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Soluble CD23 levels are elevated in the serum of patients with primary Sjögren's syndrome and systemic lupus erythematosus

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SUMMARY

The low affinity IgE receptor FcεRII (CD23) is important in several aspects of T and B cell function. In this study serum levels of soluble CD23 (sCD23) were measured in three groups: 26 female patients with systemic lupus erythematosus (SLE), 21 females with primary Sjögren's syndrome (pSS) and 25 normal healthy females. The concentration of sCD23 was determined using an enhanced chemiluminescent sandwich ELISA developed in this laboratory. Increased levels of sCD23 were observed in pSS and in SLE patients compared with controls (median 23·0 *versus* 8·6, $P < 0.0002$ and 18·1 *versus* 8·6, $P < 0.002$ respectively). While the median level of sCD23 was found to be higher in pSS than in SLE the difference was not statistically significant. Patients with SLE and pSS on glucocorticoid treatment had significantly lower levels of sCD23 than patients not on this treatment (median 28·9 *versus* 14·4, $P < 0.05$). Amongst the control patients sCD23 was inexplicably lower in the female members relative to the males (median 8·5 *versus* 12·3, $P < 0.05$). Although serum IgG and IgA levels were significantly elevated in pSS and SLE patients relative to controls there was no direct correlation between sCD23 and the serum levels of these immunoglobulins. We conclude that B cell hyperactivity which occurs in both pSS and SLE is associated with raised levels of sCD23.

Keywords soluble CD23 Sjögren's syndrome systemic lupus erythematosus enhanced chemiluminescence ELISA

INTRODUCTION

The exact role of the low affinity IgE receptor FcεRII on the surface of B cells is unclear. There is increasing evidence to suggest that CD23 may influence both antigen presentation to T cells [1], and the differentiation of B lymphocytes into immunoglobulin-secreting cells. This was originally illustrated by the loss of CD23 from the surface of resting IgM^+/IgD^+ precursor B cells after isotype switching [2]. More recently this progression has been shown to occur regardless of isotype switching [3].

Soluble CD23 (sCD23) is formed by a process involving cleavage of the transmembrane protein by a membrane-associated protease to form the stable 25-kD product [4]. It has also been suggested that some of the fragments may be autoproteolytic and capable of cleaving both intact CD23 and the intermediate species [5].

It is now recognized that sCD23 behaves as a multifunctional cytokine: in synergy with IL-1, it is responsible for the differentiation of thymocytes, myeloid cell precursors and germinal centre centrocytes and in conjunction with IL-4 it has importance in IgE regulation (reviewed in [1]).

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The biological activity of both intact CD23 [4] and/or degradation products [6] appears to extend beyond mere B cell differentiation and may include the promotion of B cell growth. This remains controversial (reviewed in [7]).

Increased B cell reactivity is a feature of several rheumatic diseases. These include rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [8]. This is manifested by an increase in the number of spontaneous immunoglobulin-secreting cells, and confirmed by the increased percentage of $CD23^-$ B cells indicating cell maturity.

Elevated levels of 25-kD sCD23 have been detected within both the serum and the synovial fluid of RA patients [8,9]. An increase in the serum concentration of sCD23 also occurs in other conditions associated with abnormalities of immunoglobulin production, e.g. hyper-IgE syndrome, B-chronic lymphatic leukaemia (B-CLL) and following bone marrow transplantation [10,11]. In atopic individuals, however, both normal and elevated sCD23 levels have been reported [12,13].

In view of these findings, we compared the serum sCD23 levels in normal healthy subjects with two autoimmune conditions which are regularly associated with hypergammoglobulinaemia: SLE and primary Sjögren's syndrome (pSS). Elevations of serum sCD23 in these conditions would provide further support for its role in the regulation of B cell activity.

PATIENTS AND METHODS

Patients

Blood samples were obtained from 26 patients with SLE and 21 patients with pSS. All patients with SLE fulfilled at least four of the 1982 revised ARA criteria for the classification of SLE [14], while those with pSS were classified according to Fox's criteria [15]. For a control group we selected 13 male and 25 female hospital employees from whom blood was obtained at the time of recruitment.

MoAbs

Two antibodies directed against different epitopes on CD23 were used. Clone EBVCS2 (a kind gift from Dr Bill Sugden, McArdle Laboratory for Cancer Research, University of Wisconsin-Madison Medical School, WI) and BU38 conjugated with horseradish peroxidase (The Binding Site, Birmingham, UK).

Assay procedure

A double monoclonal sandwich assay coupled to an enhanced chemiluminescent detection system was developed to measure sCD23. Briefly, microtitre plates (Dynatech Microlite II) were coated overnight at 4°C with a 1:2500 dilution in PBS of the anti-CD23 MoAb EBVCS2. The plates were then washed with PBS containing 0·05% Tween and blocked with 3% dried milk powder in PBS for 30 min. After being shaken dry, serum or EDTA plasma samples (100 μl) were added in duplicate. A standard curve was included on each plate using sCD23 derived from RPMI 8866 (see below). After incubation the plates were washed, and BU38 conjugated with horseradish peroxidase 1:2000 was added and incubated for a further 1 h. After final washing, Amerlite signal reagent (ADL) was added and the plates read immediately in an Amerlite Chemiluminescence Analyser.

From the luminescence values obtained, a titration curve was constructed by the method of best fit polynomial from which sample concentrations were calculated.

Interplate quality control

A standard preparation was obtained from the plasmapheresis fluid of a patient with SLE. This was added to each plate to provide a means of estimating the interplate coefficient of variation. Over the concentration range 17–170 U this varied between 11·7% and 4·7%, respectively.

RPMI 1866 cells were grown in normal tissue culture medium containing 10% fetal calf serum (FCS). Cells were collected by centrifugation and washed free of serum. The pelleted cells were then resuspended in distilled water and frozen at −80°C. sCD23 was released by thawing and sonication. The preparation was subsequently clarified by centrifugation in a microfuge and the resulting preparation aliquoted and frozen at −80°C ready for use.

Measurement of total serum immunoglobulins

IgG, IgA and IgM were determined using a Beckman Array automated nephelometer. For IgE determinations a sandwich ELISA, previously developed in this laboratory, was used [16].

Statistical analysis

The standard curve included on each plate was used to calculate the sCD23 levels. As the data obtained were not normally distributed, non-parametric statistics were used for analysis. In the first instance all groups were compared using the Kruskal–Wallis test in order to determine if at least one group had an independent data distribution. Each group was then compared in turn with the other groups using the Mann–Whitney test.

RESULTS

Initial observations revealed an unusual statistically significant increase in the sCD23 level between the age-matched male and female subjects comprising the control group ($P<0·03$, Table 1). In view of this finding all further group comparisons were restricted to the females.

The distribution of sCD23 levels detected within the SLE and pSS subjects and controls are shown in the scatter plot (Fig. 1). Median levels of sCD23 in patients with SLE (median 18·1) and pSS (median 23·0) were statistically elevated compared with the normal donors (median 8·6) ($P<0·002$ and $P<0·0002$ respectively). Although the pSS patients had the highest level of sCD23 this was not statistically elevated compared with the SLE patients.

Six patients with SLE and four with pSS were on treatment with prednisolone at the time of sampling. The effect of this treatment on the level of sCD23 is shown in Table 2. As a general finding, the median level of sCD23 was significantly reduced in the group of 10 patients on steroid therapy (median 14·4 *versus* 28·9, $P<0·05$).

There was no association between sCD23 and the levels of specific immunoglobulins for either the SLE or pSS patients

Table 1. Comparison of soluble CD23 (sCD23) levels in normal males and normal females

	n	Mean age (years)	Median sCD23	Interquartile range
Male	13	30·7	12·3*	9·5–15·2
Female	25	30·3	8·5*	6·7–11·6

sCD23 levels in arbitrary units.
* Significant difference at $P<0·03$.

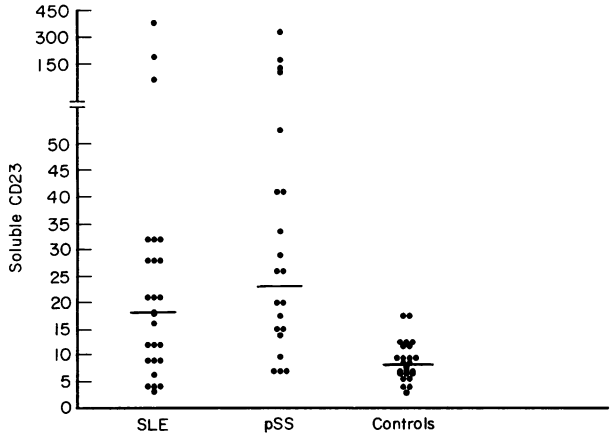

Fig. 1. Soluble CD23 (sCD23) levels measured in patients with systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS) and in normal controls. Group median values are indicated by horizontal bars.

Table 2. Comparison of soluble CD23 (sCD23) levels in systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) patients on glucocorticoid therapy with those not receiving this treatment

	n	Median sCD23	Interquartile range
Steroids	10	14·4*	6·0–20·5
No steroids	37	28·9*	17·5–40·4

sCD23 measured in arbitrary units.
* Significant difference at $P < 0.05$.

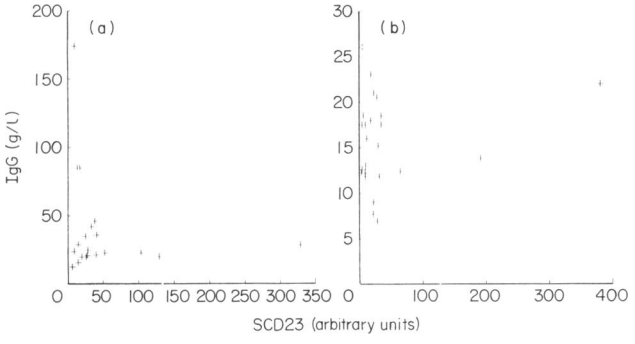

Fig. 2. (a) Scatterplot of total IgG *versus* soluble CD23 (sCD23) levels in primary Sjögren's syndrome. (b) Scatterplot of total serum IgG *versus* sCD23 levels in systemic lupus erythematosus.

Table 3. Comparison of immunoglobulin levels in primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE) patients and normal controls

Immunoglobulin class	pSS	SLE	Controls
IgG (g/*l*)	25·0 (22·2–39·0)	17·5 (12·2–18·5)	10·8 (9·3–12·5)
IgM (g/*l*)	1·3 (1·0–1·5)	1·5 (1·2–2·1)	1·75 (1·1–2·2)
IgA (g/*l*)	2·9 (2·5–3·5)	2·6 (2·0–4·1)	1·7 (1·5–2·3)
IgE (g/*l*)	30·0 (22–39)	24·0 (15–76)	23·0 (10–64)

Inter-quartile ranges are in parentheses. IgG levels in pSS *versus* controls, significant difference $P < 0.0001$; IgG levels in SLE *versus* controls, significant difference $P < 0.001$; IgA levels (pSS and SLE) *versus* control, significant difference $P < 0.05$.

(Fig. 2a, b). The level of IgG was, however, substantially raised in both these groups compared with controls ($P < 0.001$, Table 3). A less striking, yet nevertheless significant increase, was also observed for IgA ($P < 0.05$). By contrast, both IgM and IgE levels were normal and indistinguishable in all three groups (Table 3).

DISCUSSION

B cells showing spontaneous immunoglobulin synthesis are increased in the peripheral blood of patients with SLE whilst B cell hyperactivity has been observed in both the peripheral blood and bone marrow of these patients [17]. Enhanced B cell activation associated with increased numbers of immunoglobulin-secreting cells has also been noted in patients with RA [18].

In this study we have observed elevated sCD23 levels in the sera of patients with both SLE and pSS. For SLE this agrees with the preliminary finding reported by Kumagi and co-workers [8]. In addition, the patients with pSS and SLE had markedly elevated levels of IgG and increased IgA compared with normal controls.

It has recently been reported that a combination of IL-1 and sCD23 induces thymocyte maturation [19]. Furthermore, in the presence of IL-1, sCD23 inhibits apoptosis of germinal centre B cells by the induction of the bcl-2 gene [20]. It is therefore possible that raised levels of sCD23 may be important in the etiology of the hypergammaglobulinaemia associated with SLE and pSS. The absence of any direct correlation between serum sCD23 and the degree of hypergammaglobulinaemia suggests, however, that other factors are involved.

The expression of CD23 on the human monocyte line U937 has been shown to be reduced by glucocorticoid treatment [13]. Furthermore, atopic patients receiving systemic steroids had lower sCD23 levels than untreated subjects [21]. Amongst our patients with SLE and pSS, treatment with steroids was associated with a significantly lower level of sCD23. Interestingly the sCD23 level was even lower in the steroid-treated group than amongst patients with inactive disease.

The difference in sCD23 between the male and female control subjects was a little surprising. As autoimmunne conditions are more common amongst the female population, the reduced level of serum sCD23 in the female controls suggests a limited role for this protein in the initiation of the autoimmune process. It is more probable that sCD23 is involved in the continuation of the autoimmune process via B cell autostimulation. Recently a combination of recombinant 25-kD CD23 and IL-1 has been reported to provide a co-stimulatory signal for the activation of $CD4^+$ T cells [22]. A combination of all these sCD23 activities would appear, therefore, to promote the synthesis of both antigen-specific autoreactive antibodies as well as a whole host of non-specific antibodies.

As noted by Sarfati *et al.* [23], sCD23 is elevated in patients with B-CLL. The mean level of sCD23 in this condition was, in general, considerably higher than in the patients with both SLE and pSS. Conditions associated with either B cell proliferation or increased immunoglobulins appear to be associated with raised levels of sCD23. In view of the fact that immunoglobulin levels were subnormal in some of the patients with B-CLL, this would suggest that sCD23 is involved more specifically with B cell proliferation than with overall immunoglobulin synthesis.

sCD23 has been shown to be spatially associated with HLA class II molecules [24]. Furthermore, it is recognized that both SLE and pSS are associated with the HLA DR complex. It is therefore possible that HLA class II regulates either the density of cell surface CD23 or alters its susceptibility to autoproteolytic cleavage. This would introduce another facet to the already complex issue relating HLA background to predisposition to autoimmune disease. We are currently investigating variations

in sCD23 in normal subjects of different HLA class II background and autoimmune conditions such as myasthenia gravis which are associated with both HLA and non-HLA factors.

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