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Variations in serum sCD23 in conditions with either enhanced humoral or cell-mediated immunity

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SUMMARY

Soluble CD23 (sCD23) is increased by interleukin-4 (IL-4) and decreased by interferon-γ (IFN-γ). On the basis of cytokine profiles T-helper (Th) cells may be functionally divided into IL-2- and IFN-γ-secreting Th1 cells, which are involved in cell-mediated immunity (CMI), and IL-4- and IL-5-producing Th2 cells, which are involved in humoral immunity. Compared with sex-matched controls (median 8·5) we found significantly elevated levels of serum sCD23 in patients with rheumatoid arthritis (median 22·7, $P < 0{\cdot}0002$), with the highest levels detected in patients fulfilling an increasing number of the American Association revised criteria for rheumatoid arthritis. Soluble CD23 levels were also significantly raised in autoimmune thyroiditis (median 11·7, $P < 0{\cdot}02$) and myasthenia gravis (median 10·4, $P < 0{\cdot}05$). In contrast patients with either coeliac (median 6·5) or Crohn's disease (median 5·8) had reduced levels of sCD23 compared to appropriate controls (median 11·8), in both cases significant at $P < 0{\cdot}01$. Variations in sCD23 may, therefore, reflect enhanced Th1 activity in the two latter conditions in contrast to heightened Th2 activity within the three classical autoimmune conditions.

INTRODUCTION

The low-affinity receptor for IgE (FcεII, CD23) is involved in several aspects of T- and B-cell function.[1,2] Regulation of cell surface and soluble CD23 (sCD23) is maintained by the opposing actions of interleukin-4 (IL-4) and interferon-γ (IFN-γ): IL-4 up-regulates and IFN-γ down-regulates cell-surface CD23.[2] Soluble CD23 is formed by proteolytic cleavage of the transmembrane protein.[1,2] In synergy with IL-1, CD23 is a differentiating factor for early thymocytes, myeloid cell precursors and germinal centre centrocytes.[1,2] Other activities attributed to sCD23 include the encouragement of T-cell growth, inhibition of monocyte migration, triggering of histamine release from mast cells and regulation of IgE synthesis.[2] Recent evidence also confirms a co-stimulatory role of sCD23 in the activation of CD4$^+$ T cells.[3]

Previous work in mice has suggested a functional division of CD4$^+$ helper T cells into Th1 and Th2 based on their cytokine production profile. Recent work suggests a similar division in humans with Th1 cells producing predominantly IL-2 and IFN-γ and Th2 cells producing IL-3, IL-5, IL-6 and IL-10.[4] Conditions manifesting enhanced cell-mediating immunity (CMI) such as contact dermatitis are associated with increased Th1 activity while those associated with augmented humoral activity are associated with increased Th2 activity.[4,5]

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We have recently observed sCD23 to be significantly elevated in primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE).[6] These are both associated with autoantibody production, B-cell hyperactivity and hypergammaglobulinaemia, i.e. features indicative of increased Th2 activity. While the aetiology of Crohn's and coeliac disease is unclear, both manifest several features of increased CMI that are in keeping with increased Th1 activity. We therefore compared serum sCD23 levels in three classical autoimmune situations with these two latter diseases.

MATERIALS AND METHODS

Patients sera and normal controls

The number of patients and the selection criteria[7] for each test group are detailed in Table 1. For each clinical group investigated, patient sera together with appropriate normal control sera were randomly distributed on each plate so as to minimize the effect of interplate variation. In view of our previous findings,[6] which indicated a significant difference in sCD23 levels between male and female controls, serum levels of sCD23 in each of the test groups were related to a sex-matched control group randomly selected from Health Service employees.

Monoclonal antibodies

Two monoclonal antibodies recognizing distinct sCD23 epitopes were used; clone EBVCS2 (a kind gift from Dr B. Sugden,

Table 1. Clinical and laboratory selection criteria for each of the patient groups studied

Patient group	*n*	Criteria
Rheumatoid arthritis	61	ARA revised criteria[7] and rheumatoid factor positive
Autoimmune thyroiditis	13	Anti-microsomal Ab titres > 1: in 10,000 and/or anti-thyroglobulin Ab > 1:10,000
Myasthenia gravis	29	Anti-acetylcholine receptor antibodies detected $> 1 \times 10^{-9}$ M
Coeliac disease	21	Clinical symptoms, abnormal jejunal morphology and response to gluten withdrawal
Crohn's disease	17	Clinical symptoms, characteristic radiological features and bowel granuloma

University of Wisconsin-Madison Medical School, WI) was used as the capture agent, and peroxidase-conjugated BU38 (The Binding Site, Birmingham, U.K.) employed for detection.

Chemiluminescent ELISA

The details of the optimized sCD23 assay have been described previously.[6] Briefly, this utilized a microtitre method in which sCD23 in the serum samples was captured by the anti-CD23 monoclonal antibody [clone EBVCS2] and bound sCD23 detected by peroxidase-conjugated clone BU38. Enzyme activity was measured by addition of commercial Amerlite signal reagent prior to reading in an Amerlite analyser. In each case serum sCD23 levels were calculated by reference to a calibration curve included on each plate (Fig. 1).

Standardization and interplate quality control

A reference sCD23 preparation, for the construction of standard curves on each plate, was made as follows: RPMI-8866 cells were grown in tissue culture medium (RPMI-1640 with HEPES buffer) containing 10% foetal calf serum (FCS). Cells were collected by centrifugation and washed four times in culture medium without FCS. The pelleted cells were resuspended in distilled water and frozen at −80°. Thawing and sonication were used to release sCD23 and the resulting preparation was clarified by centrifugation in a microfuge, prior to aliquoting and storage at −80°. This material was assigned an arbitrary value of 1000 U/ml (1000 arb units/ml). We have previously determined the interplate coefficient of variation to be 11·7–4·7% over the log order 17–170 arb units.[6]

Assessment of assay interference by rheumatoid factors

The potential interference by rheumatoid factors was assessed in two ways. Cross-linking of the mouse coating and detection of monoclonal antibodies were tested in the following manner: plates were coated overnight at 4° with purified mouse IgG (10 μg/ml). After washing, 20 rheumatoid factor positive sera (titre 1:256 or greater) and an equal number of normal controls were added. The remaining steps of the assay, including the addition of the peroxidase-conjugated BU38 monoclonal antibody, were identical to the method described above for the detection of sCD23.

In the second experiment using the same sera, sCD23 levels were measured conventionally as described and similarly after the addition of 1% normal rabbit sera to the BU38–horseradish peroxidase (HRP).

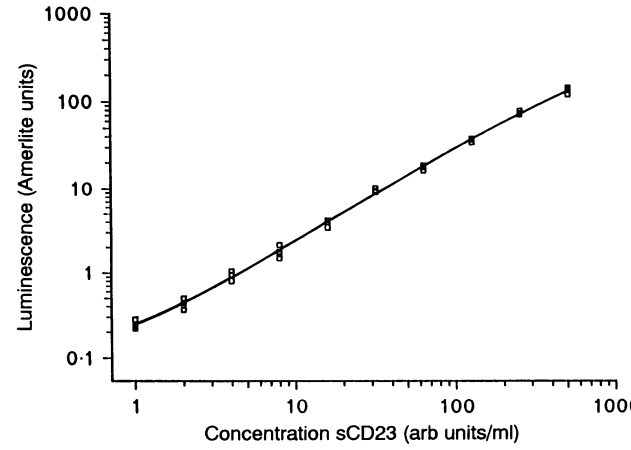

Figure 1. Dose–response curve measured as variation in chemiluminescent signal for increasing concentration of sCD23 (arb units/ml).

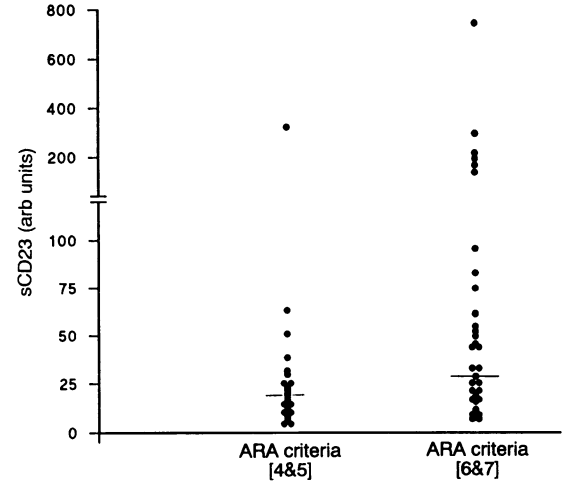

Figure 2. Comparison of sCD23 levels measured in diagnosed RA patients fulfilling differing numbers of ARA criteria. Patients with four or five criteria had significantly lower levels of sCD23 (median 16·3) compared to those fulfilling six or seven criteria (median 32·5) ($P < 0·02$).

C-reactive protein (CRP)

This was measured using an automated Beckman nephelometer.

Statistics

Group comparisons were undertaken using the Mann–Whitney test.

RESULTS

Our results show a wide variation in the concentration of serum sCD23 among the groups investigated. As the patients with autoimmune disease were predominantly females, our analysis was restricted to female patients and controls. In contrast; in coeliac and Crohn's disease the sexes were equally represented

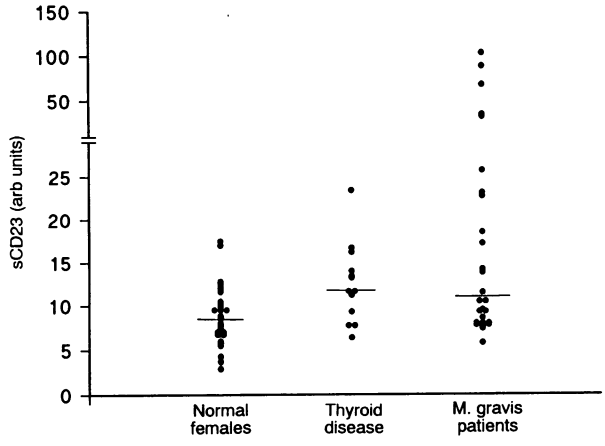

Figure 3. Scatterplot and median levels of sCD23 levels detected in thyroid disease (median 11·7) and myasthenia gravis patients (median 10·4) compared with normal female controls (median 8·5) ($P < 0.02$ and $P < 0.05$ respectively).

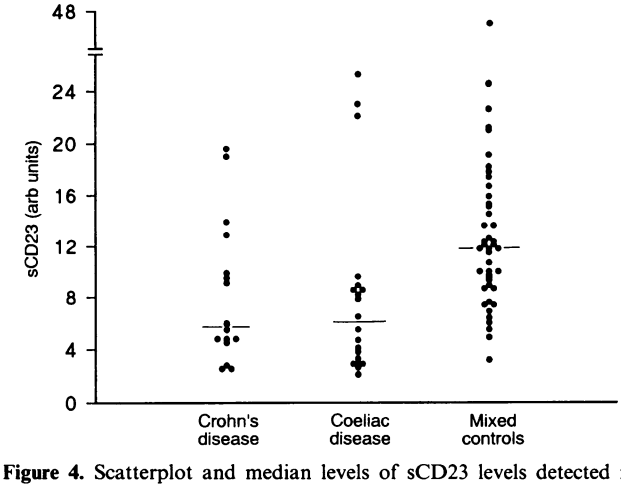

Figure 4. Scatterplot and median levels of sCD23 levels detected in Crohn's (median 5·8) and coeliac disease (median 6·5) compared with sex-matched controls (median 11·8) ($P < 0.01$ in both cases).

and comparison was undertaken with an equivalent group of mixed controls.

The highest levels of sCD23 occurred in rheumatoid arthritis (RA) patients with non-organ-specific autoimmunity [median 22·7; inter-quartile range (IQ) 12·0–52·2]. The possibility that the high levels of sCD23 detected resulted from the presence of rheumatoid factors (RF) was discounted as RF positive sera were indistinguishable from controls in their ability to cross-link mouse immunoglobulins. Furthermore the addition of 1% normal rabbit sera to the BU38–HRP conjugate produced only a small reduction (mean < 4%) in the detected enzyme activity.

Patients with RA were subsequently subdivided with respect to disease activity. For patients satisfying four or five American Rheumatism Association (ARA) criteria the sCD23 levels were lower (median 16·3) compared with patients satisfying six or seven criteria (median 32·5). This difference was statistically significant ($P < 0.02$) (Fig. 2).

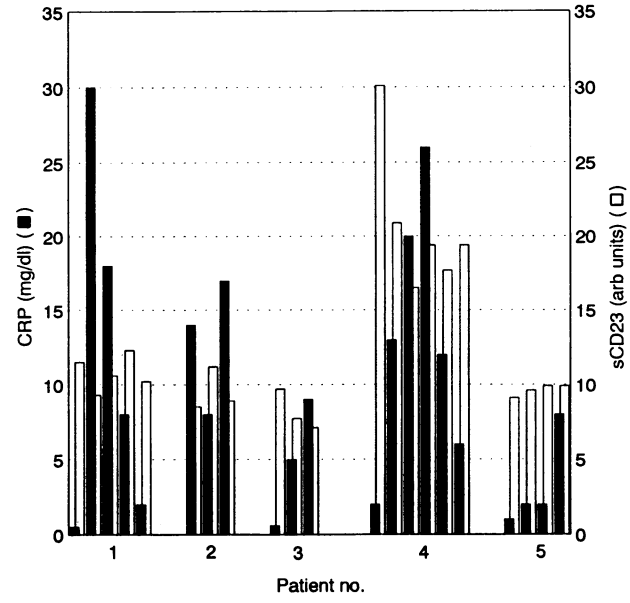

Figure 5. Variation of sCD23 with CRP in patients with infection, but without autoimmunity, monitored over 2–4 weeks.

The increase in sCD23 in autoimmune thyroditis (median 11·7) and myasthenia gravis (median 10·4) was in both cases lower than in RA but nevertheless still significantly elevated relative to female controls (median 8·5) (Fig. 3).

These findings were in direct contrast to the patients with Crohn's and coeliac disease (Fig. 4) in whom there was a significant depression of sCD23 (median levels 5·8 and 6·5, respectively) compared to the sex-matched controls (median 11·8). Normal serum protein levels in both of these groups disproved the possibility that these low levels were a consequence of hypoproteinaemia resulting from malabsorption. Amongst the patients with coeliac disease, dietary gluten exclusion produced no significant increase in sCD23 and levels remained subnormal in both treated and untreated patients.

The possibility that elevation of sCD23 represents an acute phase response was investigated by randomly selecting 20 patients without autoimmunity in whom the CRP was either normal (> 0·4 mg/dl) or elevated (> 7·0 mg/dl). There was no difference in sCD23 levels in these two groups. Sequential CRP and sCD23 estimations in five patients with infective illness, but without clinical or serological evidence of autoimmunity, are shown in Fig. 5. This confirms the absence of any correlation between sCD23 and CRP. Moreover sCD23 levels were relatively stable over the assessment period which ranged from 2 to 4 weeks.

DISCUSSION

Efforts to establish the importance of individual cytokines in specific autoimmune diseases have often produced conflicting results. This is particularly so in RA.[8,9] To this extent disease expression is determined by the net balance of cytokine activity. This is affected by several factors: the activation state of different immune cells, cytokine-binding proteins and inhibitors, and the differential expression and functional activity of cytokine receptors to mention but a few.[9,10] Assessment of the overall balance of cytokine activity would, however, be emi-

nently instructive in deciphering the intricacies of immune system involvement in different diseases.

The regulation of human CD4+ T-cell function by the opposing action of IFN-γ produced by the Th1 subset and IL-4 produced by the Th2 subset mirrors the control of sCD23 by these cytokines.[2,5] It is therefore possible that serum levels of sCD23 provide an indirect means of assessing the overall balance of Th1 and Th2 activity in various disease states. On a practical note this may allow a more precise use of immunosuppressive therapy.[11]

Systemic autoimmune disease has been induced by the repeated administration of low doses of $HgCl_2$, D-penicillamine or gold salt preparations to susceptible strains of mice, rabbits and rats.[12] Similarly autoimmunity occurs with the chronic stimulation of graft-versus-host disease (GVH) produced by the injection of parental lymphocytes into adult non-irradiated F_1 hybrid mice.[13] In each case both autoantibody production and polyclonal B-cell hyperactivity are evident and enhanced Th2 activity is suggested by increased IgE levels or IL-4 expression.[12]

Until recently low or normal levels of IgE in human autoimmune conditions with hypergammaglobulinaemia and polyclonal B-cell activation suggested a limited role for IL-4. Sarfati *et al.*[14] now describe a 16,000 MW fragment of CD23 which is capable of inhibiting IgE production. This suggests that the latter may be important in autoimmune disease. Furthermore, recent work confirms the second complement receptor (CR2, CD21) is a ligand for CD23.[15] CD21 is found on B cells, some T cells and follicular dendritic cells. Triggering of CD21 by Epstein–Barr virus (EBV), iC3b, C3d or anti-CD21 antibodies leads to B-cell activation. Increased levels of sCD23 may, therefore, encourage B-cell activation and growth by interaction with CD21. As both proteins are produced by B cells the possibility of autocrine stimulation is clearly evident.

Our results (in this study and previously) show significantly elevated levels of sCD23 both in organ-specific and non-organ-specific human autoimmune disease. This suggests an overall predominance of Th2 activity which is compatible with increased B-cell reactivity. This appears to be greater in the latter. In organ-specific disease, locally increased IFN-γ suggestive of enhanced Th1 activity may be important in the initiation of the autoimmune process. In established disease, however, our results suggest an overall predominance of Th2 activity which would appear to be necessary for the autoantibody production. A similar situation would prevail in rheumatoid synovium.

The aetiology of both Crohn's and coeliac disease is unclear. In both these conditions autoantibodies are uncommon. In Crohn's disease raised levels of IFN-γ have been recorded in both patient sera and following phytohaemagglutinin stimulation of peripheral blood mononuclear cells.[16] Histological analysis of diseased bowel, moreover, shows T-cell infiltration and granuloma formation (features of 'delayed-type' hypersensitivity). These findings are strongly suggestive of enhanced Th1 activity. In coeliac disease hypersensitivity to the gliadin fraction of gluten is considered aetiologically important and T-cell involvement is clear from analysis of jejunal biopsies.[17] These show advanced CMI damage in the destructive type III mucosal lesion.[17] On balance this suggests a predominance of Th1 activity which is confirmed by the low levels of sCD23 in these patients. In a provisional analysis of patients with ulcerative colitis (UC) we have found normal levels of sCD23 ($n=7$, median 9·4). This would suggest that the immunological abnormality in UC and Crohn's disease is different. Furthermore the low levels of sCD23 in Crohn's and coeliac disease reflect a specific immune abnormality rather than simply the gastrointestinal location of the immunological disturbance.

The low sCD23 in coeliac patients was no different in untreated or treated patients. This is consistent with coeliac patients having an innate tendency to enhanced Th1 function. Furthermore, the low serum sCD23 levels in coeliac patients may represent the basal circulating level of this protein. Increased local and systemic concentrations of IFN-γ may, however, be possible, for example, by gluten ingestion in those hypersensitive to this protein. This would produce the pathgonenic changes characteristics of increased CMI without causing further depression of sCD23. A natural tendency to Th1 activity would produce coeliac disease in people sensitive to gluten and Crohn's disease in those sensitive to other undefined antigen(s). We are currently investigating serum sCD23 levels in close relatives of patients with coeliac disease to see if enhanced Th1 activity is a heritable trait.

In autoimmune disease, however, sCD23 may be more directly involved in disease pathogenesis and levels appear to vary with disease activity and treatment with steroids.[6] Estimation of serum SCD23 may therefore be of some value in the monitoring of autoimmune disease.

Recent work confirms a close spatial association on the cell surface between CD23 and HLA-DR molecules.[18] Moreover, the expression of both proteins on the B-cell surface is up-regulated by IL-4. As CD23 is co-stimulatory for CD4+ T cells it is possible that the interaction between Th1 and Th2 cells is influenced by HLA-DR. We have documented variation in sCD23 in four conditions associated with the HLA-DR3 haplotype (this report and ref. 6). Three of these have features associated with enhanced Th2 activity (primary Sjögren's syndrome, myasthenia gravis (MG) and autoimmune thyroid disease). In the fourth condition (coeliac disease) enhanced Th1 activity is also evident. The cause of this difference is unclear. It is possible that the pattern of T-helper cell responsiveness is determined by the antigenic stimulus or, more precisely, by the combination of processed peptide and major histocompatibility complex (MHC) class II protein.

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