β_2 -Microglobulin and its binding activity in serum from patients with SLE

P. E. EVRIN AND T. STRÖM

From the Departments of Clinical Chemistry and Rheumatology, Central Hospital, Boden, Sweden

SUMMARY Repeated determinations of β_2 -microglobulin (β_2 m) and β_2 -microglobulin binding activity (β_2 m ba) in serum were performed during a follow-up of 23 patients with SLE. β_2 m was determined by a radioimmunoassay. Individual mean values were raised in 65% of the patients. The mean value for all patients, 2.9 mg/l, was significantly higher than that of the controls. The β_2 m concentrations paralleled the clinical disease activity in several cases with different disease manifestations. The β_2 m ba was determined by precipitation of 125 I-labelled β_2 m with polyethylene glycol. Increased β_2 m ba was found in 26% of the SLE patients with the 90th percentile in 40 healthy subjects being taken as the upper normal limit. There were, however, no significant differences between the mean values for β_2 m ba in the patients and the controls. Patients with systemic lupus erythematosus (SLE), suffering exacerbation of their disease, had a higher mean β_2 m ba than those with less active disease. Variations of the serum β_2 m ba occurred especially in patients with active disease but did not seem to reflect the clinical course. The β_2 m ba was recovered in the IgG peak when SLE serum was subjected to gel chromatography on Sephadex G-200.

β_2 -Microglobulin (β_2 m) is a small protein with a molecular weight of 11 800¹ and has an amino acid sequence which is related to the constant parts of heavy and light chains of immunoglobulins.^{2,3} The protein is located on the surface of nucleated cells⁴ and is a constant subunit of the HLA antigens.⁵⁻⁷

β_2 m is normally found in serum, urine, and other body fluids.^{8,9} The serum β_2 m level is normally rather constant in both sexes except for a slight increase with age.⁹ Elevated serum levels have been found in patients with a decreased glomerular filtration rate,^{10,11} in patients with SLE,¹²⁻¹⁴ and also in some rheumatic diseases such as rheumatoid arthritis,¹⁵⁻¹⁷ Sjögren's syndrome,^{18,19} and ankylosing spondylitis.²⁰

Autoantibodies directed against β_2 m have been reported in SLE by several investigators.^{14,20-23} Evidence has also been published against the presence of such antibodies.²⁴ A lymphocytotoxic effect of antibodies against β_2 m in SLE patients has been described.^{14,22}

There seem to be no reports of the long-term variation of serum β_2 m and these autoantibodies in

SLE patients. In this study the results of repeated determinations of the serum β_2 m and β_2 -Microglobulin-binding activity (β_2 m ba) are presented. A characterisation of the molecular size of the β_2 m-binding factor(s) was also performed.

Materials and methods

Twenty-three patients (21 women, 2 men; mean age 37, range 17-68 years) with a clinical diagnosis of SLE were investigated. All except one patient with a deficiency of complement C2 were antinuclear antibody (ANA) positive. Twelve patients fulfilled 4 or more of the American Rheumatism Association (ARA) criteria for classification of SLE.²⁵ In those with fewer symptoms or criteria the diagnosis had been made after observation and investigations for other connective tissue diseases, especially drug induced lupus, mixed connective tissue disease, systemic sclerosis, and rheumatoid arthritis. Latex tests for rheumatoid factor were negative for all patients. Seven patients had renal involvement (haematuria >5 erythrocytes per high-power visual field and/or proteinuria >0.2 g/l). Central nervous system (CNS) lupus (neurological or neuropsychiatric abnormality and abnormal electroence-

Accepted for publication 14 June 1983.

Correspondence to Dr P. E. Evrin, Department of Clinical Chemistry, Central Hospital, S-961 85 Boden, Sweden.

phalogram) was diagnosed in 3 cases. Thirteen patients had various locomotor and cutaneous symptoms but no involvement of major internal organs. Eighteen patients were on treatment with antimarials, corticosteroids, or azathioprine or combinations of these.

Four patients were adequately examined on only one occasion. In the other cases clinical and laboratory assessment was performed on several occasions (mean 4, range 2–11) during a mean follow-up time of 12.3 months (range 2–20 months).

At each examination blood samples were drawn for determination of serum β_2 m and β_2 m ba and serum stored at -20°C until analysis. Further laboratory tests included a full blood count, examination of urinary sediment, and determination of the serum concentrations of creatinine and the complement components C3 and C4 by a single radial immunodiffusion method (Nor-Partigen, Behring Institute). Urinary albumin was analysed by a Dipstix test, and when positive the total protein was determined.

Serum β_2 m was determined by a radioimmunoassay, Phadebas β_2 -Micro-test (Pharmacia Diagnostics). The coefficient of variation of the method during the study was 9% as calculated from values obtained in control serum analysed in each assay.

The β_2 m ba was analysed by mixing 200 μl serum with 100 μl ^{125}I -labelled β_2 m containing 0.6 ng of the protein. The tracer was the same as that used in the Phadebas β_2 -Micro-test. Incubation was then carried out for 3 hours at room temperature. Then 300 μl of bovine serum and 1.6 ml 15% polyethylene glycol (PEG) 6000 in 0.1 M phosphate buffer, pH 7.4, containing 0.05% NaN_3 was added to precipitate the labelled β_2 m bound in complexes of high molecular weight. After being mixed for a short time the samples were centrifuged at 1400 g for 30 min at $+4^\circ\text{C}$. The supernatant was discarded and the radioactivity counted in the pellet. The β_2 m ba was expressed as a percentage of the total amount of tracer added to each sample. Each sample was analysed in duplicate. One serum sample was divided in aliquots and stored at -20°C . The sample was assayed for β_2 m ba by 20 determinations in 9 separate assays during an 8-month period. A mean value of 19.4% was obtained with a coefficient of variation of 8.9%.

The molecular size of the β_2 m ba was investigated by gel chromatography on Sephadex G-200 (Pharmacia Fine Chemicals) of serum from one healthy subject and from the patient, A, with the highest β_2 m ba. The β_2 m ba in the serum samples were 5.6 and 29% respectively. 2 ml of serum was applied to the column and eluted with 0.01 M Tris-HCl, pH 7.3, containing 0.9% NaCl and 0.05% NaN_3 . The absor-

bance at 280 nm and β_2 m ba was then determined in the eluate.

Results

SERUM β_2 -MICROGLOBULIN

The mean serum β_2 m level in the patient group, as calculated from the mean values for each patient, was 2.9 mg/l, SD 0.99, range 1.58–5.61 mg/l (Fig. 1). The range for single values was 1.3–8.0 mg/l in the 108 samples analysed. Fig. 1 also shows the normal range of the assay as used in our laboratory. Fifteen of 23 patients (65%) had mean values above the

Fig. 1 Serum β_2 m. Each dot represents the mean value for one patient. Horizontal line: mean value for all patients. Shaded area: normal range.

upper normal limit. The mean value for the patients fulfilling 4 or more ARA criteria did not differ from that of the patients with fewer criteria (*t* test, $p > 0.2$). No patient had elevated serum creatinine value. The glomerular filtration rate, determined in 6 of the 7 patients with renal disease, was normal except in 2 cases, one with an endogenous creatinine clearance of 71 ml/min and another with an inulin clearance of 69 ml/min/1.73 m². The corresponding serum β_2 m values were 3.2 and 3.1 mg/l, respectively.

The mean serum β_2 m value in the patient group was significantly higher (*t* test, $p < 0.005$) than that of 36 healthy blood donors (mean 1.56 mg/l, SD 0.18). These subjects had a mean age of 41 years.

The mean serum β_2 m values did not differ significantly between the 3 clinical types of disease as defined above (*t* test, $p > 0.5$). The 2 highest individual values, 8.0 and 6.9 mg/l, were noted in one patient with myositis and arthritis and one with renal involvement with at that time a normal creatinine clearance.

The single values of serum β_2 m showed a significant positive ($p < 0.05$) linear correlation with the erythrocyte sedimentation rate ($r = 0.46$) and the β_2 m ba ($r = 0.34$). Negative correlations were found with the blood haemoglobin level ($r = -0.40$) and the number of peripheral blood lymphocytes ($r = -0.26$). No significant correlations were found between serum β_2 m and serum creatinine, C3, C4, or the numbers of peripheral blood leucocytes and platelets.

SERUM β_2 -MICROGLOBULIN BINDING ACTIVITY

The mean β_2 m ba in the patient group was 8.2%, SD 3.2, range 6.0–19.5%. The values were calculated from the mean value for each patient. Single values ranged from 5.6 to 24% in the 93 samples analysed.

The control group included 19 men and 21 women who were subjectively healthy and not receiving any drugs except for oral contraceptives in some cases. The mean age was 41.2 years, range 21–61. Fig. 2 shows the mean value and the skew distribution of the values in these controls. There was no significant ($p > 0.25$) difference between men and women. 90% of the controls had values $< 8.9\%$. If this was considered the upper normal limit, 26% of the SLE patients had elevated mean values. However, the mean value for β_2 m ba of the SLE patients was not significantly higher than that of the controls (Wilcoxon's rank sum test, $p > 0.05$). The mean value for the patients fulfilling 4 or more ARA criteria did not differ from that of the patients with fewer criteria (*t* test, $p > 0.3$).

There were no significant differences between the mean values for β_2 m ba in the 3 clinical types of

Fig. 2 β_2 m ba in the serum of controls (C) and SLE patients. For the patients each dot represents the mean value for one individual. Horizontal lines: mean values for the groups.

disease. The single values for β_2 m ba showed a significant ($p < 0.05$) positive correlation with serum creatinine ($r = 0.50$) and serum β_2 m ($r = 0.34$) and a negative one with the blood haemoglobin concentration ($r = -0.40$). The β_2 m ba did not correlate with the numbers of peripheral blood lymphocytes or serum C3 or C4.

CHARACTERISATION OF THE β_2 -MICROGLOBULIN BINDING ACTIVITY

Serum from one patient, A, with a high β_2 m ba, was

Fig. 3 Gel chromatography on Sephadex G-200 of serum from one SLE patient. The absorbance at 280 nm and the β_2 m ba were determined in the eluate.

Fig. 4 Gel chromatography on Sephadex G-200 of serum from one healthy subject with a low β_2 m ba. The absorbance at 280 nm and the β_2 m ba were determined in the eluate.

separated by gel chromatography. The peak containing the β_2 m ba eluted mainly in the same position as IgG (Fig. 3). When serum from one healthy subject with a low β_2 m ba was analysed in the same way, it did not show any peak of β_2 m ba (Fig. 4).

The β_2 m concentration was also determined by radioimmunoassay on the G-200 eluate of serum from patient A to see if the β_2 m ba could interfere in the assay. However, the only peak obtained in the assay was that corresponding to free β_2 m.

FOLLOW-UP

The disease activity, assessed clinically, was divided into 'low activity' and 'exacerbation'. Exacerbation was defined as: (1) The occurrence of nephropathy, CNS lupus, or serositis; (2) an increase of haematuria or proteinuria, with 100% or more in patients with

Fig. 5 Serum β_2 m and β_2 m ba in patients, grouped according to the disease activity. Each dot represents the mean value for one patient. Bars denote individual ranges. Horizontal broken lines denote mean values for the groups.

pre-existing renal involvement; (3) aggravation of cutaneous and/or locomotor symptoms requiring a doubling of the corticosteroid dose to at least 20 mg prednisolone a day. According to these definitions 6 patients had exacerbations.

Fig. 5 shows that the mean levels of serum β_2 m and β_2 m ba were higher in the group of patients with exacerbations than in the group with less active disease ($p < 0.05$ and $p < 0.025$ respectively). It is also seen that the levels of both β_2 m and β_2 m ba in many cases showed considerable variations, which tended to be greater in cases with exacerbations.

In 5 of the 6 cases with exacerbations elevated levels of serum β_2 m seemed to precede or parallel the increased disease activity. Two cases are shown in Figs. 6 and 7. There was also some tendency of the C3

and C4 concentrations to rise with normalising β_2 m levels. In the less active cases it was not possible to relate the serum β_2 m levels to the more subtle clinical changes.

The β_2 m ba showed great variations in 4 of the 6 patients with an exacerbating course of their disease. In these 4 patients the elevated values first tended to become normal when corticosteroid and immunosuppressive therapy was intensified. However, during sustained clinical remission rising and very high values were again noted in 2 of 3 cases. Two patients had an onset of nephropathy during the study but showed only smaller variations in the β_2 m ba. In the less active cases some elevated values were found but no correlations with the course of the disease.

Fig. 6 Patient A. Serum β_2 m, β_2 m ba, C3, and C4. Longitudinal study in a patient with lupus nephropathy. Shaded area: normal range of β_2 m, C3, and C4. Arrow ($\leftarrow$): 90th percentile of β_2 m ba in the controls.

Fig. 7 Serum β_2m , β_2m ba, C3, and C4. Longitudinal study in a patient with CNS lupus. Shaded area: normal range of β_2m , C3, and C4. Arrow ($\leftarrow$): 90th percentile of β_2m ba in the controls.

Discussion

The study comprised patients with various manifestations of SLE, except for subjects with overt renal insufficiency or severe serositis, who are not treated at the department of rheumatology. Patients fulfilling fewer than 4 ARA criteria for classification were included, as such patients constitute one part of the spectrum of SLE.²⁶

Our findings of elevated mean values of serum β_2m in 65% of SLE patients with a normal or virtually normal glomerular filtration rate are in accordance with earlier observations.¹²⁻¹⁴ As in other studies,^{13, 20} elevated β_2m levels were not related to any particular clinical subset of the disease. A finding not previously reported was the great variations of the β_2m serum level that several patients showed during the long-term follow-up. Most of these variations occurred in the group of patients with active disease, who also had a significantly higher mean value for β_2m than the less active cases. Elevated β_2m values tended to become normal during treatment. Thus in at least some patients with SLE elevated levels of serum β_2m seem to reflect an increased disease activity. Of interest in this context are the negative correlations found between the serum β_2m values and the blood haemoglobin concentrations and the numbers of

blood lymphocytes respectively, since anaemia and lymphocytopenia are associated with active SLE.²⁷

Experimental studies^{28, 29} and clinical observations¹⁸ support the concept that the high concentrations of β_2m found in serum, synovial fluid, and saliva in rheumatoid arthritis and Sjögren's syndrome are due to an increased synthesis of β_2m , presumably as a result of an activation of the immune system. The same explanation could be valid for the elevated serum levels in SLE. A decreased renal elimination, caused by complexing of β_2m and autoantibody into larger, less filtrable complexes, has also recently been proposed.²⁰

Increased serum β_2m values could also be caused by an interference of the β_2m ba in the radioimmunoassay. However, in our gel chromatography study neither complexed β_2m nor eventual antibodies against the protein seemed to react in the assay.

In accordance with earlier studies^{14, 21, 23} gel chromatography experiments with serum from one patient showed the molecular size of the β_2m ba to correspond to that of IgG. However, our method of β_2m ba is not specific for immunoglobulins. Thus it is possible that other substances capable of binding β_2m in larger complexes might also be measured in the assay.

Increased β_2m ba was found in 26% in our

material, corresponding to 21%, reported by Revillard *et al.*,¹⁴ who used a method similar to ours. When 128 samples from SLE patients and healthy subjects were analysed by our method and by that of Revillard *et al.*, the correlation coefficient between the methods was found to be 0.88. In 2 other studies^{20 21} increased values were found in 71% and 42% respectively. The discrepancies may be due to the methods used or to differences in the patients.

In our patients elevated β_2 m ba in SLE sera was not correlated with any particular clinical subset of SLE, and this is in agreement with other studies.^{14 20} During the course of the disease the β_2 m ba showed considerable variations in several patients. The mean serum β_2 m ba was higher and the variations greater in patients with exacerbations than in patients with more inactive disease. When the individual patients were studied, however, no consistent pattern emerged. Some patients with clinically well-controlled disease showed rising and even extremely high β_2 m ba, while others, developing involvement of internal organs during the study, continued to be in the normal range. The variations were in general slower than those of the serum β_2 m and seem compatible with the concept that the β_2 m ba is an antibody.

Relatively high levels of β_2 m ba were also obtained in 4 apparently healthy subjects, 3 of whom did not give any medical history of significance. The fourth subject had a father with ankylosing spondylitis, a disease which also is associated with increased β_2 m ba.²⁰ It is of interest that not only SLE patients but also some of their relatives have been shown to have lymphocytotoxic antibodies against β_2 m.²² When the control material was analysed by the method of Revillard *et al.*,¹⁴ 2 of the 4 mentioned individuals had elevated values and one a value at the upper normal limit.

Virus infection has long been regarded as a possible pathogenetic factor in SLE. It has been proposed that the anti- β_2 m antibodies are directed at altered β_2 m on the surface of cells, transformed by virus, or at β_2 m complexed with viral antigens.^{14 21} However, the mechanism for the production of β_2 m ba is unknown. Except in SLE and ankylosing spondylitis increased β_2 m ba has been demonstrated in rheumatoid arthritis.^{20 23} Our findings of increased β_2 m ba also in some healthy subjects raise the possibility that an elevated β_2 m ba might be due to a nonspecific activation of the immune system. Substances such as bacterial lipopolysaccharides and Epstein-Barr virus have been shown to induce production of autoantibodies by polyclonal activation of autoreactive B lymphocytes.³⁰ A chronic virus infection or a deficient capacity to eliminate B lymphocyte activating substances could result in a persistent autoantibody production. Individual differences in

the faculty of the immune system to respond to non-specific stimuli might be one explanation for elevated β_2 m ba in certain patients as well as in some healthy subjects.

We are grateful to Mr Eddy Rehnberg for his skilful technical assistance.

References

- Berggård I, Bearn A G. Isolation and properties of a low molecular weight β_2 -globulin occurring in human biological fluids. *J Biol Chem* 1968; **243**: 4095-103.
- Smithies O, Poulik M D. Initiation of protein synthesis at an unusual position in an immunoglobulin gene. *Science* 1972; **175**: 187-9.
- Peterson P A, Cunningham B A, Berggård I, Edelman G. β_2 -microglobulin. A free immunoglobulin domain. *Proc Natl Acad Sci USA* 1972; **69**: 1697-701.
- Evrin P E, Pertoft H. β_2 -microglobulin in human blood cells. *J Immunol* 1973; **111**: 1147-54.
- Nakamuro K, Tanigaki N, Pressman D. Multiple common properties of human β_2 -microglobulin and the common portion fragment derived from HL-A antigen molecules. *Proc Natl Acad Sci USA* 1973; **70**: 2863-5.
- Peterson P A, Rask L, Lindblom J B. Highly purified papain-solubilized HL-A antigens contain β_2 -microglobulin. *Proc Natl Acad Sci USA* 1974; **71**: 35-9.
- Grey H M, Kubo R T, Colon S M, *et al.* The small subunit of HL-A antigens is β_2 -microglobulin. *J Exp Med* 1973; **138**: 1608-12.
- Evrin P E, Peterson P A, Wide L, Berggård I. Radioimmunoassay of β_2 -microglobulin in human biological fluids. *Scand J Clin Lab Invest* 1971; **28**: 439-43.
- Evrin P E, Wibell L. The serum levels and urinary excretion of β_2 -microglobulin in apparently healthy subjects. *Scand J Clin Lab Invest* 1972; **29**: 69-74.
- Bernier G M, Cohen R J, Conrad M E. Microglobulinaemia in renal failure. *Nephron* 1968; **218**: 598-9.
- Wibell L, Evrin P E, Berggård I. Serum β_2 -microglobulin in renal disease. *Nephron* 1973; **10**: 320-31.
- Evrin P E, Wibell L. Serum β_2 -microglobulin in various disorders. *Clin Chim Acta* 1973; **43**: 183-7.
- Weissel M, Scherak O, Fritzsche H, Kolarz G. Serum β_2 -microglobulin and SLE. *Arthritis Rheum* 1971; **19**: 968.
- Revillard J P, Vincent C, Rivera S. Anti- β_2 -microglobulin lymphocytotoxic autoantibodies in systemic lupus erythematosus. *J Immunol* 1979; **122**: 614-8.
- Manicourt D, Brauman H, Orloff S. Plasma and urinary levels of β_2 -microglobulin in rheumatoid arthritis. *Ann Rheum Dis* 1978; **37**: 378-82.
- Sjöblom K G, Saxne T, Wollheim F A. Plasma levels of β_2 -microglobulin in rheumatoid arthritis. *Ann Rheum Dis* 1980; **39**: 333-9.
- Ström T, Evrin P E. β_2 -microglobulin in rheumatoid arthritis. *Ann Rheum Dis* 1981; **40**: 211-3.
- Talal N, Grey H M, Zwaifler N, Michalski J P, Daniels T E. Elevated salivary and synovial fluid β_2 -microglobulin in Sjögren's syndrome and rheumatoid arthritis. *Science* 1975; **188**: 1196-8.
- Ström T, Evrin P E, Karlsson A. Serum β_2 -microglobulin in Sjögren's syndrome. *Scand J Rheumatol* 1978; **7**: 97-100.
- Curry R, Thoen J, Shelborne C, Gaudernack G, Messner R. Antibodies to and elevations of beta-2-microglobulin in the serum of ankylosing spondylitis patients. *Arthritis Rheum* 1982; **25**: 375-80.
- Ooi B S, Ooi Y M, Pesce A J, Pollak V E. Antibodies to β_2 -microglobulin in the sera of patients with systemic lupus erythematosus. *Immunology* 1977; **33**: 535-41.

- 22 Messner R P, Horatius R, Ferrone S. Lymphocytotoxic antibodies in systemic lupus erythematosus patients and their relatives. *Arthritis Rheum* 1980; **23**: 265–71.
- 23 Falus A, Merety G, Glikmann G, *et al.* β_2 -Microglobulin-containing IgG complexes in sera and synovial fluids of rheumatoid arthritis and systemic lupus erythematosus patients. *Scand J Immunol* 1981; **13**: 25–34.
- 24 Plesner T, Wiik A. Demonstration of electrophoretic heterogeneity of serum β_2 -microglobulin in systemic lupus erythematosus and rheumatoid arthritis: evidence against autoantibodies to β_2 -microglobulin. *Scand J Immunol* 1979; **9**: 247–54.
- 25 Cohen A S, Reynolds W E, Franklin E C, *et al.* Preliminary criteria for the classification of systemic lupus erythematosus. *Bull Rheum Dis* 1971; **21**: 643–8.
- 26 Lom-Orta H, Alarcon-Segovia D, Diaz-Jouanan E. Systemic lupus erythematosus. Differences between patients who do, and who do not, fulfil classification criteria at the time of diagnosis. *J Rheumatol* 1980; **7**: 831–7.
- 27 Fries J F, Holman H R. *Systemic lupus erythematosus*. Philadelphia, London, Toronto: Sanders, 1975; 79–82.
- 28 Evrin P E, Nilsson K. β_2 -microglobulin production in vitro by human hematopoietic, mesenchymal and epithelial cells. *J Immunol* 1974; **112**: 137–44.
- 29 Karlsson F A, Groth T, Sege K, Wibell L, Peterson P A. Turn-over in humans of β_2 -microglobulin: the constant chain of HLA-antigens. *Eur J Clin Invest* 1980; **10**: 293–300.
- 30 Möller E, Ström H, Al-Balaghi S. Role of polyclonal activation in specific immune response. *Scand J Immunol* 1980; **12**: 177–82.