EXTENDED REPORT

Anti-serum amyloid component P antibodies in patients with systemic lupus erythematosus correlate with disease activity

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An appendix showing the reported clinical manifestations can be seen at <http://www.annrheumdis.com/supplemental>

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Objective: To determine the presence of raised titres of anti-serum amyloid P component (SAP) antibodies in patients with systemic lupus erythematosus (SLE) and to evaluate their correlation with clinical disease by the SLEDAI and clinical manifestations.

Methods: 452 samples were screened for raised anti-SAP antibody titres by an ELISA. Clinical measures and SLEDAI scores were independently reviewed from medical records. 21 serial samples from 7 patients with SLE were assessed for a change in anti-SAP antibody titres after treatment.

Results: Raised anti-SAP antibody titres were detected in 145/328 (44%) SLE samples. In 112 randomly selected samples, 69/112 (62%) patients had raised anti-SAP antibodies and anti-dsDNA antibody titres, whereas only 32/112 (28%) had raised anti-dsDNA antibody titres without raised anti-SAP antibody titres. The mean titre of anti-SAP antibodies in patients with active disease was higher than in patients with inactive disease and controls. SLEDAI scores, assessed in 54 patients, were raised in 26/31 (84%) patients with raised anti-SAP antibody titres. A SLEDAI score ≥ 8 was found in 16/31 (52%) patients with raised anti-SAP antibody titres but in only 5/23 (22%) patients without raised titres. No specific pattern of disease was detected in patients with or without raised titres of anti-SAP antibodies. Serial sampling from patients with active SLE and raised anti-SAP antibody titres showed that anti-SAP antibody titres decreased after treatment and correlated with clinical improvement.

Conclusion: Raised anti-SAP antibody titres detected in patients with SLE correlate with disease activity and decrease with improvement of clinical disease, and thus may serve as an additional prognostic marker.

Serum amyloid component P (SAP) is a member of the pentraxin family of proteins.¹ It is a highly conserved plasma protein named for its universal presence in amyloid deposits, and a normal component of a number of basement membranes, including the glomerular basement membrane.² It is the single normal circulating protein with specific calcium dependent binding to DNA and chromatin in physiological conditions.³ When bound to ligand, SAP forms a stable decamer with two pentameric rings.⁴ The SAP-DNA binding interaction involves a decapeptide around Arg120.⁵ SAP is a constitutive protein (40 µg/ml) synthesised by hepatocytes.⁶ It is located on chromosome 1q23, in proximity to one of the putative genes associated with systemic lupus erythematosus (SLE).⁷

SAP interacts with nuclear ligands, including chromatin and snRNP, is important in the handling of chromatin exposed by cell death, and has a role in the clearance of nuclear ligands from apoptotic and necrotic cells.^{8–9} SAP binds in vivo both to apoptotic cells, the surface blebs of which bear chromatin fragments, and to nuclear debris released by necrosis.⁹

The pentraxin protein family (SAP and C reactive protein (CRP)) activates complement through the classical pathway and participates in the opsonisation of particulate ligands and bacteria.³ Both CRP and SAP have nuclear transport signals that facilitate their entry into the nuclei of intact cells.³ In the mouse, SAP binds to the FcγRI and FcγRIII

receptors and therefore can activate the complement system.¹⁰ During the acute phase response, C5a generated as a consequence of complement activation acts in concert with interleukin 6 and/or interleukin 1β to promote up regulation of the SAP gene in a murine model.¹¹ SAP binds directly to apoptotic cells in the early¹² and late stages¹³ of apoptosis. This interaction could mitigate against deposition of these antigens in tissue and autoimmune reactivity.

Mice with targeted deletion of the SAP gene spontaneously develop antinuclear autoimmunity and severe glomerulonephritis, a phenotype resembling SLE.^{2 14 15} The SAP knock-out mice also have enhanced anti-DNA responses to immunisation with extrinsic chromatin.²

SLE is an autoimmune disease whose multifactorial aetiology and diverse mechanisms have yet to be established. One postulated mechanism of dysregulation of the immune system is the abnormal clearance of apoptotic cells.^{16–18} The physiological display of nuclear antigens in apoptotic bodies offers a plausible mechanism whereby the autoantigens prominent in SLE are exposed to the immune system, thereby stimulating an autoimmune response.¹⁷ One postulation is that once chromatin is released into the circulation, SAP binds the chromatin and sequesters it from the antigen driven immune response.²

Abbreviations: BSA, bovine serum albumin; CASQ, clinically active and serologically quiescent; CRP, C reactive protein; ELISA, enzyme linked immunosorbent assay; OD, optical density; PBS, phosphate buffered saline; SACQ, serologically active but clinically quiescent; SAP, serum amyloid P; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index

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In humans, SAP is predominantly a physiological protein, whereas in the mouse it is an acute phase reactant protein (as is CRP in humans).¹⁴ Abnormal levels of SAP in humans are not reported. In addition, no genetic polymorphisms have been detected. SAP levels do not fluctuate with disease activity.^{19–20} However, SAP-DNA complexes are lower in patients with SLE than in healthy controls.²¹ Incomplete clearance of apoptotic cells may lead to increased levels of apoptotic bodies, stimulating the increased production of autoantibodies during active disease.¹⁴ We speculate that anti-SAP antibody titres may be raised in patients with SLE and hence influence clinical disease. Binding of anti-SAP antibodies to SAP might affect the function of SAP and therefore lead to abnormal clearance of persistent antigens and the propagation of autoimmunity.

In this study, our primary aim was to determine the presence of raised titres of anti-SAP antibodies in patients with SLE. Our secondary aim, if such raised titres were found, was to evaluate their correlation with clinical disease by the SLE Disease Activity Index (SLEDAI) and clinical manifestations.

PATIENTS AND METHODS

Patients and controls

Laboratory features of 328 consecutive and unselected patients with SLE were evaluated in a multicentre cross sectional study. All fulfilled four or more of the American College of Rheumatology revised criteria for the classification of SLE.²² Control samples, matched for age and sex ($n = 124$), were obtained from normal blood donors at the Israel National Blood Bank. Clinical findings were assessed in 189 patients with SLE.

Serum samples evaluated for the presence of raised titres of anti-SAP antibodies

The 328 consecutive samples analysed for the presence of raised titres of anti-SAP antibodies were from patients with SLE attending four lupus/rheumatology clinics: 54 from the Israel Lupus Clinic (group I), 135 from Germany (group II), 88 from Italy (group III), and 51 from Slovakia (group IV). Serial samples ($n = 21$) measured from seven patients with SLE attending the Israel Lupus Clinic (group I) were assessed separately for change in anti-SAP antibody titres after treatment.

Clinical assessment

Clinical findings were assessed in 189 patients with SLE attending two outpatient clinics between the years 2000 and 2001. All patients had documented files and underwent a medical interview and physical examination by a rheumatologist. A serum sample from each patient was collected for anti-SAP antibody testing by enzyme linked immunosorbent assay (ELISA). Disease activity was assessed using the SLEDAI score²³ for 54 patients with SLE (group I), and correlation with raised anti-SAP antibody titres was evaluated. Serial samples from seven patients with SLE with active disease were assessed separately for change in anti-SAP antibody titres before and after treatment and the correlation with clinical improvement. In 135 randomly chosen patients with SLE (group II), 31 clinical manifestations (appendix, see <http://www.annrheumdis.com/supplemental>) reported in the clinic at any time from the diagnosis were determined and correlated with raised anti-SAP antibody titres. Clinical and serological characteristics collected in a protocol form were transferred to a computerised database program (Excel-Microsoft Office).

Clinical assessment was not available for 139 patients with SLE.

Table 1 Raised anti-SAP antibody titres in patients with SLE

	No of patients	No (%)
I. Israel*	54	32 (59)
II. Germany*	135	31 (22)
III. Italy	88	61 (69)
IV. Slovakia	51	21 (41)
SLE clinics total	328	145 (44)
Control	124	3 (2)

*Serum samples with available clinical parameters.

Laboratory variables

Samples were screened for anti-SAP and anti-dsDNA titres with an ELISA.

Detection of anti-SAP antibody titres

Nunc plates (96 well) were coated with SAP (Sigma Chemical, St Louis, MO, USA) 5 µg/ml in Dulbecco's phosphate buffered saline (PBS) with calcium (Biological Industries, Beit Haemek, Israel), and kept overnight at 4°C. The plates were blocked with 3% bovine serum albumin (BSA) for 1 hour at 37°C. Serum samples were added at a dilution 1:200, in PBS supplemented with Ca²⁺/1% BSA for 4 hours at room temperature. The binding was probed with antihuman IgG and IgM conjugated to alkaline phosphatase, and incubated for 1 hour at 37°C. An appropriate substrate was added. Washing between steps was performed using PBS with 0.05% Tween-20. The plates were read at 405 nm optical density (OD) by ELISA Titertek. A value was considered significantly raised when the OD reading for anti-SAP levels was above three standard deviations (3SD) of the mean OD reading for the control group. The specificity of binding was confirmed by inhibition assay.

Detection of anti-dsDNA antibody titres by ELISA

We have described previously the detection of anti-dsDNA antibodies.²⁴

Inhibition assay

Total IgG was affinity purified on a protein G column (Pharmacia, Upsala, Sweden) from five patients with SLE (four patients with raised levels of anti-SAP and one patient who did not have circulating anti-SAP antibodies). The affinity purified IgG was further purified on a dsDNA-cellulose column (Pharmacia), and tested for anti-dsDNA activity. Anti-SAP binding of the affinity purified IgG was tested at different concentrations (0.05–50 µg/ml) to define the 50% binding to SAP by ELISA. IgG, at a concentration of 50% binding to SAP, was incubated with different concentrations of SAP (0–50 µg/ml) overnight at 4°C. The mix was centrifuged at 14 000 rpm for 20 minutes. The presence of free anti-SAP antibodies in the supernatant was assayed by transferring the supernatants to SAP coated 3% BSA blocked ELISA plates. After a 4 hour incubation at room temperature, the bound anti-IgG antibodies were probed by antihuman IgG conjugated with alkaline phosphatase, and an appropriate substrate. Between each step, extensive washings with PBS-Tween 0.05% were performed. The percentage inhibition was defined according to:

$$\% \text{ Inhibition} = ((\text{OD of sample with no competitor} - \text{OD of sample with competitor}) / \text{OD of sample with no competitor}) \times 100$$

Statistical analysis

The Pearson linear correlation and the two tailed p value were used to assess the correlation between raised anti-SAP antibody levels and clinical disease. The receiver operating

Figure 1 Titres of anti-SAP antibodies in 189 patients with SLE; 54 patients with SLE with clinically active disease (group I—Israel), 135 patients with SLE with clinically inactive disease (group II—Germany), and in 54 healthy controls.

characteristics curve analysis was performed for the evaluation of raised anti-SAP antibody levels and the SLEDAI score. Student's *t* test was used for group comparisons when appropriate.

RESULTS

Prevalence of anti-SAP antibodies

In a multicentre cohort study, 452 samples (328 patients with SLE, 124 normal controls) were screened for anti-SAP antibody titres. Raised anti-SAP antibody titres were found in 145 (44%) (range 22%–69% for the different clinics) of 328 SLE samples, compared with 2% of the control group ($p < 0.001$) (table 1). The mean titre of anti-SAP antibodies in patients with active disease ($n = 54$) was higher than in patients with inactive disease ($n = 135$) and in controls ($n = 39$) (mean (SD) OD at 405 nm 0.34 (0.11) *v* 0.115 (0.05) *v* 0.08 (0.04), respectively; fig 1). In a randomly selected group of 112 patients, 69 (62%) had raised both anti-SAP antibody and anti-dsDNA antibody titres, whereas only 32 (28%) had raised anti-dsDNA antibody titres without raised anti-SAP antibody titres.

IgG and IgM isotypes of anti-SAP antibodies were measured in 149 SLE serum samples. The IgG isotype was raised more frequently than the IgM isotype (88/149 (59%) and 61/149 (41%), respectively), but this was not statistically significant. Only 15 patients with SLE had both IgG and IgM isotypes.

Competition assay in five patients (four patients with raised titres of anti-SAP antibodies compared with one patient without raised titres) showed that anti-SAP and anti-dsDNA antibodies are distinct (figs 2 and 3).

Relationship between anti-SAP antibodies and clinical features and disease activity

Clinical assessment of samples from 54 patients with SLE was performed using the SLEDAI score. Patient demographics included 47 (87%) women, and the mean age was 44.7 years (range 18–73). The receiver operating characteristics curve analysis was performed for evaluation of raised anti-SAP antibody levels and the SLEDAI score. The best value for defining patients with mild-moderate disease compared with severe disease was a SLEDAI score of 7. Patients with SLEDAI score >7 were considered to have severe disease. The SLEDAI score was divided into remission (score = 0), mild disease (score 1–4), moderate disease (score 5–7), or severe disease (score ≥ 8). Thirty-one patients (57%) had raised anti-SAP antibody titres. SLEDAI scores were raised (score >0) in 26 (84%) patients with raised titres of anti-SAP antibodies. Raised anti-SAP antibody titres correlated with disease activity. A SLEDAI score of ≥ 8 , indicating severe disease, was found in 16/31 (52%) patients with raised

Figure 2 SAP dose dependent binding of protein G and dsDNA-cellulose affinity purified total IgG from four lupus patients positive for SAP (patients 1–4) and one lupus patient negative for SAP recognition (patient 5). The binding was assayed by ELISA. Data presented as OD at 405 nm, mean (SD) of three experiments.

Figure 3 Inhibition of binding of affinity purified IgG from lupus patients to SAP by SAP. Total IgG affinity purified from five lupus patients incubated with different concentrations of SAP. Patients 1–4 had raised titres of anti-SAP antibodies, but patient 5 did not. The inhibition of the binding was calculated as the percentage inhibition of total IgG to SAP by SAP. The data are presented as mean (SD) of three experiments.

anti-SAP antibody titres while only 5/23 (22%) patients without raised titres of anti-SAP antibodies had a high SLEDAI score. The average SLEDAI score in patients with raised anti-SAP antibody titres was 6.87. Figure 4 represents the percentage of patients with or without raised anti-SAP antibody titres compared with SLEDAI scores (0–4 indicating remission or mild disease, 5–7 indicating moderate disease, ≥ 8 indicating severe disease).

Although raised anti-SAP antibody titres correlated with severe disease activity (SLEDAI >7), no correlation was detected between raised anti-SAP antibody titres and specific clinical manifestations described in the SLEDAI score.

The positive predictive value was 76.2% for the probability that more severe disease is found in the presence of raised titres of anti-SAP antibody titres. The negative predictive value was 54.5% for the probability that there is mild-moderate disease in the presence of raised titres of anti-SAP antibody titres. The positive and negative likelihood ratios were 2.37 and 0.62, respectively.

In 135 randomly selected patients with SLE, the distribution of 31 clinical manifestations reported in the clinic at anytime from the diagnosis was assessed (appendix; see

Figure 4 SLEDAI scores for 54 patients with SLE, comparing the percentage of patients with and without raised anti-SAP antibody titres. Remission and mild disease: SLEDAI 0–4; moderate disease: SLEDAI 5–7; severe disease: SLEDAI ≥ 8 .

<http://www.annrheumdis.com/supplemental>). All patients were women. The mean (SD) age at serum sampling was 46.4 (13.5) years. Raised anti-SAP antibody titres in 34 (25%) patients with SLE did not show a specific pattern when compared with patients without raised anti-SAP antibody titres. The distribution of clinical manifestations in patients with raised anti-SAP antibody titres was in order of prevalence: arthritis (26/34), malar rash (19/34), photosensitivity (18/34), discoid lesions (17/34), myalgia (14/34), neuropathy (14/34), nephritis (7/34), depression (9/34). Other manifestations were less commonly found.

Serial sampling ($n = 21$) of seven patients with SLE with clinically active disease by SLEDAI who had not been previously treated and had initially raised anti-SAP antibody titres showed a decrease in anti-SAP antibody titres upon treatment (fig 5).

DISCUSSION

In this study we examined the concept that patients with SLE may have raised titres of SAP antibodies and that these autoantibodies may influence disease activity. As far as we know, we are the first multicentre group to assess the levels of anti-SAP antibodies in patients with SLE. We found that raised anti-SAP antibody titres were significantly higher in a large number of patients with active SLE (defined by SLEDAI) than in patients with inactive disease and healthy controls. The different subgroups were valuable for comparing the frequency of anti-SAP antibody titres in different patient groups.

The main aim of this study was to investigate the presence of raised titres to anti-SAP antibodies in SLE in comparison with a control group. For this reason, we chose to evaluate a large number of samples taken from patients with SLE of European and Israeli populations. Upon finding an increased frequency of these antibodies in patients with SLE, we chose then to compare the presence of anti-SAP antibody titres with (a) disease activity using SLEDAI (group I) and (b) with specific clinical manifestations (group II). The clinical information was available in two groups only. For the most part group I had active disease and group II had inactive disease. The difference in the nature of the clinics cannot be fully explained, except that the clinic from group I was a new clinic and hence had more newly diagnosed patients who had not received treatment.

Figure 5 Serial sampling of anti-SAP antibody titres in seven patients on 2–4 occasions. All patients had been recently diagnosed with SLE or had a flare. The first samples were taken before treatment. The OD for 3SD of the mean controls was 0.154.

In group I, 59% of patients had raised anti-SAP antibody titres. Raised anti-SAP antibody titres correlated with raised SLEDAI scores. In another group of 135 patients, where 31 clinical manifestations were assessed, most patients were in remission, which may explain the relatively low frequency (22%) of raised titres of anti-SAP antibody titres found. Patients with SLE may have non-active clinical disease but still have raised autoantibody titres. This is supported by a prospective cohort study where patients who were serologically active but clinically quiescent (SACQ) in three consecutive clinic visits were analysed for the development of a clinical flare over the subsequent year and were evaluated for predictive factors for flare before and during their SACQ period. Forty six patients with episodes of SACQ had a clinical flare within 1 year, while 60 did not. No predictive factors for flare were found either during or before the SACQ period. Hence, a significant population of patients with SLE are SACQ and must be followed up over time and treated only on the basis of clinical criteria.²⁵

Conversely, patients may be clinically active and serologically quiescent (CASQ). In another study, 514 patients in a single clinic, who on at least three consecutive visits had clinical activity in the absence of a low complement and raised DNA binding, were followed up for 4 years. Demographics, disease characteristics, and treatment for the CASQ periods, as well as the previous and subsequent disease course were analysed. Sixty two patients had at least one episode of CASQ lasting a mean (SD) of 9.8 (6.4) months. During these periods, patients showed evidence of clinical disease activity with a high SLEDAI score. Major organ involvement occurred in 43 patients. Of the 58 patients who had follow up after their last CASQ defining visit, nine remained CASQ for 39 (23) months. Of the remaining 49 patients, 23 became inactive, 21 became clinically and serologically active, and 5 were serologically active but clinically quiescent (SACQ). This study supports the suggestion that clinical laboratory correlation in SLE is a heterogeneous relationship. The majority of patients have clinical-serological concordance. However, a minority of patients have discordance between clinical and serological status, and are either SACQ or CASQ. Therefore, monitoring both clinical and serological features in patients with SLE is important.²⁶

A increase of anti-SAP antibody titres correlated with disease activity measured by the SLEDAI, making them potentially a good marker for active disease.

Serial sampling of patients provided important information about the change in anti-SAP antibody titres correlating with clinical improvement and treatment. Interestingly, two representative patients with SLE and secondary APS presenting with organic brain syndrome manifested as cognitive dysfunction, initially had raised titres of anti-SAP antibody titres before treatment, which decreased after treatment with prednisone and intravenous immunoglobulin or intravenous immunoglobulin alone.^{27,28} Neither patient had raised titres of anti-dsDNA, but antinuclear antibody was positive, and complement levels were low. The decrease in anti-SAP antibody titres correlated with an increase in complement levels and with clinical improvement. We suggest that a test for anti-SAP antibody titres may be ancillary to the test for anti-dsDNA antibody titres for detection of disease activity. However, owing to the small patient sample, further evaluation is necessary.

The clinical association with raised anti-SAP antibody titres suggests that these antibodies may have a role in the aberrant clearance of nuclear debris during apoptosis. The SAP-anti-SAP antibody complex may explain the normal levels of SAP detected in the serum, and possibly suggest abnormal function of the protein.²⁰

We established an ELISA for anti-SAP antibody detection in serum samples. No differences in the data were obtained in the presence or absence of Ca²⁺ ions, perhaps owing to dilutions of the serum samples. Purified IgG bound SAP with no dependency on Ca²⁺ ions. SAP molecules in the samples did not interfere with anti-SAP binding.

Two populations of anti-SAP antibodies exist. One population is cross reactive with dsDNA, and the second does not react with dsDNA. Figure 2 represents the population which is not dependent on dsDNA, exhibiting the dose dependent specific binding of anti-SAP antibodies to SAP.

In conclusion, we were able to show that raised titres of anti-SAP antibodies are present in patients with SLE, and hence may play a part by binding to SAP and altering its function. Raised anti-SAP antibody titres were detected in 145/328 (44%) patients with SLE. There was a significant correlation between raised anti-SAP and anti-dsDNA titres. Raised anti-SAP antibody titres correlated with disease activity measured by the SLEDAI. Anti-SAP antibody titres decreased as clinical disease improved. We propose that in patients with SLE, raised anti-SAP antibody titres may serve as an additional prognostic marker.

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