Changes in plasma β_2 microglobulin concentrations after allogeneic bone marrow transplantation

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SUMMARY Plasma concentrations of β_2 microglobulin (B_2M), the light chain of the class I major histocompatibility complex, were measured serially in 26 patients undergoing allogeneic bone marrow transplantation (BMT). The concentrations fell after conditioning treatment, and recovered when the marrow was transplanted. Bacterial infection did not influence B_2M concentration, but nine of 22 episodes of acute graft versus host disease were associated with raised concentrations. Increased plasma B_2M concentrations were also a feature of eight episodes of chronic graft versus host disease, and these fell after treatment. Reactivation of herpes simplex, varicella zoster, or cytomegalovirus infections were also accompanied by raised B_2M concentrations. Three patients with cytomegalovirus pneumonitis had high concentrations of plasma B_2M , the rise starting between five and 22 days before onset of symptoms. Although it is non-specific, serial measurement of plasma B_2M in patients undergoing BMT may be clinically useful in monitoring chronic graft versus host disease.

Beta₂microglobulin (B_2M), a low molecular weight non-glycosylated protein (11.8 kD) which forms the light chain of the class I major histocompatibility complex (MCH I), is present on the surface membrane of most nucleated cells.¹ The B_2M /MHC I complex is required for the recognition of target antigen by cytotoxic T cells.² Modulation of expression of the complex may have a role in immunological surveillance,³ and in organ rejection after transplantation.⁴ Raised concentrations of serum B_2M have been found in various haematological neoplasms,⁵ notably plasmacytic⁶ or monocytic⁷ proliferations, and are also increased in certain virus infections⁸ and the acquired immune deficiency syndrome (AIDS).⁹ We studied the changes in plasma B_2M concentrations after allogeneic bone marrow transplantation (BMT), with particular reference to graft versus host disease (GvHD) and viral infection, which are important causes of ensuing morbidity and mortality.¹⁰

Patients and methods

Twenty six consecutive patients undergoing allogeneic BMT were studied (table). Citrated venous blood samples were taken before conditioning treat-

ment, and about three times a week during the patients' stay in hospital. Extra samples were taken if an important clinical event occurred, and at each out-patient visit. Plasma was separated within three hours of collection and stored at minus 20°C.

Batches were assayed for B_2M by radial immunodiffusion using antiserum obtained from Atlantic Antibodies and calibrated using the Pharmacia Beta₂-micro radioimmunoassay 100 (Pharmacia Diagnostics AB, Uppsala, Sweden). The normal range for plasma B_2M is 0.8 to 2.4 mg/l. As plasma B_2M concentrations are increased by renal glomerular dysfunction,¹¹ samples with a creatinine concentration of greater than 140 μ mol/l were excluded from analysis. Concentrations of the acute phase reactant protein, C-reactive protein, were measured on the same samples by radial immunodiffusion using antisera obtained from Unipath Laboratories, Bedford, England, and standards provided by Behringwerke, Marburg-Lahn, West Germany.

Pre-transplant conditioning consisted of cyclophosphamide 60 mg per kg on two consecutive days followed by total body irradiation. Twenty two patients each received three daily fractions of about 3.3 Gy at a midline dose rate of 0.06 Gy/minute; cases 25 and 26 received 7.2 Gy and 7.45 Gy, respectively, in single fractions at a mid line dose rate of 0.16 Gy/minute; cases 8 and 12 were not irradiated. Bone

Table Details of patients

Case No	Age	Sex	Diagnosis
3	28	Female	Secondary acute myeloid leukaemia
4	20	Female	Acute lymphoblastic leukaemia, first complete remission
5	30	Female	Acute lymphoblastic leukaemia, first complete remission
6	8	Female	Acute myeloid leukaemia, first complete remission
7	27	Male	Acute lymphoblastic leukaemia, first complete remission
8	2	Female	Congenital neutropenia
9	33	Male	Chronic granulocytic leukaemia, first chronic phase
10	7	Male	Acute lymphoblastic leukaemia, first complete remission
11	14	Male	Acute lymphoblastic leukaemia, first complete remission
12	36	Male	Acute myeloid leukaemia, first complete remission
13	20	Female	Acute myeloid leukaemia, second complete remission
14	20	Female	Acute myeloid leukaemia, first complete remission
15	16	Male	Acute lymphoblastic leukaemia, first complete remission
16	20	Female	Chronic granulocytic leukaemia, first chronic phase
17	41	Male	Chronic granulocytic leukaemia, first chronic phase
18	21	Male	Chronic granulocytic leukaemia, first chronic phase
19	22	Male	Acute myeloid leukaemia, first complete remission
20	3	Male	Acute lymphoblastic leukaemia, first complete remission
21	1	Female	Acute myeloid leukaemia, first complete remission
22	35	Female	Acute myeloid leukaemia, third complete remission
23	33	Female	Chronic granulocytic leukaemia, first chronic phase
24	16	Female	Acute lymphoblastic leukaemia, first complete remission
25	20	Male	Acute lymphoblastic leukaemia, second complete remission
26	11	Female	Acute myeloid leukaemia, second complete remission
27	24	Male	Acute myeloid leukaemia, first complete remission
28	35	Male	Chronic granulocytic leukaemia, first chronic phase

marrow was harvested from a histocompatible sibling and infused within four hours of collection. Cases 3 to 26 received cyclosporin A as prophylaxis against GvHD. In cases 27 and 28 the donor marrow was treated with the rat monoclonal antibody Campath¹¹² and cyclosporin A was omitted. Bacterial infection was diagnosed when a culture grew organisms or when there was a combination of fever, strong clinical suspicion, and a serum concentration of C-reactive protein greater than 40 mg/l.¹³ When possible, the diagnosis of GvHD was confirmed by biopsy of the appropriate organ.

Results

EARLY POST-TRANSPLANT PERIOD

All patients showed falls in plasma B₂M concentrations after conditioning treatment, with the nadir at about day 8 (day 0 being the day of transplantation). The combination of cyclophosphamide and total body irradiation produced a more noticeable depression in plasma B₂M (mean 0.9 (SD 0.3 mg/l)) than did cyclophosphamide alone (1.5 (0.1) m/l).

During the second week after BMT the mean

plasma B₂M concentrations rose steadily to within the normal range. Six confirmed episodes of bacterial infection (fig 1) were associated with high concentrations of C-reactive protein (79.7, (20.1) mg/l) and a normal concentration of plasma B₂M (1.4, (0.6) mg/l).

Twenty two discrete episodes of acute GvHD (fig 2) were typified by modest increases of C-reactive protein concentrations in plasma (15.3 (14.3) mg/l), while plasma B₂M concentrations were increased in nine of the 22 samples (overall mean 2.4 (0.9) mg/l. Most episodes of acute GvHD were preceded by a rising plasma B₂M that fell in response to treatment with high doses of steroids given intravenously.

During the first 30 days after BMT most patients showed a weak positive correlation between total white cell count and plasma B₂M concentrations (mean correlation coefficient (r) = 0.3 (0.3), range -0.3 to +0.6).

LATER POST-TRANSPLANT PERIOD

Episodes of bacterial infection after day 30 were uncommon and characterised by high C-reactive protein, and normal B₂M concentrations in plasma (data not shown).

Samples were obtained at the onset of eight epi-

β_2 microglobulin concentrations after allogeneic bone marrow transplantation

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Fig 1 Comparison of plasma β_2 microglobulin and C-reactive protein concentrations in patients with graft versus host disease or infections.

Fig 2 Changes in plasma β_2 microglobulin concentrations in patient with chronic graft versus host disease.

Fig 3 Plasma β_2 microglobulin concentrations in three patients (cases 3, 7, and 14) with fatal cytomegalovirus pneumonitis; arrow indicates onset of symptoms.

sodes of chronic GvHD; six affected skin alone, and two both skin and liver. Raised concentrations of B₂M in plasma (4.4 (1.1) mg/l), and minimally raised concentrations of C-reactive protein (7.6 (13.5) mg/l, median 10.0 mg/l) were seen (fig 1). Treatment with corticosteroids and azathioprine produced a rapid fall in plasma B₂M concentrations and these reflected the activity of the disease (fig 2).

VIRAL INFECTIONS

In three of four episodes of herpes simplex viral infection and in both episodes of herpes varicella zoster reactivation there were slightly raised concentrations of B₂M in plasma (2.7, (0.4) mg/l, and 3.3 (0.7) mg/l, respectively (fig 1).

Three patients developed fatal cytomegalovirus pneumonitis (cases 3, 7, and 14). All showed a pro-

nounced rise in plasma B₂M concentrations (fig 3) starting 5, 16, and 22 days, respectively, before the onset of clinical symptoms. Renal glomerular function was normal during that period, and all three patients developed bone marrow depression and severe lymphopenia in the terminal stages. Case 16 recovered spontaneously from a less severe respiratory illness associated with bilateral pulmonary infiltrates and a positive early cytomegalovirus antigen detection test on bronchoalveolar lavage fluid. The plasma concentration of B₂M rose to 3.6 mg/l and returned to normal with clinical recovery.

Pre-treatment plasma B₂M concentrations were not significantly different between those patients (n = 12) who were positive for cytomegalovirus antibody (2.1, (0.9) mg/l) compared with those who were negative (n = 14; 1.7 (0.7) mg/l) before transplantation.

Discussion

The B₂M present in plasma and tissue fluids is believed to be shed from the surface membrane of nucleated cells.¹ After conditioning treatment for BMT there is a pronounced fall in plasma B₂M concentrations that correlates with the intensity of treatment. Underlying causes could include destruction of lymphoid tissue or other cells which are significant B₂M producers—for example, haemo-poietic precursors and cells of the mononuclear phagocyte system.^{7 14} Concentrations rise as marrow engraftment occurs.

Episodes of acute GvHD are often preceded by a rise in plasma B₂M, and concentrations above normal were recorded in nine of the 22 episodes studied. Acute GvHD is mediated by alloreactive donor T lymphocytes, and the onset is usually heralded by a rise in the number of T lymphocytes of the suppressor cytotoxic subset.¹⁵ The rise in plasma B₂M concentrations and subsequent fall after high doses of steroids, is consistent with its origin being in a steroid sensitive lymphocyte subset. T lymphocytes are hyperproducers of B₂M in vitro¹⁶ and the T suppressor lymphocytosis of infectious mononucleosis is accompanied by high serum B₂M concentrations.¹⁷

Moderately raised plasma B₂M concentrations are a feature of chronic GvHD. Again, the rapid fall in response to steroids or azathioprine suggests that lymphocytes are the main source of B₂M. Raised B₂M concentrations have been reported in several autoimmune disorders such as rheumatoid arthritis,¹⁸ systemic lupus erythematosus,¹⁹ and Sjögren's syndrome,²⁰ which have features in common with chronic GvHD. Hepatocellular damage is not the single cause of these changes, as concentrations do not rise in patients with hepatitis A or drug induced hepatitis.¹⁷ Theoretically, a rise in serum B₂M concentration could be a feature of leukaemic relapse after BMT, especially in monocytic or myelomonocytic variants,²¹ although we have not found this.

Raised plasma B₂M concentrations during episodes of herpes simplex virus, herpes varicella zoster, and cytomegalovirus reactivation are similar to those seen in patients who are not immunocompromised.⁸ Both primary infection and reactivation of cytomegalovirus cause an absolute increase in suppressor/cytotoxic T lymphocytes^{22 23} which may be a cause of high plasma B₂M concentrations. Natural killer cell activation mediated by interferon occurs in response to both murine²⁴ and human²⁵ cytomegalovirus, and natural killer cells have high concentrations of surface B₂M (much of it together with non MCH determinants).²⁶ Alpha and gamma interferon induce B₂M-MCH-I expression on a variety of

cell types,²⁷ possibly resulting in increased shedding of B₂M into plasma. Recent studies showing an association between GvHD and immune responses to cytomegalovirus are also of interest in the light of our findings. It is remarkable that BMT patients can sustain such high plasma concentrations of B₂M without renal dysfunction, but with pancytopenia, bone marrow failure, and gross depletion of lymphoid tissue (as confirmed at necropsy); this suggests hyperproduction by certain lymphoid cell clones.

In conclusion, measurement of plasma B₂M concentrations after allogeneic BMT provides an indicator of some of the complex immunological processes occurring in these patients, especially those related to GvHD and viral infections. Plasma B₂M concentrations are clearly non-specific clinically, but we have found them helpful in the monitoring of chronic GvHD when combined with routine laboratory tests and measurement of concentrations of acute phase reactant proteins in serum.

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