Phagocyte-specific S100A8/A9 Protein Levels During Disease Exacerbations and Infections in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective:*** S100A8 and S100A9 are calcium binding proteins of the S100 family highly expressed in neutrophils and monocytes. S100 proteins are novel ligands of TLR4 important in modulating inflammation. High levels of S100A8/A9 found in human inflammatory diseases are a marker of disease activity in rheumatoid arthritis (RA) and juvenile rheumatoid arthritis (JRA). We determined levels of S100A8/A9 in sera of patients with systemic lupus erythematosus (SLE) and analyzed their relation to clinical variables of disease activity.

Methods. A group of 93 patients with SLE were studied over a period of 3 years, and 143 serum samples were analyzed. S100A8/A9 serum concentrations were determined by a sandwich ELISA. Sera from 10 primary Sjögren's syndrome (pSS) patients and 50 healthy volunteers were used as controls. Correlations to SLEDAI, ANA, anti-dsDNA, WBC, CH50, C4, and CRP were made. In addition, infections were recorded in all SLE patients.

Results. Serum levels of S100A8/A9 were significantly ($p = 0.04$) higher in SLE patients (1412 ± 664 ng/ml) versus healthy controls (339 ± 35 ng/ml) and pSS patients (400 ± 85 ng/ml). The only significant correlation ($r = 0.219$; $p = 0.015$) was found was between S100A8/A9 and SLEDAI. Further, SLE patients with concomitant infections had higher serum levels of S100A8/A9 (39300 ± 13375 ng/ml) than those without infections (1150 ± 422 ng/ml).

Conclusion. Serum levels of S100A8/A9 are significantly raised in SLE versus pSS patients and healthy controls and can be correlated to a disease activity index. S100A8/A9 is a more relevant marker of infection in SLE patients. (First Release Sept 15 2009; J Rheumatol 2009;36:2190–4; doi:10.3899/jrheum.01302)

Key Indexing Terms:

S100A8/A9	INFLAMMATORY DISEASES	NEUTROPHILS
INFECTION	SYSTEMIC LUPUS ERYTHEMATOSUS	SLEDAI

Cells of the phagocytic lineage such as neutrophils and monocytes play an important role in the inflammatory response of various diseases. They display, depending upon their stage of activation, pro- as well as anti-inflammatory properties. S100A8 and S100A9 are 2 calcium-binding proteins belonging to the S100 family[1-4]. These proteins are specifically secreted by human monocytes after activation of protein kinase C[5]. Their expression in leukocytes is specifically restricted to neutrophils and in the early stages of differentiation of monocytes.

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In inflammatory conditions, S100A8 and S100A9 are expressed by infiltrating monocytes and neutrophils but not in resting tissue macrophages and lymphocytes[6]. Non-covalently associated complexes of both molecules play a role in calcium dependent modulation of cytoskeletal-membrane interactions. Calcium dependent translocation of S100A8 and S100A9 from the cytoplasm to the plasma membrane correlates with the inflammatory activation of these cells as shown by increased levels of cytokines such as tumor necrosis factor or interleukin ß, and by activation of the respiratory burst[4]. Expression of S100A8 and S100A9 by myelomonocytic cells correlates with disease activity in murine models of experimental inflammation and several human inflammatory diseases (rheumatoid arthritis, juvenile rheumatoid arthritis, renal allograft rejection, and inflammatory bowel diseases)[7-10]. Recently, S100A8 and S100A9 were identified as novel endogenous ligands of Toll-like receptor 4 promoting systemic endotoxin-induced shock as well as inflammatory arthritis[11]. The physiological mechanisms triggering the release of these proteins have not yet been identified. Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disorder characterized by the systemic involvement of several organs. Inflam-

matory markers of disease activity in SLE are not very specific. We aimed to measure sera levels of S100A8/A9 in SLE patients and to determine if S100A8/A9 could be a reliable marker of disease activity in SLE.

In the present study, we show that the serum concentrations of S100A8/A9 in SLE are significantly higher than in controls. In addition, we also demonstrate higher levels of S100A8/A9 in SLE patients presenting with infection.

MATERIAL AND METHODS

Inclusion of patients and controls. Included in the study were 93 outpatients seen at the Erasme Hospital. All patients fulfilled 4 or more of the American Rheumatism Association (ARA) classification criteria for SLE[12]. Blood was collected for analysis one week before the consultation and disease activity was assessed according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)[13]. Fifty healthy volunteers and 10 patients with pSS served as controls. Further, sera from 8 patients (without SLE) with severe infections and 6 SLE patients presenting with intercurrent infections were also collected. Disease flare was identified by a SLEDAI score > 4, whereas infection was determined by hemocultures, urinalysis and sputum microbiology when indicated.

Laboratory variables. Laboratory investigations included blood cell counts, erythrocyte sedimentation rate (ESR), CRP (high sensitivity), hemoglobin concentration, antinuclear antibody (ANA), anti-dsDNA antibodies, antibodies to extractable nuclear antigens (ENA) and complement levels (CH50, C3, and C4).

Serum was kept at –20˚C and analyzed for S100A8/A9 by sandwich ELISA technique as described[7]. Serum concentrations of S100A8 and S100A9 were assessed by a sandwich ELISA that allows detection of the S100A8/S100A9 heterodimer as described[5]. S100A8 and S100A9 form noncovalently associated complexes in the presence of calcium that are detected by ELISA system used (inhouse assay). The ELISA was therefore calibrated with the native S100A8/S100A9 complex and data expressed as ng/ml S100A8/S100A9. The sensitivity of the assay was < 0.5 ng/ml. Mann–Whitney U-test was used to determine significant differences between distinct groups.

CRP (high sensitivity) was measured by a turbimetric latex assay (DiaSys Diagnostic systems GmbH, Germany). Briefly, the latex particles coated with anti-CRP are agglutinated when they react with samples that contain CRP. The latex particle agglutination is proportional to the concentration of CRP and is measured by turbimetry. The lower limit of detection of the assay was 0.03 mg/dl.

Statistical analysis. Statistical analysis was performed with GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA, USA). The Mann-Whitney nonparametric test was used for comparisons between SLE patients and controls. Spearman's rank correlation tested for the relationship between S100A8/A9 and the clinical and biological variables. All values are expressed as the mean ± SEM. P values < 0.05 were considered as statistically significant.

RESULTS

The mean age of SLE patients was 44 ± 9.8 years. There were 90 women and 3 men. At the time of examination, 81% of patients were ANA positive, 65% had anti-dsDNA antibodies, 7% had anti-sm, 27% had anti-SSA, 8% had anti-SSB, 9% had anti-RNP antibodies, and 5% of the patients were positive for rheumatoid factor. The initial characteristics of the SLE patients are shown in Table 1.

Six patients with SLE presented a documented infection at the time of consultation.

Table 1. Characteristics of the SLE patients.

No. patients	93
Age, yrs	44 ± 9.8
Sex, female/male	90/3
Disease duration, yrs	12.7 ± 1.3
Medication	
Methylprednisolone	46
Hydroxychloroquine	48
Immunosuppressants	22

The mean value of SLEDAI was 4.6 ± 0.7. The mean age of the patients suffering from SS were 46 ± 5.4 years (10 women). These patients presented no inflammatory markers in the sera and had no clinical signs of ongoing inflammation such as arthritis or serositis. The healthy control group consisted of 26 men and 24 women and had a mean age of 16 years (median 1.2–34.3 yrs).

The serum levels of S100A8/A9 were significantly higher in the SLE patients (1412 ± 664 ng/ml) versus healthy controls (339 ± 35 ng/ml) and pSS patients (400 ± 85 ng/ml) (Table 2).

There was significant correlation between S100A8/A9 and the SLEDAI (r = 0.219; p = 0.015), while no significant correlation was found between S100A8/A9 and other laboratory variables such as ESR, CRP, platelets, and lymphocytes (Table 3).

We also compared and analyzed serum levels of S100A8/A9 in SLE patients with disease flare and SLE patients who were in remission (SLEDAI < 4). In SLE patients with disease flare, the levels of S100A8/A9 were significantly higher (p = 0.04) as compared to those who were in remission and to the healthy control group (Table 2).

Six patients with SLE presented documented infections (Table 4). Serum levels of S100A8/A9 in these patients (39300 ± 13375 ng/ml) were significantly higher (p < 0.001) than in SLE patients who were not infected (1150 ± 422 ng/ml). We therefore determined serum concentrations of S100A8/A9 in 8 patients with severe infections (4 septic shock, 3 septicemia, 1 leptospirosis) and compared them to SLE patients presenting infection. Levels of S100A8/A9 patients with severe infections (6268.7 ± 3245.9 ng/ml) were significantly higher as compared to the control group (p < 0.05).

Further, we compared S100A8/A9 to CRP to detect lupus flares and infections. S100A8/A9 was a better marker than CRP for the evaluation of disease activity (Table 2) while both CRP and S100A8/A9 are reliable markers of infection. However, S100A8/A9 with a cutoff value of 8100 ng/ml (set on the basis that it was the lowest value from SLE infected patients) is a better marker than CRP (value > 1 mg/dl)[14] to detect infections (chi-square, p = 0.0002).

DISCUSSION

We determined serum levels of S100A8/A9 in SLE patients

Table 2. Serum levels of S100A8/A9 and CRP in healthy controls, in SLE patients in remission, in SLE patients with flare and SLE patients with infection.

	SLE In Remission (n = 69)	SLE with Flare (n = 18)	SLE with Infection (n = 6)	Control (n = 50)
S100A8/A9, ng/ml	552 ± 44	4694 ± 3559*	39300 ± 13375[§]	339 ± 35
CRP, mg/dl	0.592 ± 0.08	0.76 ± 0.24**	7.6 ± 2.76[#]	< 0.5

*p < 0.001, Mann-Whitney test, SLE group with flare vs SLE group in remission; [§]p < 0.001, Mann-Whitney test, SLE group with flare vs SLE group with infection; **p > 0.05, Mann-Whitney test, SLE group with flare vs SLE group in remission; [#]p = 0.009, Mann-Whitney test, SLE group with flare vs SLE group with infection.

Table 3. Spearman's rank correlation coefficients (r) between laboratory variables of disease activity, number of patients in each analysis (n), and significance levels (p).

	PNN	WBC	CRP	ESR	C3	C4	CH50	dsDNA	SLEDAI
S100A8/A9									
r	0.46	0.198	0.257	0.297	0.112	0.213	–0.11	0.063	0.219
n	70	70	66	66	46	44	60	78	120
p	0.001	0.10	0.045	0.015	0.46	0.16	0.346	0.585	0.015
PNN									
r		0.858	0.251	0.127	0.451	0.387	–0.04	–0.385	–0.1
n		70	65	65	38	39	45	57	71
p		0.0001	0.199	0.357	0.004	0.016	0.772	0.007	0.405
WBC									
r			0.169	0.025	0.545	0.612	0.138	–0.137	–0.23
n			42	70	28	28	46	62	70
p			0.284	0.839	0.007	0.001	0.237	0.296	0.052
CRP									
r				0.363	0.357	0.476	0.478	–0.082	0.125
n				42	17	15	28	36	65
p				0.018	0.159	0.07	0.069	0.63	0.32
ESR									
r					0.098	–0.25	–0.20	0.001	0.15
n					26	26	46	62	70
p					0.635	0.220	0.184	0.992	0.215
C3									
r						0.732	0.563	–0.509	–0.381
n						42	44	33	46
p						0.0001	0.0001	0.002	0.009
C4									
r							0.598	–0.662	–0.551
n							43	33	44
p							0.0001	0.0001	0.0001
CH50									
r								–0.492	–0.503
n								53	65
p								0.0001	0.0001
dsDNA									
r									0.618
n									78
p									0.0001

PNN: polynuclear neutrophils; WBC: white blood cells; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SLEDAI: SLE Disease Activity Index.

Table 4. Clinical characteristics of 6 patients with SLE presenting with infection.

	S100A8/A9	CRP (mg/dl)	SLEDAI	Infection
Patient 1	8100	3.7	12	Pneumonia
Patient 2	8200	4.2	15	Pyelonephritis
Patient 3	12000	5.8	12	Urinary tract
Patient 4	69600	3.1	4	Endocarditis
Patient 5	70400	21	5	Salpingitis
Patient 6	67500	6.2	47	Encephalitis

and correlated them to the SLEDAI. Previous studies have shown that S100A8/A9 is a marker of disease activity in several inflammatory diseases such as RA[5,7-10]. Our study confirms S100A8/A9 as a potential marker of disease activity in SLE. The other classical markers of inflammation such as ESR and CRP do not correlate with the SLEDAI.

It is established that CRP levels are not high in SLE flares, making it a very poor marker for monitoring disease activity. Persistent mild increased levels are observed in some patients with Jaccoud arthropathy[15]. Elevated levels of CRP are found in sera of SLE patients presenting intercurrent infections [16,17] and in lupus associated serositis and arthritis[18]. Interestingly, the mean levels of CRP in SLE patients with infections were significantly higher than in those without infections[19], but the range of values overlapped considerably implying poor discriminative value[20]. Why CRP levels remain low despite high disease activity is still a matter of debate. Antibodies against CRP have been detected in SLE patients but the detected antibodies do not bind native pentameric CRP and do not correlate with CRP levels[21]. Moreover, it has also been shown that low levels of CRP in SLE patients with active disease might result from defective production rather than increased consumption[20]. However, in active SLE with renal flare, very low levels of CRP might reflect increased consumption by immune complexes[22]. It was recently shown that the unresponsiveness of CRP in SLE flare might be related to abnormal interleukin 6 response[23].

Because the classical markers of inflammation are neither specific nor sensitive for detecting SLE flare or infection, we hypothesized that S100A8/A9 was a better parameter. S100A8/A9 secreted by activated phagocytes in inflammatory conditions appeared to be a better marker of disease activity as compared to acute phase proteins synthesized by the liver. The 2 calcium binding proteins, of myeloid origin, form heterocomplexes in the cytosol of granulocytes, monocytes and early differentiated macrophages. Their intracellular function pertains to cell homeostasis, but these molecules become DAMP (damage associated molecular patterns) once they are released from activated phagocytes in trigger to cell stress.

S100A8 and S100A9 are highly upregulated in several inflammatory diseases such as rheumatoid arthritis, glomerulonephritis, and vasculitis[24]. A pivotal role of S100 protein in inflammatory processes has been highlighted by the identification of a new inflammatory syndrome characterized by very high levels of these molecules[25]. More recently, S100A8/A9 have been shown to be endogenous ligands of Toll-like receptor-4 and play an important role in innate immunity[11].

In our study, we found higher S100A8/A9 levels in SLE patients versus controls and significant correlation (although weak) with SLEDAI. It was paradoxical that only a weak correlation appeared. This could be explained by the fact that most of the SLE patients in our group did not have high disease activity. A previous study by Haga, *et al*, showed significantly increased levels of S100A8/A9 in SLE patients and a significant correlation to SLEDAI[14]. In that study, there were higher levels of S100A8/A9 in SLE patients presenting with arthritis. Very high levels of S100A8/A9 in RA and in SLE patients with arthritis might indicate that these proteins might also be a more specific marker of arthritis. In our study, we had only 3 patients presenting with arthritis and they presented significantly higher levels of S100A8/A9. Several experimental models of arthritis have shown a predominant link between S100A8/A9 and synovitis. There is a strong correlation between the extent of inflammation and the levels of S100A8/A9 in immune complex mediated as well as antigen–induced mice[26]. In patients with rheumatoid arthritis, psoriatic arthritis, and juvenile idiopathic arthritis, high levels of expression of S100A8/A9 have been found in the inflammatory lesions in the synovium. The sera levels of these proteins in inflammatory arthritis correlate with the extent of local inflammation[5,7].

Interestingly, we found that levels of S100A8/A9 were much higher in SLE patients presenting with infection as compared to those without infection. This result is in accordance with the known release of S100A8/A9 in many inflammatory diseases. The CRP levels of the SLE patients presenting with intercurrent infections were also elevated. However, S100A8/A9 was much more sensitive than CRP (cutoff value 1 mg/dl) in detecting underlying infections using a cutoff value of 8100 ng/ml. In the korean study by Suh, *et al*[27], the presence of CRP levels higher than 5 mg/dl was associated with intercurrent infections in SLE patients. Higher values of CRP in SLE patients might therefore be indicative of infections in SLE.

Levels of S100A8/A9 are predominantly determined by the activation or turnover of neutrophils, and to a lesser extent by monocytes since their concentration in the latter is 5 times lesser[28]. The higher values of the S100A8/A9 in SLE might result from active secretion of neutrophils at the site of inflammation. We would therefore be expecting that sera levels of S100A8/A9 would correlate with levels of polynuclear neutrophils (PNN). We, however, did not observe any correlation between these 2 variables. This could be explained by the fact that the levels of S100A8/A9 in SLE do not reflect the number of phagocytes at the

inflammatory sites but imply other pathogenic factors pertaining to disease activity.

We also found that the classical anti-dsDNA and complement (CH50, C4) presented a strong correlation with SLEDAI, as described in the literature[29].

In our present study, we found that S100A8/A9 were elevated in sera from patients with SLE presenting with disease flare. However, only a weak correlation was found with SLEDAI as compared to classical complement and anti-dsDNA antibodies. As such, routine dosage of S100A8/A9 does not seem indicated to monitor disease activity in SLE. Very high levels of S100A8/A9 were detected in lupus patients with intercurrent infections. S100A8/A9 is a more relevant marker of infection than disease flare in SLE. The quantification of S100A8/A9 might be useful in those patients where the distinction between infection and lupus flare is difficult. A larger prospective trial is required to determine cutoff values of S100A8/A9 for detecting infections in patients with SLE.

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