Beta-2 Microglobulin—An Immunogenetic Marker of Inflammatory and Malignant Origin

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ABSTRACT

Major contributions have been made in the last few years to the knowledge of the structure, fate and cellular origin of beta-2 microglobulin (B₂M); but the question of its function still remains unclear. The concept of B₂M being simply a marker of renal physiology seems less satisfactory when reference is made to its relationship to the immunogenetic system. Although assay of B₂M cannot be considered as a specific diagnostic tool, it still may be regarded as a useful parameter in monitoring inflammatory, malignant, and auto-immune disease activity.

Introduction

Beta-2 microglobulin (B₂M) was isolated in 1964 by Berggard⁷ from the urine of patients with Wilson’s disease or chronic cadmium poisoning. Its characterization in 1968 determined that it consisted of a single polypeptide chain of about 100 amino acids.⁴ Research regarding this particular microprotein has evolved along two lines. The first, mostly clinical, established its importance as a marker of renal function.²⁴ The more recent, more fundamental, led to the discovery of its close association with immunoglobulins and cell surface histocompatibility antigens.²² Although the two areas overlap, various aspects of beta-2 microglobulin and its relationship to the immunogenetic system will be reviewed.

Structure and Function

Beta-2 microglobulin is a protein of low relative molecular weight (Mᵣ 11800) that is present in small amounts of normal urine, serum, and other biological fluids. It is structurally related to immunoglobulin G²⁵ and to the cell-surface histocompatibility antigens. It is synthetized by all nucleated cells and is present on their surface. Its biological role remains unclear. At least 30 different microproteins originating from plasma have been identified since 1964 in the urine of healthy individuals.¹³ With its 11800 molecular weight and a 16 Å size, B₂M is one of the small sized proteins ideally designed to pass freely through the glomerular sieve. Some physicochemical properties of B₂M are listed in table I.
The small size of B2M has made it a suitable candidate for determining the sequence of the amino acid residues that make up the peptide (figure 1). From studies of the amino acid sequences of B2M, it appears that the arrangement of the 100 amino acids of this moiety bears a very striking homology with the constant domains of the heavy and light chains of the immunoglobulins, especially with the CH3 domain. However, the circulating B2M does not stem from a splitting of immunoglobulins. In fact it has been shown to be present on the surface of lymphocytes and later on the surface of almost all nucleated circulating cells, mesenchymal and epithelial cells as well. Although there is a significant amino acid homology between B2M and the constant domains of IgG, B2M does not react with antisera to the heavy or light chains of the immunoglobulins. In table II are described various immunologic properties of B2M.

**Clinical Chemistry of B2M**

Beta-2 microglobulin circulates in the blood at detectable levels and is also present in urine, saliva, colostrum, and amniotic fluid. In healthy subjects, the concentration of B2M in serum fluctuates within a narrow range of values, from 900 to 2500 µg per L. The urine range is 6.0 to 300 µg per L. These values appear to be independent of the immunoassay used. Determination of B2M in human urine or serum was initially by single radial immunodiffusion, but this method is not sensitive enough for detecting B2M in

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**TABLE I**

<table>
<thead>
<tr>
<th>Physiochemical Properties of Beta-2 Microglobulin</th>
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<tr>
<td><strong>Electrophoretic mobility at pH 8.6</strong></td>
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<tr>
<td><strong>Isoelectric pH</strong></td>
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<tr>
<td><strong>Molecular weight</strong></td>
</tr>
<tr>
<td><strong>Sedimentation coefficient (S_{20w})</strong></td>
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<tr>
<td><strong>Carbohydrate content</strong></td>
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<td><strong>N_2 content</strong></td>
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<td><strong>Molecular stokes radius</strong></td>
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**Figure 1.** Structural characteristics of Beta-2-microglobulin. (Taken from Poulik, M. D. and Bloom, A. D. J. Immunol. 110: 1430-1433, 1973.)

Amino-acid residues: 100
BETA-2 MICROGLOBULIN—AN IMMUNOGENETIC MARKER

...reference population urine without a preliminary concentration step. Radioimmunoassay (RIA) methods were then developed which can accurately measure B2M in normal urine or serum without sample preconcentration.

The two available radioimmunoassay (RIA) methods for B2M are the solid phase RIA technique of Evrin and the double-antibody method of Shuster et al. Solid phase RIA has also been commercialized.* The disadvantages of the radioisotope methods (short shelf-life, cost, health hazards, time of assay [six to eight hours]) have been previously discussed. A lymphocytotoxicity inhibition technique for determining B2M has recently been proposed, but the method is less accurate and sensitive than RIA and gives a semi-quantitative estimate. Bernard has recently published a highly sensitive method for the determination of B2M in human urine or serum based on the direct agglutination by B2M of latex particles on which an antibody against B2M is absorbed. The agglutination is quantitated by counting the remaining unagglutinated particles or by turbidimetry.

Interindividual variations in B2M, still unexplained, exist, but in a given reference individual the level is stable. Serum levels of B2M are independent of body mass or sex but are slightly increased in elderly persons. The narrow reference range of values is higher in malignant disorders, particularly in multiple myeloma and monocytic leukemia. Elevated values of serum B2M are considered a very sensitive indicator of the impairment of glomerular filtration rate. Healthy subjects excrete very small quantities of B2M in urine (6 to 300 μg per L) but its excretion increases considerably in tubular dysfunction, as observed in chronic cadmium poisoning, Fanconi’s syndrome, Balkan nephropathy, and Wilson’s disease. In occupational medicine, B2M in urine is the most sensitive test for early detection of tubular damage from excessive exposure to cadmium. Recent reports have also implicated increased B2M in cerebrospinal fluid of patients with leukemia or lymphoma and early relapse in the central nervous system. Increased B2M has been reported in Crohn’s disease, Sjogren’s syndrome, and rheumatoid arthritis.

**Beta-2 Microglobulin and Its Relationship to the Immunogenetic System**

Welsh et al demonstrated that lymphocytes in culture produce B2M and this production could be stimulated by mitogenic substances. Peterson showed that B2M is part of the major histocompatibility cell-bound human leukocyte antigen (HLA) complex responsible to a great extent for immuno-rejection of transplanted organs and tissues in non-genetically related hosts. Although conflicting data, mainly owing to the methodology of cell-bound HLA solubilization, have appeared, the proposed structure of HLA antigens has been postulated. Human leukocyte antigen is composed of two glycopeptide chains, so-called heavy chains, carrying the antigenic specificity and linked together through a S-S-bond embedded in the cell surface membrane. Each of the heavy chains is linked, seemingly through a non-covalent bond, to a so-called light chain which is precisely B2M. The heavy chain has allotype variants whereas the light chain has not; in other words, different HLAs bear the same B2M. It appears that HLA is always bound

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**TABLE II**

<table>
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<tr>
<th>Immunologic Properties of Beta-2 Microglobulin (B2M)</th>
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<tr>
<td>Amino acid homology and structural similarity to CH3 region of IgG</td>
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<tr>
<td>Antigenically distinct from IgG</td>
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<tr>
<td>B2M does not bind with Staph A protein</td>
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<tr>
<td>B2M is non-covalently linked to major histocompatibility antigens (HLA) on the surface of nucleated cells</td>
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to B2M and the same situation exists in the equivalent histocompatibility system of the mouse. A common ancestor gene coding for the 100 amino acid sequence of immunoglobulins and surface antigens has been suggested.

**B2M in Solid Malignancies**

Several studies indicate that in patients with a wide variety of malignant tumors, serum B2M levels may be abnormally elevated as compared to those of age-matched controls. In primary bronchial cancer, 44 percent of cancer patients had elevated serum levels when compared to their controls which had an elevated B2M in 11 percent of cases. Serum B2M levels were measured by RIA in patients with various malignant neoplasms, ascites, and also with definite or suspected hepatoma showing variable levels of serum alpha-fetoprotein. Elevated B2M levels (over 3.5 mg per L) were found in various malignant neoplasms, especially in multiple myeloma (67 percent) and hepatoma (60 percent). It is interesting to note that the ascites/serum ratios of B2M were significantly higher in patients with malignant ascites than in those with non-malignant ascites. Beta-2 microglobulin also correlated well with alpha-fetoprotein assays in those patients with defined or suspected hepatoma (r = 0.72, p < 0.001).

Serum B2M levels were measured in 210 cancer and control patients to assess the significance of this marker. Subjects included patients with breast and gastrointestinal cancer, corresponding control patients in both categories (39 women with benign breast disease and 21 gastrointestinal controls with benign gastrointestinal disease) and healthy volunteers. The composition of these groups allowed an assessment of the relative importance of changes related to cancer, benign disease, age, and sex. A significant rise in serum B2M levels with advancing age was demonstrated in the control subjects. Mean levels were also consistently higher in females than in males of each patient group. After statistical correction for these age and sex effects, mean values for B2M remained significantly higher in each of the cancer groups than in their benign controls. Patients with more advanced breast cancer had higher levels of serum B2M than those subjects with early disease. This was true for patients with stomach cancer compared to those with colorectal carcinoma. A possible interpretation of these findings is that B2M concentrations increased with increasing tumor bulk and, therefore, this determination may be of value in the treatment and management of cancer patients.

Studies relating differences in the cell surface of malignant cells with normal cells suggest that solid and superficial basal cell carcinomas lack immuno-reactive B2M on the cell surface. This finding was in contrast to studies on normal epidermis and that of various non-malignant dermatoses, including basal cell papillomas.

The concentrations of immuno-reactive B2M in cerebrospinal fluid (CSF) and cystic fluid of central nervous system tumors (gliomas, craniopharyngiomas) were also shown to have an increased concentration of B2M in cystic fluid of embryonic tumors (craniopharyngiomas) as contrasted to low grade astrocytomas.

**B2M in Leukemia and Lymphoma**

Serum B2M is usually elevated in malignancies of the beta lymphocyte lineage, including B-cell lymphomas, chronic lymphocytic leukemias, Waldenstrom's disease, and multiple myeloma. Normal B2M levels have been reported in non-malignant monoclonal gammopathies. This latter finding suggests the use of serum B2M to differentiate idiopathic benign dysgammaglobulinemia in patients with monoclonal gammopathies from B-cell malignancies (i.e., myelomas, Waldenstrom disease, chronic lymphoid leukemias, and most lymphomas). In
untreated Hodgkin’s disease B2M concentrations were shown to be correlated with the stage of the disease spread. Long-term observation of non-Hodgkin’s lymphomas also indicates that two groups can be discerned which reflects changes in the plasma proteins. The first group demonstrated increased acute phase reactant proteins during the active disease. Serum B2M was occasionally increased. In remission these subjects showed a normal protein profile. A second group was typified by a chronic elevation of B2M and sedimentation rate (ESR) but had normal C-reactive proteins. Chronic lymphocytic leukemia also showed an increased B2M level and normal acute phase proteins.

B2M in Chronic Inflammatory Diseases and Polyclonal Lymphocyte Activation

Certain inflammatory diseases involving polyclonal lymphocyte activation are associated with increased B2M synthesis. Elevated serum B2M levels have been reported in rheumatoid arthritis, Sjogren’s syndrome, systemic lupus erythematosus, sarcoidosis, Crohn’s disease, and angioimmunoblastic lymphadenopathy. Plasma and urinary levels of B2M have been studied in patients suffering from rheumatoid arthritis (RA). Despite normal renal glomerular function in all patients, 50 percent had supranormal plasma B2M and 30 percent showed elevated urinary output. This latter finding appears to be associated with higher plasma levels. The plasma B2M concentrations also paralleled the patients’ lymphocytosis and “joint count.” It was suggested that these findings reflect the increased total mass and/or membrane turnover of lymphoid tissue in RA.

In Sjogren’s syndrome, 38 percent of patients with clearly defined disease had elevated serum B2M. These patients had a higher mean age and greater frequency of the complete triad of Sjogren’s including elevated antinuclear antibodies. Elevated serum B2M was also associated with increased complications in Sjogren’s syndrome. Salivary levels of B2M have proven useful in evaluating the degree of inflammation in Sjogren’s. Striking elevations were seen in patients with associated renal or lymphoproliferative complications. The data suggest that determination of B2M in saliva may provide a simple, non-invasive technique for estimation, treatment, and evaluation of therapy of local inflammation in autoimmune disease.

Summary

The role of B2M and, for that matter, of the histocompatibility antigens is uncertain. Because of the resemblance of B2M to segments of the IgG molecule and its location on lymphocyte surfaces, it has been hypothesized that it functions in such aspects as recognition of antigens and interactions between T and B lymphocytes. Several reports indicate that in patients with a wide variety of malignant tumors, serum B2M levels may be abnormally elevated when compared to controls. This finding was more commonly observed in metastatic malignancies. The elevation of serum B2M in malignancies of the Beta lymphocyte type has helped to differentiate idiopathic benign monoclonal gammopathies from B cell cancer. Serum B2M has also been shown to correlate with the stage of certain malignant diseases. Assay of B2M appears useful in monitoring recurrent disease activity and reflecting primary monoclonal or polyclonal activation and proliferation in the lymphoid system.

References


