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Serum Beta-2-microglobulin Binding Activity in Monoclonal Gammopathy: Correlative Study and Clinical Significance

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Abstract—*Serum β2m binding activity (S β2m-BA) was determined by a polyethylene glycol exclusion test of radiolabeled human β2m in 185 serum samples from 62 patients with multiple myeloma (MM). Elevated S β2m-BA was found in more than half of the samples from IgG myeloma taken before treatment or during progression of the disease but not during the plateau-phase. Conversely, elevated S β2m-BA was found in only one case of IgA myeloma, one case of monoclonal gammopathy of undetermined significance and none of the Bence Jones myelomas. S β2m-BA appears to be related to disease progression in IgG myeloma. The activity is supported by minute amounts of serum autoantibodies which are distinct from the monoclonal component. S β2m-BA was independent from serum β2m levels.*

INTRODUCTION

BETA-2-MICROGLOBULIN (β2m) is a low-molecular-weight protein (mol. wt, 11,800) synthesized by all nucleated cells and originally isolated from human urine [1]. β2m is, in fact, the light chain of histocompatibility antigen present on the membranes of most cells [2]. Serum β2m (S β2m) is the free form of β2m [3]. It is noteworthy that high S β2m levels were found in patients with lymphoproliferative diseases, especially of B cell lineage, including multiple myeloma (MM) [4–11]. Recently, we have shown that S β2m levels were highly correlated with the myeloma cell mass and that for patients evaluated following induction chemotherapy, there was also a clear correlation between S β2m levels and the magnitude of tumor regression or progression [12].

Increased binding activity directed towards free β2m (Sβ2m-BA) was detected in patients with systemic lupus erythematosus (SLE) by Ooi *et al.* [13] and more recently by ourselves [14]. Assays of S β2m-BA on protein fractions obtained by chromatography showed the presence of increased S β2m-BA with 7 S fractions. Subsequently, this 7 S material reactive to β2m was demonstrated to be IgG [13, 14]. Finally, in patients with SLE we have shown that such an IgG binding activity to free β2m represented a component of the low-avidity antibodies reactive to lymphocyte surface usually called 'cold lymphocytotoxins' [14]. Increased binding activity was also found in patients with rheumatoid arthritis [15].

More recently, IgM anti-β2m antibodies were found in patients with leprosy by Bahr *et al.* [16]. In fact, the nature of this binding activity remains unclear and the term of 'autoantibodies' is still discussed.

In this study we have evaluated the S β2m-BA of 62 patients with MM in comparison with that of (a) normal controls (NC) and (b) patients with monoclonal gammopathies of undetermined significance (MGUS). In MM, S β2m-BA was correlated with the main presenting features and the clinical status of patients. We have been more especially interested in patients with MM since this disease is often associated with both (a) high serum levels of free β2-m in relation to an increased synthetic rate and/or a frequent renal failure [17] and (b) low levels of polyclonal immunoglobulins, due to a depressed synthesis in connection with suppressor cells [18], in contrast

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to high levels of a monoclonal immunoglobulin, most frequently of IgG or IgA type.

MATERIALS AND METHODS

Patients

Serum β2m levels and S β2m-BA were evaluated in 62 patients with MM (i.e 185 serum samples), using the diagnostic criteria of the Southwest Oncology Group [19]. The mean age (yr) was 62 ± 12 (extremes, 30–85) and the sex ratio M/F = 1/1. Thirty patients had IgG myeloma, 15 IgA and 16 pure Bence Jones only. Furthermore, 1 patient had IgD myeloma. Sixty-six percent had kappa subtype and 34% lambda light chain subtype. Hypercalcemia (defined as adjusted serum calcium level >2.625 mmol ‰) [20] was noted in 18% of cases. According to the clinical staging system of Durie and Salmon [21], 15% of patients were stage I, 32% stage II and 53% stage III. Seventy-five percent of patients had normal creatinine levels (defined as levels 107 μmol ‰ in males and 99 μmol ‰ in females) and 25% abnormal levels. Only 8% of our patients had serum creatinine levels >150 μmol ‰ (= stage B) [21]. One-third of the patients were studied both at the time of diagnosis and at various intervals during induction chemotherapy. Two-thirds of the patients were studied only during treatment, at the time of primary treatment failure, in remission with plateau-phase or relapse. At initial staging the myeloma cell mass (i.e. TBMC) and during treatment the TBMC changes were evaluated according to the programs of Salmon and Wampler [22], considering changes in body weight, [^{131}I]-albumin plasma volume and serum monoclonal protein rates. Response to chemotherapy was defined as a ⩾50% regression of the TBMC and relapse as a ⩾50% increase of myeloma cell mass over the remission levels. In responsive patients the plateau-phase was defined as a ⩾6 months steady-state phase without any significant changes in TBMC [23]. Patients were treated every 5 weeks with intermittent courses of a combination of cycle non specific drugs (melphalan, cyclophosphamide) plus prednisone with or without vincristine.

S β2m levels and S β2m-BA were simultaneously assessed in 37 controls (= healthy blood donors) and in 9 MGUS (=12 serum samples), 7 of IgG type and 2 of IgA type. These asymptomatic patients were selected on the following criteria: (1) low and stable monoclonal protein levels (⩽1.5 g % for IgG, ⩽1.0 g % for IgA, follow-up at ⩾2 yr); (2) bone-marrow plasmocytosis ⩽10% without atypical plasma cells; (3) normal plasma-cell bone marrow acid phosphatase activity [24]; and (4) absence of urine monoclonal component ('Bence Jones protein') and of reduced levels of normal serum immunoglobulins [19].

Methods

Assay for Sβ2m. S β2m levels were measured by a radioimmunoassay developed in our laboratory using a coprecipitation technique, as previously described [25].

S β2m-BA. The method has been described in another report [14]. Briefly, β2m was purified from the urine of renal transplanted patients, as previously described [26]. Highly purified β2m was labeled with ^{125}I using the chloramine T method [25] and diluted (10 ng/ml) in borate buffer 0.125 M, NaCl 0.075 M, pH 8.3. Fifty microliters of this buffer supplemented with Tween 20 (1.6%) were mixed with 100 μl of serum and 50 μl of 125 I-β2m (0.5 ng). After 1 hr at room temperature 1 ml of PEG (6000 MWT, Merck, 10% in borate buffer) was added and the mixture left for 40 min in melting ice, then centrifuged (2300 *g*, 20 min). Supernatants were then discarded and the radioactivity of the pellet counted in a gamma counter. For each serum the results were expressed as percentage precipitation (defined as ratios of precipitation of the tested sample divided by the radioactivity precipitable by trichloracetic acid). Optimal concentration of PEG (10%) and dilution of serum (1:4) were determined in preliminary experiments using rabbit anti-β2m antisera diluted in normal human serum.

Statistical studies. For statistical analysis we used the Wilcoxon *W* test, the chi-square method (χ^2 test for 2 × 2 contingency tables) with the Yates correction when necessary. Bivariates analyses (= linear correlation) were performed with the Pearson correlation coefficient.

RESULTS

As illustrated in Table 1, S β2m-BA levels were not significantly different in MM and MGUS in comparison with normal controls. Only 1 serum from MGUS and 21 from MM had a binding activity greater than 3 standard deviations from the mean. The 21 positive measurements were found in the sera of 12 patients, 1 with an IgA MM and the others with IgG MM.

Sera of patients who were studied repeatedly over a long period of time were classified according to the type of the monoclonal component and the patients' clinical status. As shown in Table 2, β2m-BA was elevated in sera from 13 patients with IgG MM, taken either before treatment or during progressive disease (including patients with primary failure and relapse after more than 6 months of remission).

Table 1. Comparison of serum beta-2-microglobulin binding activity (S β2m-BA) in normal controls, patients with monoclonal gammopathies of undetermined significance (MGUS) and patients with multiple myeloma (MM)

Clinical status (No. of serum samples)	Serum beta-2-microglobulin binding activity S β2m-BA (mean values ± S.D.)	% abnormal S β2m-BA values (i.e. values ⩾ 3.87*)
Normal controls ($n = 56$)	2.98 ± 0.55	(0/56)
Monoclonal gammopathies of undetermined significance ($n = 12$)	2.92 ± 0.69	(1/12)
Multiple myeloma ($n = 185$)	3.31 ± 2.08†	(21/185)‡

*3.87: upper normal limit of 3 standard deviations from the mean of normal values.
†Not significantly different to normal controls and to monoclonal gammopathies of undetermined significance (MGUS).
‡21 abnormal values in 12 distinct patients.

Table 2. Serum β-2-microglobulin binding activity (S β2m BA): relation to clinical status and immunoglobulin type

Clinical status	MM Ig type	Samples Tested	Samples Positive	S β2m-BA (mean ± S.D.)
Pretreatment	IgG	9	5	3.99 ± 0.84*
	IgA	7	0	2.83 ± 0.58†‡
	BJ	4	0	2.88 ± 0.32†‡
Plateau-phase	IgG	34	0	2.89 ± 0.31‡
	IgA	20	0	2.87 ± 0.40‡
	BJ	19	0	2.48 ± 0.27†‡§‖
Progressive disease	IgG	14	8	4.46 ± 1.30*
	IgA	15	0	2.86 ± 0.25‡
	BJ	22	0	2.70 ± 0.28‡

*Significantly different from normal controls ($P < 0.02$, Wilcoxon W test).
†Significantly lower than IgG type, of the same clinical status ($P < 0.05$, Wilcoxon W test).
‡Not significantly different from normal controls.
§Significantly lower than IgA type ($P < 0.001$, Wilcoxon W test).
‖Significantly different from BJ pre-treatment values ($P = 0.05$, Wilcoxon W test) and BJ with progressive disease ($P < 0.02$).

Abnormal β2m-BA was never observed in treated patients during the plateau-phase. Patients with MM of BJ type had significantly lower β2m-BA values than the other types during the plateau-phase.

Two patients had highly elevated S β2m-BA: 1 sample was obtained from a 62-yr-old female with IgGκ progressive MM despite treatment. Two other samples from the same patient, not included in this study, confirmed the high S β2m-BA (20%). Four sequential samples were obtained from a second patient, a 72-yr-old female with IgA MM; all values ranged between 12 and 15% during progressive disease.

A possible relationship between S β2m-BA and serum IgG levels was studied in the group of patients with IgG MM. No such correlation was found (Fig. 1). However, if patients were classified in two groups according to their serum IgG levels, a tendency towards an elevation of S β2m-BA in

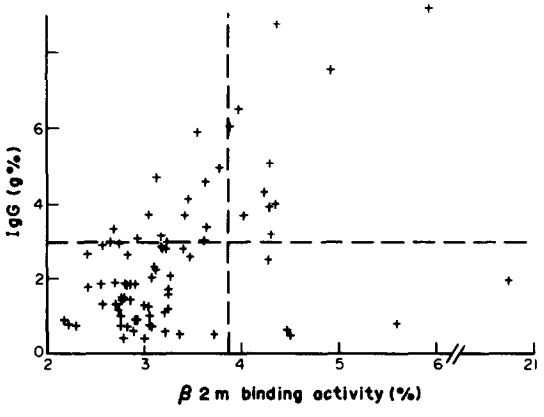

Fig. 1. Relation between β2m BA and IgG levels. The dashed lines indicate the upper limit of normal values.

patients with IgG levels greater than 3 g % was noticed (Table 3). Such a relationship might depend on the cut-off points and in any case remained of only borderline significance.

Among the 185 serum samples, 27 were obtained from patients with renal insufficiency and S β2m levels greater than 3 mg/l. This group includes 9 sera from IgG MM and 18 from IgA or BJ MM. None of them had elevated S β2m-BA.

Forty-five samples were obtained from patients with high tumor mass and S β2m levels greater than 3 mg/l, including 20 sera from IgA or BJ MM with normal S β2m-BA except in the case discussed above. Conversely, 13 of the 25 sera from IgG MM had an elevated S β2m-BA. Taking all the 185 sera together, no correlation was found between serum β2m levels and S β2m-BA. When the same relationship was studied within each series of IgG, IgA or BJ type, no correlation was found either.

Finally, the possible relationship of S β2m-BA to tumour mass was studied. No such correlation could be demonstrated in IgG, IgA or BJ MM.

DISCUSSION

In this study S β2m-BA was found to be elevated only in certain types of MM. Elevation was observed in only one case of IgA myeloma among the 15 studied, in 0 of the 15 BJ MM and 1 among 12 MGUS. Conversely, 11 of the 31 patients with IgG MM had elevated Sβ2m-BA in one or several occasions. Furthermore, SB2m-BA appears to be related to disease activity within the group of IgG MM. Indeed, elevated values were found in more than half of pretreatment samples and in sera taken during progressive disease but in none of the 34 samples obtained during a plateau-phase as defined by Durie *et al.* [23].

Therefore determination of S β2m-BA in patients with IgG MM may provide a new biological criterion related to disease activity. This parameter might be useful in patients with elevated pretreatment values. Further studies will be needed to determine whether S β2m-BA might represent a useful index of the patient's response to treatment.

Unlike other biological markers, S β2m-BA appears to be loosely related to tumor mass and not precisely correlated with serum IgG levels in patients with IgG MM. No correlation (positive or negative) was found between serum β2m levels and S β2m-BA. Although the precise proportion of free and bound β2m have not been measured in MM sera, it is noteworthy that high S β2m-BA may be associated with normal or elevated serum levels of free β2m. A comparable situation can be found in rheumatoid arthritis, in which the same serum may contain monomeric and complexed IgG as well as rheumatoid factor.

The characterization of S β2m-BA in MM will be described in another report [Vincent *et al.*, submitted]. The β2m binding factor is clearly distinct from the monoclonal component, as shown by the following: (1) the β2m binding capacity is much too low to be accounted for by the antibody specificity of the monoclonal component; (2) fractionation experiments performed with the only serum from IgA MM with high S β2m-BA showed that this activity was associated with IgG but not with IgA; (3) the serum from IgG MM was passed on β2m immunoadsorbent. All S β2m-BA was removed by this procedure but most of the IgG, including the monoclonal component, were not bound to the immunosorbent. A minute amount of IgG antibodies with β2m-BA could be eluted from the column; and (4) finally, labeled β2m was found to form large complexes greater than 200,000 daltons when mixed with IgG MM serum, indicating that at least two determinants of the molecule were complexed

Table 3. Influence of IgG levels on the S β2m-BA of IgG myeloma patients

IgG levels in serum (g %) (No. of patients)	Serum beta-2-microglobulin binding activity (S β2m-BA)	
	Normal	Abnormal*
<3 ($n = 21$)	17	4 (19%)
⩾3 ($n = 10$)	3	7 (70%)†
Total ($n = 31$)	20	11

*Abnormal values: values greater than 3.87, the upper normal limit of 3 standard deviations from the mean of normal values.

†Significantly different to patients with low serum IgG levels (i.e. <3g%): $P < 0.02$, χ^2 method with Yates correction—contingency table 2 × 2.

with antibody combining sites, thus ruling out the monoclonality of the antibody.

The background level of S β2m-BA in normal serum could be attributed to the presence of natural auto-antibodies of the IgG class [Vincent *et al.*, submitted]. The precise amount of such antibody is difficult to determine, but it can be estimated to represent less than 1 in 10,000 IgG molecules. Therefore, myeloma with such antibody activity should be quite rare in contrast with other MM with other auto-antibody activity [27].

Increased production of autoantibodies to β2m is likely to be attributed to an immunoregulatory defect. Indeed, such autoantibodies are elevated in autoimmune diseases such as systemic lupus erythematosus [13, 14] and rheumatoid arthritis [15, 28, 29].

Although less frequently than in autoimmune diseases, elevated S β2m-BA was also found in leprosy [16], acute viral hepatitis, chronic active hepatitis and a few cases of inflammatory bowel diseases [Vincent and Revillard, unpublished data]. The precise immunoregulatory disorders responsible for the increased production of these autoantibodies are still unknown. The data presented in this report showing that S β2m-BA is reduced in BJ MM during the plateau-phase but increased in active IgG MM may help to clarify the mechanisms of this disorder.

Autoantibodies to β2m have been shown to cause platelet aggregation [30], whereas heterologous antibodies of the same specificity are known to affect various lymphocyte responses [26]. It is therefore possible that β2m autoantibodies represent an additional factor contributing to some disorders in MM patients.

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