Serum Levels of Soluble CD40 Ligand at Flare and at Remission in Patients with Systemic Lupus Erythematosus

MARIA URQUIZU-PADILLA, EVA BALADA, FINA CORTÉS, EDUARDO HERMOSILLA PÉREZ, MIQUEL VILARDELL-TARRÉS, and JOSEP ORDI-ROS

ABSTRACT. ***Objective.*** To perform a prospective evaluation of soluble CD40 ligand (sCD40L) levels according to the activity of systemic lupus erythematosus (SLE).

Methods. Two serum samples were taken from 53 patients with SLE: at flare and at remission. Clinical and biological measures (sCD40L levels were measured by a commercial ELISA) were evaluated in both situations.

Results. Patients with SLE had significantly lower median levels of sCD40L during flare than during remission [3365 (6157) vs 7125 (4122) pg/ml; $p < 0.001$]. The multivariate analysis to explain those patients with lower values of sCD40L during flare than during remission included 3 variables: 2 related to flare (prednisone dose received ≤ 15 mg/day and platelet counts > 192,000 × 10^6/l) and one related to lower changes in SLE Disease Activity Index (SLEDAI) score. We regrouped patients with the 2 characteristics related to flare as Group 4, and the others were Group 123. All patients with low SLEDAI scores at flare had statistically significant lower sCD40L levels during flare than during remission. When flare SLEDAI scores were higher than the 50th percentile, patients of Group 123 showed the same behavior and even more diminished levels of sCD40L during flare than patients of Group 123 with low SLEDAI scores ($p = 0.023$); and patients of Group 4 showed no differences in the values of sCD40L between flare and remission ($p = 0.241$).

Conclusion. sCD40L plays a biologically active role, with decreased levels at flare at low SLEDAI scores. At high SLEDAI scores there are mechanisms that involve platelets and that are inhibited by high doses of prednisone that lead to increased serum values of sCD40L at flare. (First Release April 1 2009; J Rheumatol 2009;36:953–60; doi:10.3899/jrheum.080978)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
TUMOR NECROSIS FACTOR
DISEASE ACTIVITY
SCD40L

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology. Serum of patients with SLE contains a variety of autoantibodies, which form immune complexes that precipitate in the tissues and cause various tissue disorders. The clinical presentations of the disease vary, and the course and prognosis are characterized by remissions and flares.

From the Systemic Autoimmune Diseases Research Laboratory, Vall d'Hebron Research Institute, Hospital Vall d'Hebron, Barcelona, Spain.

Dr. Urquizu-Padilla was supported by funds provided by the Spanish Ministerio de Sanidad y Consumo via the Instituto de Salud Carlos III (CM04/00034).

M. Urquizu-Padilla, MD, PhD; E. Balada, PhD; F. Cortés, MD, PhD; E. Hermosilla Pérez, Statistical Department; M. Vilardell-Tarrés, MD, PhD, Professor; J. Ordi-Ros, MD, PhD, Systemic Autoimmune Diseases Research Laboratory, Vall d'Hebron Research Institute, Hospital Vall d'Hebron.

Address reprint requests to Dr. M. Urquizu-Padilla, Research Unit in Systemic Autoimmune Diseases, Vall d'Hebron Research Institute, Hospital Vall d'Hebron, Passeig Vall d'Hebron 119-129, 08035 Barcelona, Spain. E-mail: mariaurquizu@gmail.com

Accepted for publication December 2, 2008.

The binding of the CD40 ligand (CD40L) molecule on the T-helper cell surface to the B cell surface CD40 is a molecular event that mediates direct T cell help for B cell activation and differentiation, rescuing B cells from apoptosis, and leading to antibody production[1].

CD40L, also known as TRAP, gp39, and CD154, is a member of the tumor necrosis factor (TNF) family. It is a transmembrane protein distributed in leukocyte cells (T and B lymphocytes, basophils/mast cells, eosinophils, monocytes/macrophages, and natural killer cells), and in non-leukocyte cells (platelets, epithelial cells, endothelial and smooth muscle cells). Like CD40 (its receptor), CD40L is primarily distributed on cells of the vasculature; nevertheless, in contrast to CD40, quiescent cultured cells and non-diseased tissue do not usually express the ligand constitutively. After the interaction between CD40 and CD40L takes place, a signal transduction begins that results in a broad variety of immune and inflammatory responses[1]. CD40L has also been described in a soluble form, which is functional as well, since it also has the CD40 ligation domain[2].

The autoimmune disease best characterized in terms of

CD40L dysregulation is SLE. Under normal circumstances the immune system allows only transient expression of CD40L. However, patients with SLE express abnormally high levels of CD40L on both T and B cells and the overall number of CD40L-positive cells is increased[3-5]. Importantly, the increased expression of CD40L on SLE T cells was shown to induce higher levels of CD80 on co-cultured B lymphocytes[5] and to produce a pathogenic variety of antinuclear antibodies *in vitro*[3]. It does seem probable that the overexpression of CD40L on T cells of patients with SLE contributes to the pathogenesis of the disease.

Programmed cell death has been suggested to be involved in the clonal elimination of self-reactive lymphocytes for the normal function of the immune system. Interaction with membrane-bound self-antigens may eliminate self-reactive B cells by apoptosis. Antigen-receptor-mediated B cell apoptosis is blocked when a signal is transduced via the CD40 molecule on the B cell surface[6]. Although blockage of CD40-CD40L interactions decreases B cell activity in murine models[7], human trials in SLE have been unsuccessful to date, either as a result of lack of efficacy[8] or increased adverse events including thrombotic events[9].

Soluble CD40L (sCD40L) has been reported to be elevated in the serum of patients with SLE compared to controls[10-13]. The elevated sCD40L levels were found to be functional in that they were shown to increase expression of accessory molecules on B cells[11,12]. The levels of sCD40L correlated with anti-double-stranded DNA (anti-dsDNA) antibody titers[12]. When patients were classified with regard to the SLE Disease Activity Index (SLEDAI) score (higher than 9 vs 2–9), higher levels of sCD40L were found in those patients whose disease was more active. In terms of activity, other authors have associated the highest levels of sCD40L with the highest European Consensus Lupus Activity Measurement (ECLAM) score[10]. Vakkalanka, *et al*[11] found levels of sCD40L significantly higher in patients with severe disease (central nervous system, nephritis, or proteinuria > 1 g/day), compared with moderate (proteinuria < 1 g/day or serositis) or mild disease. Nevertheless, it should be pointed out that, to date, no study has performed a followup of the values of sCD40L in the same patients with SLE during flare and during remission. We performed such a study to determine the variations of sCD40L serum levels between flare and remission in a cohort of 53 patients with SLE, and we also established the relationship with other measures linked to SLE.

MATERIALS AND METHODS

Patients. Fifty-three consecutive patients with SLE who had a flare of the disease (46 women, 7 men) were included in our study. All patients fulfilled at least 4 of the American College of Rheumatology revised criteria for the classification of SLE[14]. SLE activity was assessed by the SLEDAI[15] score. A flare of the disease was defined as any clinical event directly attributable to disease activity leading to a SLEDAI score ≥ 6 that would require an escalation of treatment[16]. All patients were followed up periodically for a median period of 219 days [interquartile range (IQR) 655] until they achieved remission. The minimum period was 29 days and the maximum was 1,877 days. Clinical remission was considered when the SLEDAI score was ≤ 6 and, simultaneously, when this value meant a 50% decrease from the flare score. At the time of the study, demographic and SLE-related data were collected including disease status, laboratory findings, clinical symptoms at flare and during remission, and treatment received at the time of the sample collection and to treat the flare. Subjects' written consent was obtained according to the Declaration of Helsinki, and the study design conformed to standards currently applied in Spain[17].

Sample collection. A serum sample was obtained at the time of the flare and at the remission period. Whole blood was collected into Vacutainer tubes that were centrifuged at 3000 rpm for 20 min at room temperature. Serum was separated from cells immediately after centrifugation and stored at –40°C until analyzed. The levels of sCD40L were measured using a commercial quantitative sandwich ELISA kit (Human sCD40 Ligand: R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's instructions.

Statistical analysis. Continuous variables were expressed as either means ± standard deviation (SD) or as medians (IQR) if data were skewed; categorical variables were expressed as numbers and percentages.

To evaluate differences in proportions of variables in the 2 stages of the disease (flare and remission) we used the McNemar test. To evaluate continuous variables in the 2 stages of the disease we used the paired sample t-test for normal variables and the Wilcoxon matched-pairs signed-rank test for non-normal variables.

Chi-squared test was used to evaluate differences in proportions in non-paired groups. Either the Mann-Whitney U-test (for no normal variables) or the independent samples t-test (for normal variables) for equality of means (along with Levene's test for equality of variances) was used to compare means between non-paired groups. Kruskal-Wallis H was used to compare means between non-paired groups with non-normal variables. Spearman's rank correlation was used to examine the relationship between 2 continuous variables.

To create a model to explain the behavior of sCD40L, we applied a binary stepwise logistic-regression approach (method forward:LR) to choose the significant variables that could explain the dependent variable "sCD40L in flare lower than sCD40L in remission," including as covariates all the significant variables obtained in the univariate analysis. All confidence intervals were computed at the 95% level.

p values (2-tailed) less than 0.05 were considered statistically significant. All analyses were performed with the Statistical Package for Social Sciences (SPSS) software, version 12.0.

RESULTS

Clinical and laboratory measures of patients with SLE. We studied 53 SLE patients. Most were women (87.1%) and of Caucasian origin (87.1%). The 7 remaining patients were Latin American (5), Gypsy (1), and Arab (1). The mean age at diagnosis of SLE was 25.6 ± 11.0 years (range 10–50 yrs); and at the time of study 31.1 ± 12.4 years (range 11-61 yrs). Flares consisted mainly of renal (64.2%), musculoskeletal (49.1%), and mucocutaneous involvement (43.4%). Twenty-seven patients (50.9%) had several organs affected at the time of the flare. No patient had any change in immunosuppressive treatment in order to treat the flare at the time the flare sample was taken. Disease status, laboratory findings, and clinical symptoms are detailed in Table 1.

Patients with SLE during flare had lower sCD40L levels than those in remission. Thirty-nine of the 53 patients had statistically significant lower levels of sCD40L at flare than

Table 1. SLE status, laboratory findings, and clinical symptomatology.

Characteristic	Flare	Remission	p
Disease status			
Time from diagnosis, yrs, mean ± SD	5.4 ± 5.3		
Time from flare, days, median (IQR)		219.0 (655)	
Previous flares[1], median (IQR)	3.0 (3)		
SLEDAI, mean ± SD	14.3 ± 5.7	2.6 ± 1.8	< 0.001
SLICC > 0, n (%)	10 (18.9)	13 (24.5)	0.250
Laboratory findings			
Leukopenia[2], n (%)	4 (7.5)	1 (1.9)	0.375
Lymphopenia[3], n (%)	28 (52.8)	21 (39.6)	0.167
Thrombopenia[4], n (%)	3 (5.7)	0 (0.0)	< 0.001
≤ 192,000 × 10^9/l platelets, n (%)	18 (34.0)	5 (9.4)	0.002
High anti-dsDNA[5], n (%)	44 (86.3)	39 (76.5)	0.180
Ratio anti-dsDNA[6], median (IQR)	5.6 (19.0)	3.6 (9.3)	0.002
Low complement C3[7], n (%)	31 (58.5)	11 (20.8)	< 0.001
Low complement C4[8], n (%)	27 (50.9)	11 (20.8)	< 0.001
Low CH50 activity[9], n (%)	27 (61.4)	10 (19.6)	< 0.001
Hyperfibrinogenemia[10], n (%)	40 (75.5)	39 (73.6)	1.000
Pathological creatinine[11], n (%)	8 (15.1)	9 (17.0)	1.000
Pathological proteinuria[12], n (%)	33 (62.3)	11 (21.8)	< 0.001
Clinical symptomatology			
Renal, n (%)	34 (64.2)	6 (11.3)	
Neurological, n (%)	2 (3.8)	0 (0.0)	
Musculoskeletal (arthritis or myositis), n (%)	26 (49.1)	0 (0.0)	
Serositis, n (%)	10 (18.9)	0 (0.0)	
Mucocutaneous lesions, n (%)	23 (43.4)	1 (1.9)	

[1] Previous flares: any flare with SLEDAI ≥ 6 was considered (including the current flare); [2] leukopenia (according to SLEDAI): < 3,000 × 10^6 cells/l; [3] lymphopenia: < 1,500 × 10^6 cells/l; [4] thrombopenia (according to SLEDAI): < 100 × 10^6 cells/l; [5] high anti-dsDNA titers (by ELISA): above normal range for testing laboratory; [6] Ratio anti-dsDNA: patient's level/upper level of normality; [7] low complement C3: < 85 mg/dl; [8] low complement C4: < 10 mg/dl; [9] low CH50 activity: < 34 U/ml; [10] hyperfibrinogenemia: > 3.17 g/l; [11] pathological creatinine: Female > 1.1 mg/dl, male > 1.3 mg/dl; [12] pathological proteinuria: > 500 mg/dl/24 h. IQR: interquartile range; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics.

compared with the sample obtained for the same individual at the remission timepoint [3365 (6157) vs 7125 (4122) pg/ml; p < 0.001; Figure 1]. When sCD40L levels were analyzed according to the type of flare the same trend was observed (data not shown).

Predictive model of sCD40L values lower at flare than at remission. We investigated what variables characterized the group of patients whose sCD40L levels were lower at flare than at remission, with negative values in the variable "sCD40L differences" (defined as the difference between sCD40L levels at flare minus sCD40L levels at remission).

In the univariate analysis we considered all the variables included in our study (type of flare, treatment applied, SLEDAI, other antibodies, etc.) and others built from them. We found statistically significant relationships between negative "sCD40L differences" and the variables described in Table 2. After applying a multivariate analysis (Table 2), only 3 variables persisted: SLEDAI at remission ≥ 20% of score at flare, prednisone doses at flare higher than 15

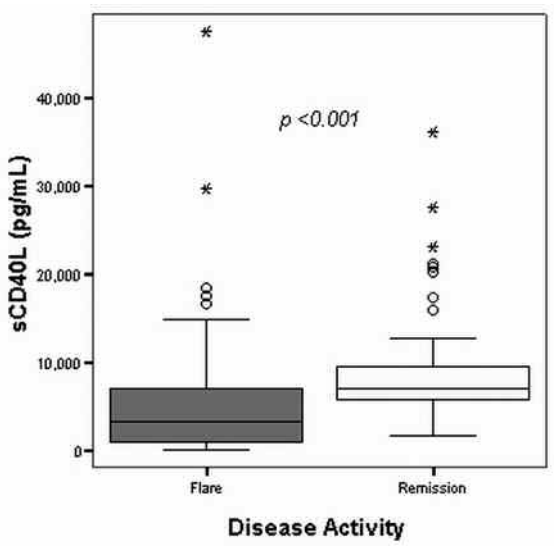

Figure 1. sCD40L levels and disease activity. At flare, patients had lower sCD40L values than at remission.

Table 2. Crude and adjusted analysis for negative sCD40L differences (values at remission higher than at flare).

Variables	n (%)	Crude OR	95% CI	p	Adjusted OR	95% CI	p
Mean age ≥ 34 yrs	21 (39.6)	5.7	1.1–28.9	0.024			
Time from flare to remission > 550 days	16 (13.2)	8.1	1.0–68.6	0.041			
SLEDAI in remission ≥ 20% of flare	22 (41.5)	15.2	1.8–127.5	0.002	17.9	1.9–172.2	0.013
Flare							
Platelets ≤ 192,000 × 10^6/l	18 (34.0)	10.0	1.2–84.5	0.020	9.6	1.0–98.1	0.056
Prednisone doses > 15 mg/day	17 (32.1)	9.0	1.1–76.2	0.022	14.2	1.4–141.5	0.023
Remission							
Hyperfibrinogenemia (> 3.7 g/l)	39 (73.6)	4.6	1.2–17.3	0.033			

Crude odds ratio (OR) univariant; adjusted OR = multivariant: model with 3 variables. SLEDAI: SLE Disease Activity Index.

mg/day, and platelet counts ≤ 192,000 × 10^6/l during flare (we chose this value because all the patients with positive "sCD40L differences" had > 192,000 × 10^6/l platelets, as shown in Figure 2).

Analysis of the predictive model to achieve a physiopathogenic explanation. Taking into account the final predictive model, we established 4 groups of patients regarding platelet counts and prednisone doses at flare (Table 3). Patients at flare without platelets ≤ 192,000 × 10^6/l and without prednisone > 15 mg/day were defined as Group 4. Patients with both characteristics were in Group 1. Groups 2 and 3 consisted of patients that had only 1 of the 2 aforementioned characteristics.

We analyzed changes of values of sCD40L between flare and remission in each group and we detected a homogeneous behavior in Groups 1, 2, and 3 that was different from Group 4. Values of sCD40L were lower at flare than at remission in Group 1 [749 (2418) vs 8023 (9603) pg/ml; p = 0.043], Group 2 [3263 (3571) vs 6770 (3456) pg/ml; p = 0.034], and Group 3 [3055 (3786) vs 7408 (3515) pg/ml; p = 0.002]. Analyzed together as Group 123 we observed the same behavior: 2616 (3671) vs 7084 (3545) pg/ml; (p < 0.001). Group 4 did not show statistically significant differences between values at flare and at remission [3882 (8118) vs 7125 (4956) pg/ml; p = 0.378].

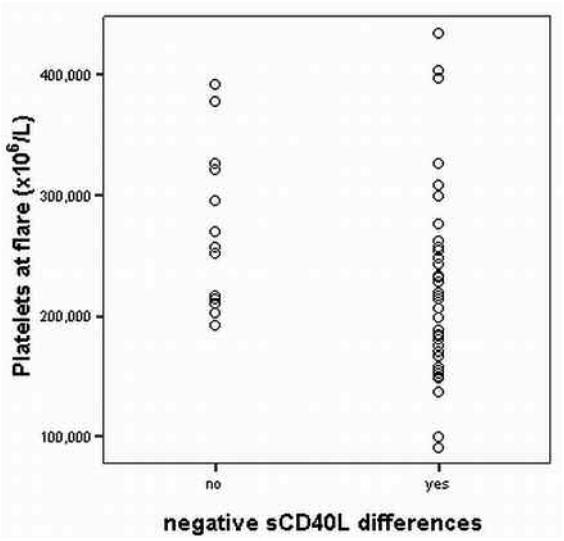

Figure 2. Platelets at flare and sCD40L differences. Only 1 patient with positive sCD40L differences had platelet counts ≤ 192,000 × 10^6/l.

Table 3. Regrouping criteria and number of patients with negative sCD40L differences in each group.

Characteristic	Groups (n) 1 (n = 5)	2 (n = 12)	3 (n = 13)	4 (n = 23)
Platelets in flare ≤ 192,000 × 10^6/l	Yes	No	Yes	No
Prednisone in flare > 15 mg/day	Yes	Yes	No	No
Negative sCD40L differences (n)	5	11	12	11

Moreover, we tested for a SLEDAI at remission ≥ 20% than that of flare in each group, and we realized that it only allowed the detection of negative "sCD40L differences" in Group 4.

Since the homogeneous behavior in the values of sCD40L between flare and remission and a SLEDAI at remission ≥ 20% of flare only allowed detecting negative "sCD40L differences" in Group 4, we decided to combine the 3 groups (1, 2, and 3) in one group: the 123 group.

We analyzed whether the differences of values of sCD40L between groups were related to values during flare or during remission. The Kruskal-Wallis H-test showed that there were differences between values of sCD40L during flare (p = 0.011) but not during remission (p = 0.591). Thus, we had to study the situation during flare to establish a physiopathogenic model. The final predictive model took into account 2 variables of flare (both of them used to construct the groups) and a variable that was built considering the per-

centage of the SLEDAI change between flare and remission. Patients with a SLEDAI at remission ≥ 20% of that at flare had lower scores of SLEDAI during flare (11.73 ± 3.97 vs 16.13 ± 6.05; p = 0.004) and higher scores of SLEDAI during remission (4.05 ± 1.36 vs 1.61 ± 1.20; p < 0.001) than patients with a SLEDAI in remission < 20% than that of flare. Thus, in order to evaluate the relationship between the SLEDAI score during flare in each group and the levels of sCD40L, we made 2 groups of patients regarding the median SLEDAI value (50th percentile) in each group, considering as a unique group patients of Groups 1, 2, and 3 together. In Group 123, the 50th percentile was 14 (IQR = 8), and in Group 4, it was 12 (IQR = 8).

We analyzed changes in sCD40L levels between flare and remission in each group, according to the SLEDAI score. Patients with low SLEDAI score (less than or equal to median) had lower sCD40L values at flare than at remission independently of the group classification: for Group 123, 4143 (4778) versus 7418 (3673) pg/ml (p = 0.005); and for Group 4, 3079 (6515) versus 6933 (6029) pg/ml (p = 0.033). Among these patients with high SLEDAI scores (higher than the median), Group 123 showed lower sCD40L values at flare than at remission, 1717 (2785) versus 6525 (4565) pg/ml (p = 0.001); and Group 4 showed no statistically significant differences between sCD40L values at flare and at remission, 9050 (12,687) versus 7801 (7091) pg/ml (p = 0.241). These results are shown in Figure 3.

We also evaluated values of sCD40L only during flare. First, we compared values in each group (Group 123 or 4) regarding the SLEDAI median score; second, we compared values in each SLEDAI score category (higher/lower or equal to median) in both groups. At flare, patients in Group 123 showed high SLEDAI values (higher than the median) at lower sCD40L values than those with low SLEDAI values (less than or equal to median): 1717 (2785) vs 4143 (7778) pg/ml (p = 0.023). There was a negative correlation between sCD40L and the SLEDAI score in this group (r = –0.387, p = 0.042). However, patients in Group 4 did not show statistically significant differences in sCD40L values at flare between high and low SLEDAI scores: 9050 (12,687) versus 3079 (6515) pg/ml (p = 0.063). There was a positive correlation between sCD40L levels and the SLEDAI score in this group (r = 0.475, p = 0.022). At low SLEDAI scores (SLEDAI less than or equal to the median), both groups (Group 123 and 4) showed sCD40L values that were similar at flare [4143 (7778) vs 3079 (6515) pg/ml; p = 0.630]; but at high SLEDAI scores (SLEDAI greater than the median) lower sCD40L values were detected in Group 123 than in Group 4 [1717 (2785) vs 9050 (12,687) pg/ml; p = 0.004].

Other analysis. We did not find any correlation between sCD40L levels and counts of lymphocytes, leukocytes, anti-dsDNA antibody titers, C3, C4, and CH50 levels, either at flare or at remission. We did not find a significant correlation between the doses of prednisone received and the lymphocyte counts.

DISCUSSION

In our study, we found lower levels of sCD40L at flare than at remission. To our knowledge, this is the first study taking serial samples to analyze levels of sCD40L from the same patient with SLE at 2 different timepoints of the disease. Only one patient with serial samples has been examined to date[12]. However, the authors did not specify the patient's disease activity at any time.

We constructed a model to determine why sCD40L

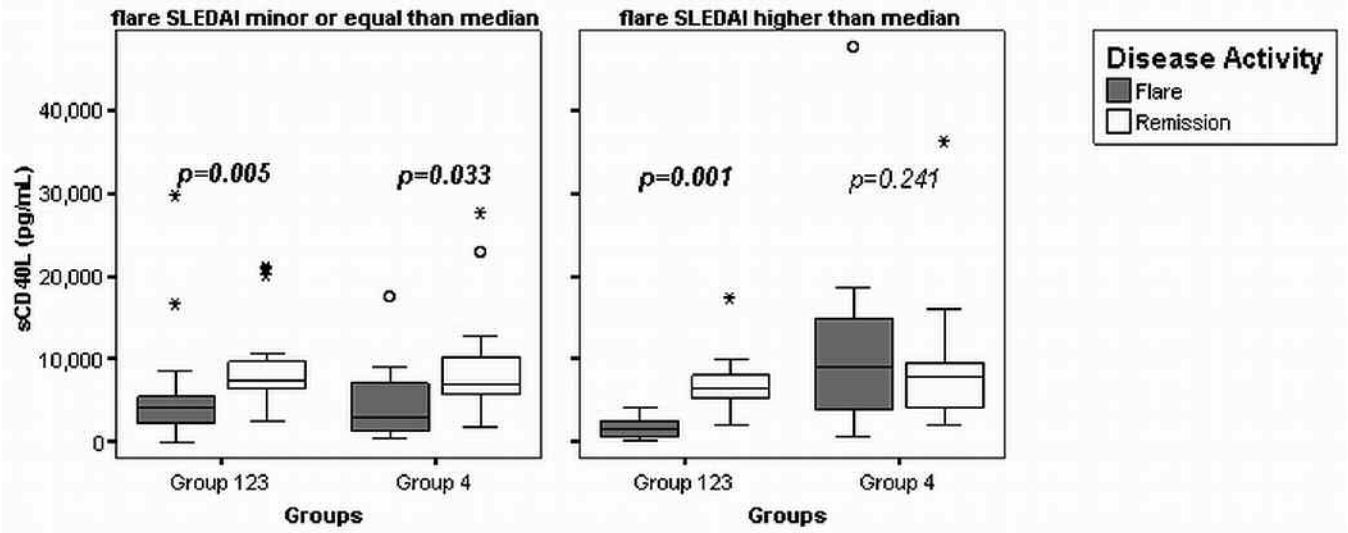

Figure 3. sCD40L values at flare and at remission (pg/ml) for the different groups according to SLEDAI score. We grouped all those patients with prednisone doses ≥ 15 mg/day and/or platelets ≤ 192,000 × 10^6/l in 1 group (Group 123), and the remaining patients in Group 4. In patients with low SLEDAI scores, sCD40L levels were lower at flare than at remission in both groups. In patients with high SLEDAI scores, in Group 123 we observed lower levels at flare than at remission; nevertheless, these differences were not detected among those patients in Group 4.

values were lower at flare than at remission, and we found that this variation was significantly associated by multivariate analysis with changes in the SLEDAI between flare and remission, low counts of platelets (≤ 192,000 × 10^6/l) during flare and high prednisone doses during flare (> 15 mg/day). To determine why these variables could explain the lower levels of sCD40L observed at flare, we analyzed the data more closely, grouping those patients who had low platelet counts (≤ 192,000 × 10^6/l) and/or who had received high prednisone doses (> 15 mg/day) as Group 123 and the rest as Group 4. We also subdivided the patients into 2 groups according to a high or low SLEDAI score during flare.

We found decreased levels of sCD40L during flare when compared to values in remission always at low SLEDAI scores, and at high SLEDAI scores only in patients receiving high doses of prednisone and/or low platelet counts (Group 123; in this group of patients the values at flare at high SLEDAI scores decreased even more than those of patients of the same group at low SLEDAI scores). At high SLEDAI scores, in Group 4 there were no differences of sCD40L values between flare and remission. In these patients receiving normal prednisone doses and having normal platelet counts, the values of sCD40L were higher than the values in patients with low SLEDAI scores of the same group, and they almost reached statistical significance. In this Group 4 there was a positive correlation between sCD40L levels and the SLEDAI score.

Besides ours, there are only 3 studies about sCD40L levels in SLE and disease activity. The relationship between sCD40L and SLE disease activity was assessed by Kato, *et al*[12]. They determined the levels of sCD40L in only 26 patients with SLE, who had different degrees of disease activity. They identified 2 groups of patients according to their SLEDAI score: score 2–9 (n = 16) and score ≥ 10 (n = 10). They found that patients with the highest SLEDAI had higher mean levels of sCD40L than patients with less active disease. Goules, *et al*[10] found a median ECLAM score of 2.5 in patients with sCD40L values above the cutoff value (n = 8) and a median ECLAM score of 2.0 in patients with low sCD40L levels (n = 15). They did not apply statistical analysis to evaluate this difference. Platelet levels at the time of the sample collection and prednisone doses were not assessed in these studies. Since our Group 4 showed higher sCD40L values at flare when the SLEDAI scores were high, as well as a positive correlation between both variables, we can postulate that perhaps most of the patients in their studies had been receiving prednisone doses < 15 mg/day and had platelet counts ≥ 192,000 × 10^6/l. Another study[13] grouped patients according to an ECLAM > 3 (n = 36; considered active) and ≤ 3 (n = 29; low active). They did not find statistically significant differences between the 2 groups, but, as we did, they found higher values in a subgroup with low activity disease (sCD40L 7.73 ± 7.8 ng/ml) than in a subgroup with high activity disease (sCD40L 7.1 ± 5.0 ng/ml).

Platelets[18] and lymphocytes[12] contain preformed CD40L, and their activation causes the shedding of this mediator. The cleavage of CD40L from the cell surface is the main origin of sCD40L. Several studies have suggested that sCD40L is active biologically and is able to induce B cell activation or differentiation[2,3,5,19]; it can also induce antigen-presenting cells to express immune accessory molecules[2,20,21].

In view of our data, we may hypothesize the following explanation for SLE flares: at low activity, sCD40L is consumed and there is not inflammatory stimulus high enough to produce more CD40L. At high SLEDAI scores if there are enough platelets and not too much prednisone has been received, it is possible that the high inflammatory stimulus triggers platelets and lymphocyte activation, thus leading to increased levels of sCD40L to compensate the consumption. In the other cases, when patients have low platelet counts and/or are receiving high doses of corticosteroids, levels of sCD40L decrease even more than in patients with low SLEDAI scores. Hence, we can suggest that the more active the disease is, the more consumption of sCD40L is achieved; and this consumption is only compensated with more production of sCD40L at high SLEDAI scores if there is not too much prednisone present and there are enough platelets.

We found platelets a limiting factor for increased levels of sCD40L at high SLEDAI scores. Therefore, at high degrees of SLE inflammation, activated platelets are a source of sCD40L. Activated platelets produce and release large amounts of sCD40L[18]. A correlation between sCD40L levels and platelet activation in patients with chest pain has been described[22]. Low platelet counts are believed to be related to immunological destruction in SLE. Nevertheless, none of our patients had autoimmune thrombopenia.

We also found that high doses of prednisone were a limiting factor for the increased levels of sCD40L at high SLEDAI scores. Prednisone could act modifying potential sources of sCD40L (as cells had to be activated in order to express CD40L on surface). As we know, CD40L is broadly distributed in vascular cells, but surface expression occurs only in activated cells. Glucocorticoids may inhibit T cell proliferation[23], T cell-dependent immunity, and the expression of genes encoding cytokines [i.e., interleukin 1 (IL-1)[24], IL-2, IL-6[25], interferon-α, and TNF-α][26]. Corticosteroids also produce nonspecific antiinflammatory effects as well as antiadhesion effects that may further contribute to immunosuppression. It has been reported that glucocorticoids upregulate CD40L mRNA in human peripheral blood mononuclear cells and surface-protein expression on them, in a glucocorticoid-receptor-dependent manner[27]. In our study we observed that patients who were taking prednisone doses higher than 15 mg/day at high SLEDAI scores

had lower values of sCD40L during flare than patients who were receiving lower doses. Perhaps at this dose the effects of prednisone are the result of a direct interaction with biologic membranes and do not act over cellular receptors. Some authors postulate 3 distinct therapeutically relevant effects of corticoids[28]: genomic, specific nongenomic, and unspecific nongenomic. Genomic effects are mediated by cytosolic receptors that alter the expression of specific genes. Specific nongenomic effects are mediated by steroid-selective membrane receptors. Unspecific nongenomic effects occur only at high glucocorticoid dosages and seem to result from direct interactions with biological membranes, but have little effect on protein synthesis. Further, it has been shown *in vitro* that the administration of prednisone inhibits, in a dose-dependent way, the activity of monocytes as antigen-presenting cells, demonstrating inhibition of the proliferative lymphocytic response[29], causing (among other effects) a decrease in the number of peripheral T lymphocytes[30].

In patients with SLE, T lymphocytes have been suggested as the main source of sCD40L. Previous reports demonstrated that the absolute numbers of blood T cells that express surface CD40L are increased in patients with active lupus[3-5]. Moreover, T cells from patients with SLE have constitutive or exaggerated expression of CD40L in culture[3-5]. Levels of sCD40L transcripts in SLE blood lymphocytes correlated with the relative concentrations of sCD40L found in SLE plasma[12]. We did not find any association between levels of sCD40L and blood counts of lymphocytes. Nevertheless, we may take into account that prednisone may work by decreasing lymphocyte numbers, and consequently reducing an identified source of sCD40L in patients with SLE. Since we did not find any correlation between the doses of prednisone taken at the time of blood draw and the lymphocyte counts, it is possible that if this relationship exists it may be related to the cumulative doses of prednisone taken in the previous weeks.

Our study suggests that sCD40L may contribute to the pathogenesis of SLE and seems to play an active role during flare. From our results it seems that the low activity of the disease during flare leads to a consumption of sCD40L levels, which at high SLEDAI scores is compensated if there are enough platelets and not too much prednisone is being received; otherwise sCD40L levels at high SLEDAI scores decrease even more. Thus, at high SLEDAI scores there seem to be mechanisms regarding platelet counts and inhibited by high doses of prednisone that increase the levels of sCD40L. Studies are required to determine the way by which both factors work to alter the levels of sCD40L, and to provide better comprehension of the effect that sCD40L may have on SLE.

REFERENCES

1. Schonbeck U, Libby P. The CD40/CD154 receptor/ligand dyad. Cell Mol Life Sci 2001;58:4-43.
2. Mazzei GJ, Edgerton MD, Losberger C, et al. Recombinant soluble trimeric CD40 ligand is biologically active. J Biol Chem 1995;270:7025-8.
3. Desai-Mehta A, Lu L, Ramsey-Goldman R, Datta SK. Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. J Clin Invest 1996;97:2063-73.
4. Devi BS, Van Noordin S, Krausz T, Davies KA. Peripheral blood lymphocytes in SLE — hyperexpression of CD154 on T and B lymphocytes and increased number of double negative T cells. J Autoimmun 1998;11:471-5.
5. Koshy M, Berger D, Crow MK. Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. J Clin Invest 1996;98:826-37.
6. Tsubata T, Murakami M, Nisitani S, Honjo T. Molecular mechanisms for B lymphocyte selection: induction and regulation of antigen-receptor-mediated apoptosis of mature B cells in normal mice and their defect in autoimmunity-prone mice. Philos Trans R Soc Lond B Biol Sci 1994;345:297-301.
7. Early GS, Zhao W, Burns CM. Anti-CD40 ligand antibody treatment prevents the development of lupus-like nephritis in a subset of New Zealand black x New Zealand white mice. Response correlates with the absence of an anti-antibody response. J Immunol 1996;157:3159-64.
8. Kalunian KC, Davis JC Jr, Merrill JT, Totoritis MC, Wofsy D. Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2002;46:3251-8.
9. Kawai T, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. Nat Med 2000;6:114.
10. Goules A, Tzioufas AG, Manousakis MN, Kirou KA, Crow MK, Routsias JG. Elevated levels of soluble CD40 ligand (sCD40L) in serum of patients with systemic autoimmune diseases. J Autoimmun 2006;26:165-71.
11. Vakkalanka RK, Woo C, Kirou KA, Koshy M, Berger D, Crow MK. Elevated levels and functional capacity of soluble CD40 ligand in systemic lupus erythematosus sera. Arthritis Rheum 1999;42:871-81.
12. Kato K, Santana-Sahagun E, Rassenti LZ, et al. The soluble CD40 ligand sCD154 in systemic lupus erythematosus. J Clin Invest 1999;104:947-55.
13. Ciferska H, Horak P, Hermanova Z, et al. The levels of sCD30 and of sCD40L in a group of patients with systemic lupus erythematodes and their diagnostic value. Clin Rheumatol 2007;26:723-8.
14. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
15. 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.
16. Ramsey-Goldman R, Isenberg DA. Systemic lupus erythematosus measures: British Isles Lupus Assessment Group (BILAG), European Consensus Lupus Activity Measurement (ECLAM), Systemic Lupus Activity Measure (SLAM), Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), and Systemic Lupus International Collaborating Clinics/American College of Rheumatology-Damage Index (SLICC/ACR-DI; SDI). Arthritis Rheum 2003;49:S225-33.
17. Vollmann J, Winau R. Informed consent in human experimentation before the Nuremberg code. BMJ 1996;313:1445-9.
18. Henn V, Steinbach S, Buchner K, Presek P, Kroczek RA. The inflammatory action of CD40 ligand (CD154) expressed on

activated human platelets is temporally limited by coexpressed CD40. Blood 2001;98:1047-54.
19. Graf D, Muller S, Korthauer U, van Kooten C, Weise C, Kroczek RA. A soluble form of TRAP (CD40 ligand) is rapidly released after T cell activation. Eur J Immunol 1995;25:1749-54.
20. Wiley JA, Geha R, Harmsen AG. Exogenous CD40 ligand induces a pulmonary inflammation response. J Immunol 1997;158:2932-8.
21. Jumper MD, Nishioka Y, Davis LS, Lipsky PE, Meek K. Regulation of human B cell function by recombinant CD40 ligand and other TNF-related ligands. J Immunol 1995;155:2369-78.
22. Heeschen C, Dimmeler S, Hamm CW, et al. Soluble CD40 ligand in acute coronary syndromes. N Engl J Med 2003;348:1104-11.
23. Arya SK, Wong-Staal F, Gallo RC. Dexamethasone-mediated inhibition of human T cell growth factor and gamma-interferon messenger RNA. J Immunol 1984;133:273-6.
24. Knudsen PJ, Dinarello CA, Strom TB. Glucocorticoids inhibit transcriptional and post-transcriptional expression of interleukin 1 in U937 cells. J Immunol 1987;139:4129-34.
25. Zanker B, Walz G, Wieder KJ, Strom TB. Evidence that glucocorticosteroids block expression of the human interleukin-6 gene by accessory cells. Transplantation 1990;49:183-5.
26. Franchimont D. Overview of the actions of glucocorticoids on the immune response: a good model to characterize new pathways of immunosuppression for new treatment strategies. Ann NY Acad Sci 2004;1024:124-37.
27. Jabara HH, Brodeur SR, Geha RS. Glucocorticoids upregulate CD40 ligand expression and induce CD40L-dependent immunoglobulin isotype switching. J Clin Invest 2001;107:371-8.
28. Buttgereit F. Mechanisms and clinical relevance of nongenomic glucocorticoid actions. Z Rheumatol 2000;59 Suppl:II/119-23.
29. Gerrard TL, Cupps TR, Jurgensen CH, Fauci AS. Hydrocortisone-mediated inhibition of monocyte antigen presentation: dissociation of inhibitory effect and expression of DR antigens. Cell Immunol 1984;85:330-9.
30. Slade JD, Hepburn B. Prednisone-induced alterations of circulating human lymphocyte subsets. J Lab Clin Med 1983;101:479-87.