-α levels in Systemic Lupus Erythematosus. *Arthritis Rheum* 2011; 64: 2947-52.
14. MASON JC: Statins and their role in vascular protection. *Clin Sci* 2003; 105: 251-66.
15. ASCER E, BERTOLAMI MC, VENTURNELLI ML *et al*.: Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. *Atherosclerosis* 2004; 177: 161-6.
16. MERONI PL, LUZZANA, VENTURA D: Anti-inflammatory and immunomodulating properties of statins. An additional tool for the therapeutic approach of systemic autoimmune disease? *Clin Rev Allerg Immunol* 2002; 23: 263-77.
17. FERREIRA GA, NAVARRO TP, TELLES RW *et al*.: Atorvastatin therapy improves endothelial-dependent vasodilation in patients with systemic lupus erythematosus: a 8 weeks controlled Trial. *Rheumatology* (Oxford) 2007; 46: 1560-65.
18. FERREIRA GA, TEIXEIRA AL, SATO EI: Atorvastatin therapy reduces interferon-regulated chemokine CXCL9 plasma levels in patients with systemic lupus erythematosus. *Lupus* 2010; 19: 927-34.
19. HOCHBERG MG: Update the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
20. BOMBARDIER C, GLADMAN DD, UROWITZ MB, CARON D, CHANG CH: Derivation of the SLEDAI: a disease activity index for lupus patients: The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630-40.
21. GLADMAN DD, UROWITZ MB, GOLDSMITH CH *et al*.: The reliability of the Systemic Lupus International Collaborating Clinics/ American College of Rheumatology Damage Index in patients with systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 809-13.
22. SCHOTTE H, WILLEKE P, TIDOW N *et al*.: Extended haplotype analysis reveals an association of TNF polymorphisms with susceptibility to systemic lupus erythematosus beyond HLA-DR3. *Scand J Rheumatol* 2005; 34: 114-21.
23. JIMÉNEZ-MORALES S, VELÁZQUEZ-CRUZ R, RAMÍREZ-BELLO J *et al*.: Tumor necrosis factor-alpha is a common genetic risk factor for asthma, juvenile rheumatoid arthritis, and systemic lupus erythematosus in a Mexican pediatric population. *Hum Immunol* 2009; 70: 251-6.
24. MCHUGH NJ, OWEN P, COX B *et al*.: MHC class II, tumor necrosis factor alpha, and lymphotoxin alpha gene haplotype associations with serological subsets of systemic lupus erythematosus. *Ann Rheum Dis* 2006; 65: 488-94.
25. POSTAL M, APPENZELLER S: The role of tumor necrosis factor-alpha (TNF-α) in the pathogenesis of systemic lupus erythematosus. *Cytokine* 2011; 56: 537-43.
26. ARINGER M, SMOLEN JS: Complex cytokine effects in a complex autoimmune disease: tumor necrosis factor in systemic lupus erythematosus. *Arthritis Res Ther* 2003; 5: 172-7.
27. ARINGER M, ZIMMERMANN C, GRANINGER WB *et al*.: TNF-α is an essential mediator in lupus nephritis. *Arthritis Rheum* 2002; 46: 3418–9.
28. SVENUNGSSON E, GUNNARSSON I, FEI G *et al*.: Elevated triglycerides and low levels of high-density lipoprotein as markers of disease activity in association with up-regulation of the tumor necrosis factor /tumor necrosis factor receptor system in systemic lupus erythematosus. *Arthritis Rheum* 2003; 48: 2533-40.
29. YAP DY, LAI KN: Cytokines and their roles in the pathogenesis of systemic lupus erythematosus: from basics to recent advances. *J Biomed Biotechnol* 2010; 2010: 365083.
30. GABAY C, CAKIR N, MORAL F *et al*.: Circulating levels of tumor necrosis factor soluble receptors in systemic lupus erythematosus are significantly higher than in other rheumatic diseases and correlate with disease activity. *J Rheumatol* 1997; 24: 303-8.
31. ALESSANDRI AL, SOUZA AL, OLIVEIRA SC *et al*.: Concentrations of CXCL8, CXCL9 and sTNFR1 in plasma of patients with pulmonary tuberculosis undergoing treatment. *Inflamm Res* 2006; 55: 528-33.
32. MOURA AS, CARMO RA, TEIXEIRA AL *et al*.: Soluble inflammatory markers as predictors of liver histological changes in patients with chronic hepatitis C virus infection. *Eur J Clin Microbiol Infect Dis* 2010; 29: 1153-61.
33. ARINGER M, SMOLEN JS: Tumor necrosis factor and other proinflammatory cytokines in systemic lupus erythematosus: a rationale for therapeutic intervention. *Lupus* 2004; 13: 344-7.
34. ARINGER M, SMOLEN JS: Efficacy and safety of TNF-blocker therapy in systemic lupus erythematosus. *Expert Opin Drug Saf* 2008; 7: 411-9.
35. ARINGER M, FEIERL E, STEINER G *et al*.: Increased bioactive TNF in human systemic lupus erythematosus: associations with cell death. *Lupus* 2002; 11: 102-8.
36. HOTAMISLIGIL GS, SHARGILL NS, SPIEGELMAN BM *et al*.: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; 259: 87-91.
37. GREEN A, DOBIAS SB, WALTERS DJ *et al*.: Tumor necrosis factor increases the rate of lipolysis in primary cultures of adipocytes without altering levels of hormone-sensitive lipase. *Endocrinology* 1994; 134: 2581-88.
38. ORMSETH MJ, SWIFT LL, FAZIO S *et al*.: Free fatty acids are associated with metabolic syndrome and insulin resistance, but not inflammation in SLE patients. *Lupus* 2013; 22: 26-33.
39. ILOWITE NT, SAMUEL P, GINZLER E, JACOBSON MS: Dyslipoproteinemia in pediatric systemic lupus erythematosus. *Arthritis Rheum* 1988; 31: 859-63.
40. BORBA EF, BONFA E: Dyslipoproteinemias in systemic lupus erythematosus: influence of disease, activity, and anticardiolipin antibodies. *Lupus* 1997; 6: 533-9.
41. FROSTEGARD J, ULFGREN AK, NYBERG P *et al*.: Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. *Atherosclerosis* 1999; 145: 33-43.
42. SVENUNGSSON E, FEI G-Z, JENSEN-URSTAD K *et al*.: TNF-alpha: a link between hypertriglyceridemia and inflammation in SLE patients with cardiovascular disease. *Lupus* 2003; 12: 451-61.
43. TU H, LI Q, XIANG S *et al*.: Dual effects of statins therapy in systemic lupus erythematosus and SLE-related atherosclerosis: the potential role for regulatory T cells. *Atherosclerosis* 2012; 222: 29-33.
44. LAWMAN S, MAURI C, JURY EC *et al*.: Atorvastatin inhibits autoreactive B cell activation and delays lupus development in New Zealand black/white F1 mice. *J Immunol* 2004; 173: 7641-6.
45. WU K,TIAN S,ZHOU H,WU Y: Statins protect human endothelial cells from TNF-induced via ERK5 activation. *Biochem Pharmacol* 2013; 85: 1753-60.