Solid phase radioimmunoassay for β_2-microglobulin; a sensitive index for renal allograft evaluation

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β_2-Microglobulin (β_2M), a low molecular weight protein of unknown function, was first isolated from the urine of patients with renal tubular disorders.[1] It has been shown to be identical to a polypeptide noncovalently bound to the larger HLA molecule on the cell surface of nucleated cells and to have a close structural homology with the C_H3 region of the IgG heavy chain.[2,3] This protein has been shown to be present on platelets, mononuclear cells and polynuclear white blood cells, but not on mature erythrocytes.[4] It has also been found in human serum, cerebrospinal fluid, saliva, and colostrum.[5] In serum it is present as a free monomer, which is filtered freely through the glomerular membrane, almost completely reabsorbed in the proximal renal tubules, and catabolized so that only relatively small quantities are excreted in the urine.[6,7] Its diagnostic use in several types of renal disease has been reported.[6-9]

We report our experience with radioimmunoassay for β_2M and its use in monitoring human renal allograft function. Because serum creatinine is commonly used for this purpose, we measured β_2M and serum creatinine serially in eight renal allograft patients in an attempt to determine the relative sensitivity of these two tests of allograft function.

Materials and methods

Radioimmunoassay for β_2M. The reagents for the assay were purchased from Pharmacia, Piscataway, New Jersey. The antibody was bound to Sephadex as the solid phase. In a typical radioimmunoassay protocol, 100 μl of each standard 3 to 96 μg/l was added to the appropriately marked tubes. Also, 100 μl of serum specimen already diluted to 1:200 was added. The 100 μl of antibody Sephadex complex was added while continuously stirring it. This was followed with 100 μl of ^{125}I solution approximately 30,000 to 60,000 cpm. The tubes were tightly capped and rotated for 3 hours at room temperature. Then 2 ml of isotonic saline was added to each tube and centrifuged at 2000 rpm for 5 minutes. The supernatant was aspirated and the pellet was counted. The interassay variation obtained with this assay was well within 5% to 10% at three different concentrations. The analytical recovery experiments showed an average recovery of 93%, 100%, and 98%, respectively, at three different concentrations of β_2M added to normal human plasma. In 45 normal subjects, the mean serum β_2M was 1.44 $\pm$ 0.35 μg/ml with a range of 0.74 to 2.1 μg/ml. There were no significant differences in the levels obtained at different decades of age (up to age 50) or between the male and female groups.

Serum creatinine. This was measured by the Technicon Autoanalyzer System and the normal range with this procedure was 0.7 to 1.4 mg/dl.

Patients. To evaluate the usefulness of serial determinations of serum β_2M in renal transplant patients, serum β_2M was measured before and at intervals after the renal transplant in eight patients. At the same time a serum creatinine level was also obtained. The *Table* lists the age, sex, and status of the transplant in these patients. Of eight patients, two had irreversible rejection and six had a successful renal transplant, one from an HLA-identical sibling donor. All the patients had been treated with 1 g of methylprednisolone in divided doses and azathioprine at the time of transplantation; the methylprednisolone was given again at the time of graft rejection. All patients were maintained on a tapering dose of prednisone and up to 1.5 mg/kg of azathioprine according to clinical conditions.

Results

Case 1. *Figure 1* illustrates the β_2M and creatinine levels in a 26-year-old woman with end-stage renal failure secondary to diabetes mellitus who received an HLA-identical kidney transplant from her brother which functioned well. Her pretransplant β_2M and serum creatinine were 17 μg/ml and 6 mg/dl, respectively. Within a week after transplantation both had decreased to

Table. Clinical characteristics of transplant patients

Case no.	Age/sex	Renal disease	Donor	Transplant status*
1	26 F	Diabetic nephropathy	Brother	Functioning
2	59 M	Chronic glomerulonephritis	Son	Functioning
3	35 F	Pyelonephritis	Cadaver	Functioning
4	30 M	Pyelonephritis	Cadaver	Functioning
5	47 M	Pyelonephritis	Cadaver	Functioning
6	34 F	Chronic renal failure (uncertain etiology)	Cadaver	Functioning
7	37 F	Diabetic nephropathy	Cadaver	Rejected
8	38 M	Chronic glomerulonephritis	Cadaver	Rejected

* Indicates one-year posttransplant status.

normal levels of 2.1 μg/ml and 1.1 mg/dl, respectively. She was discharged but required readmission for a urinary tract infection and rejection. At this time, her serum β_2M level was elevated (3.8 μg/ml) and the serum creatinine was normal (1.2 mg/dl). Over the next 3 days the serum creatinine progressively rose to 2.0 mg/dl. She was treated with additional prednisone and antibiotics with a subsequent decrease in β_2M and creatinine to 2.8 μg/ml and 0.8 mg/dl, respectively.

Case 2 (*Fig. 1*). A 59-year-old man who had kidney failure due to glomerulonephritis received a kidney transplant from his son. The high β_2M (60 μg/ml) and creatinine (17.5 mg/dl) dropped posttransplant, and at the time of discharge the patient had β_2M of 3.5 μg/ml and creatinine of 1.3 mg/dl. The patient was readmitted later for rejection symptoms and was found to have a further increase in β_2M (8.7 μg/ml) and serum creatinine (2.8 mg/dl). Treatment of the rejection was followed by a subsequent decrease in both β_2M and creatinine.

Case 3 (*Fig. 1*). A cadaver kidney transplant was performed in a 35-year-old woman with kidney failure due to pyelonephritis. She had a benign postoperative course, and 2 weeks posttransplant, her β_2M was 1.9 μg/ml and creatinine was 1.3 mg/dl. A subsequent rise in β_2M to 2.8 μg/ml was observed and was followed by a rise in serum creatinine from 1.2 to 2.0 mg/dl and clinical rejection. She was treated with 2 g of methylprednisolone intravenously, which resulted in a drop of β_2M to 2.1 μg/ml and creatinine to 1.4 mg/dl.

Case 4 (*Fig. 1*). A 30-year-old man with a long history of chronic renal failure due to pyelonephritis received a cadaveric kidney transplant. Pretransplant β_2M and serum creatinine levels (44 μg/ml and 14 mg/dl, respectively) were very high. One week posttransplant, his serum creatinine returned to normal (1.5 mg/dl) but the β_2M was still above normal limits (3.1 μg/ml). A further increase in β_2M was followed by a mild clinical rejection with a rise in serum creatinine to 2.6 mg/dl in the second week posttransplant requiring treatment with 2 g of methylprednisolone. Rejection recurred in the third and also fourth weeks in association with sustained elevation of β_2M between 4 and 5 μg/ml. These were reversed by appropriate therapy.

Case 5 (*Fig. 2*). A 47-year-old man re-

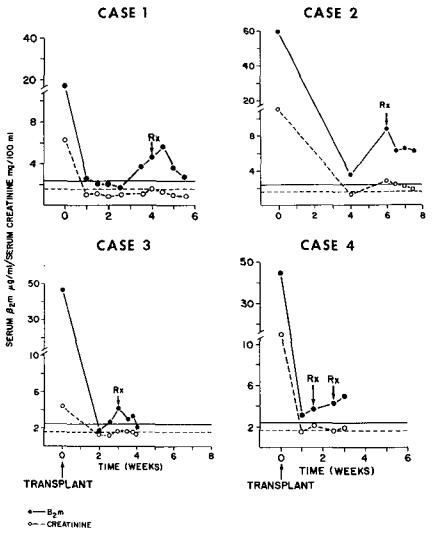

Fig. 1. Serial changes in serum β_2M and serum creatinine in renal allograft patients (cases 1 through 4). Rx indicates the treatment for rejection with methylprednisolone.

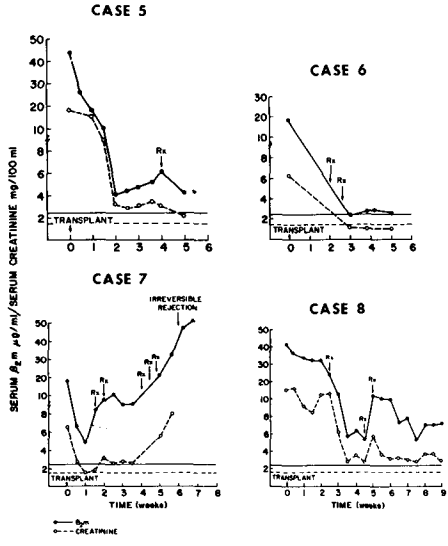

Fig. 2. Serial changes in serum β_2M and serum creatinine in renal allograft patients (cases 5 through 8). Rx indicates the treatment for rejection with methylprednisolone.

ceived a cadaveric kidney transplant that functioned after a week of acute tubular necrosis. His β_2M and serum creatinine decreased and stabilized in the second postoperative week at 4.1 μg/ml and 3.2 mg/dl, respectively. Because of leukopenia, azathioprine was discontinued for 10 days. This led to a rise in β_2M to 4.8 μg/ml followed by a rise in serum creatinine from 2.7 to 3.8 mg/dl. Again, treatment with 2 g of methylprednisolone caused a significant drop in β_2M from a peak of 6.0 to 4.2 μg/ml and creatinine to 2.2 mg/dl. The patient is doing well on maintenance doses of immunosuppressive therapy.

Case 6 (*Fig. 2*). A 34-year-old woman with end-stage renal disease of uncertain etiology did not have nephrectomy prior to transplantation. No tissue diagnosis was made for her original disease. She received a cadaveric kidney transplant, which functioned immediately, but two urinary tract infections developed and a biopsy specimen showed evidence for cellular rejection. She was treated with 4 g of methylprednisolone. This led to a decrease in serum β_2M to 2.5 μg/ml and creatinine to 1.1 mg/dl within 4 weeks after transplantation.

Case 7 (*Fig. 2*). This 37-year-old woman with chronic renal failure secondary to diabetic nephropathy received a cadaveric kidney transplant. Her β_2M remained elevated (4.8 μg/ml) one week posttransplant, though the creatinine had fallen to 1.5 mg/dl. A further rise in β_2M to 8.4 μg/ml was followed by elevation of the serum creatinine to 3.2 mg/dl. Despite improvement in the serum creatinine levels to 2.2 mg/dl after rejection therapy, there was only a minimal decrease in β_2M. Subsequently, there was a continuous rise in β_2M and irreversible rejection unresponsive to a total of 6 g of methylprednisolone.

Case 8 (*Fig. 2*). A 39-year-old man with end-stage renal disease due to nephrosclerosis received a cadaveric renal transplant that had a prolonged period of acute tubular necrosis and cellular rejection within the first several weeks after transplantation. While on hemodialysis the patient had an extremely slow rate of decrease in β_2M during the first 2 weeks posttransplant; the levels for β_2M and creatinine dropped to 32 μg/ml and 12.4 mg/dl, respectively, from pretransplant levels of 42 μg/ml and 15.8 mg/dl. As the diuretic phase began in the third week and with treatment for rejection, the β_2M and creatinine decreased to 5.7 μg/ml and 2.9 mg/dl, respectively. A subsequent rise in serum creatinine was followed by further treatment for rejection. In the eighth week posttransplant, his β_2M and creatinine were stabilized but were elevated (7.3 μg/ml and 2.8 mg/dl, respectively). Although 3 months later the serum creatinine was as low as 2.1 mg/dl, the β_2M at that time was 8.6 μg/ml. The allograft eventually underwent irreversible rejection 8 months after transplantation.

Discussion

Elevated β_2M levels have been reported in end-stage kidney failure,[7] and a direct correlation between β_2M and serum creatinine has been observed pretransplant.[6,7] In the present study, very high pretransplant β_2M levels were associated with high levels of serum creatinine. Studies utilizing serial determinations of serum β_2M measured by a radioimmunodiffusion method[8,9] have demonstrated a significant decrease in serum β_2M after transplant even in the anuric phase. The decrease that occurs with viable cadaver allografts having oliguric tubular necrosis indicates the catabolic role of transplanted kidneys on β_2M. However, the serial evaluation of both β_2M and serum creatinine after transplantation has not been described. In our experience, increase in β_2M often preceded the increase in serum creatinine and clinical evidence of graft rejection. A considerable decrease in β_2M and serum creatinine was observed following treatment for rejection in all cases except two in which irreversible rejection developed. In some cases β_2M never reached the normal range, although the serum creatinine was near or

less than 2.0 mg/dl. All these patients showed a further rise in β_2M followed by a rise in serum creatinine that necessitated treatment for rejection. Sustained elevations of β_2M greater than 6 μg/ml even in the presence of near normal serum creatinine levels, in retrospect, was a predictor of chronic progressive rejection. Also, patients with irreversible rejection showed a relatively slow rate of decline in serum β_2M. These features indicate the significance of β_2M in monitoring renal allograft function and suggest the increased sensitivity that β_2M may have over serum creatinine measurements.

Summary

A radioimmunoassay for serum β_2M was evaluated for its clinical usefulness in monitoring renal transplantation patients. In eight patients, β_2M was measured serially before and after transplantation and was correlated with serum creatinine. The pretransplant levels of β_2M were high (mean 36.6 $\pm$ 16.5 μg/ml), but decreased after successful renal transplantation at variable rates. In three patients, β_2M decreased to a normal range and subsequently rose. This increase preceded the increase in serum creatinine and clinical evidence of acute rejection. In five patients posttransplant, β_2M was above normal despite a normal serum creatinine level in three and showed a progressive increase, which was followed by a later rise in serum creatinine. A sustained decrease in serum β_2M was observed after rejection therapy with methylprednisolone in all these patients except two in whom irreversible rejection developed.

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