Changes in Serum and Urine Lysozyme Activity after Kidney Transplantation: Influence of Graft Function and Therapy with Azathioprine

Geza Horpacsy, Jörg Zinsmeyer, Karsten Schröder, and Moritz Mebel

We investigated changes in lysozyme activity in serum and urine of kidney-transplant patients, and found that the production and catabolism of lysozyme in such patients differs markedly from that in normal subjects. Resumption of graft function decreases the high serum lysozyme activity by increasing the rate of catabolism in the transplant; at the same time, however, the production is inhibited by therapy with azathioprine. Changes in serum lysozyme activity correlate well with leukocyte count; thus its determination might be useful in monitoring immunosuppression. The urinary excretion of the enzyme, although not specific to rejection, is a good index of the degree of tubular damage.

Additional Keyphrases: monitoring immunosuppression • assessing granulocyte turnover, proximal tubular dysfunction • diagnosis of acute leukemia • renal disease

Lysozyme (EC 3.2.1.17), a widely investigated protein of low molecular weight (14 500), is normally present in plasma in a concentration of about 5.6–9.4 mg/liter (1–3). Only trace amounts of active enzyme are excreted in the urine. Lysozymuria, by definition, is characterized by an urinary lysozyme concentration exceeding 1.9 mg/liter (2).

The turnover of lysozyme is very rapid. It is produced in neutrophil granulocytes, monocytes, and macrophages of the reticuloendothelial system (1, 4, 5). Bone marrow may be an important source of the enzyme, owing to the presence and probable breakdown of neutrophil granulocytes in this organ (6).

The presence of lysozyme in the secretions of various serous glands as well as in polymorphonuclear granulocytes, monocytes, and macrophages, and in Kupffer cells of the liver conforms with the widely held view that lysozyme participates in the nonspecific defense system in man (7, 8). In the presence of immunoglobulins and complement, the enzyme is effective against a broad variety of bacteria, including some pathogens (9).

The enzyme is mainly degraded in the kidney. It is believed to be filtered through the glomeruli and resorbed in the proximal tubules, and most likely is broken down within the lysosomes of the proximal tubular cells (1, 7, 10). These facts suggest the usefulness of determining lysozyme in blood and urine as an aid to diagnosis in hematology and nephrology. In fact, lysozyme measurement is very valuable for detecting disturbed granulocyte turnover and defects in the function of the proximal tubules (1, 11). Serum and urinary lysozyme determination has also recently been introduced as a useful test in the differential diagnosis of acute leukemia (12–14). There are many reports of changes in serum and urinary lysozyme activity in various kidney diseases and after bilateral nephrectomy (1, 2, 11, 15–19). The behavior of lysozyme activity in serum and urine has also been investigated in patients undergoing chronic hemodialysis and kidney transplantation (20, 23), and it was found that lysozymuria is related to tubular damage, unrelated to renal vascular changes in the grafts, and is of limited value in the diagnosis of rejection (21).

We undertook to investigate: (a) the changes of the serum lysozyme activity in persons receiving the immunosuppressive drug azathioprine and during resumption of kidney function; (b) the value of urinary lysozyme determination in the prognosis of recovery of physiological graft function, in the estimation of the degree of kidney damage, and in the diagnosis of rejection; and (c) lysozyme turnover, assessing indirectly any changes in the production, catabolism, and elimination of the enzyme.

Materials and Methods

The investigation was done in 150 patients who underwent cadaver kidney transplantation between May 1973 and December 1976 at the Kidney Transplantation Centre, Berlin–Friedrichshain. Standard surgical procedures were used. Routine oral therapy for immunosuppression was with azathioprine (1.0 tp 3.0 mg/kg body weight) and prednisolone in various dosages.

Acute rejection episodes were handled by increasing

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Table 1. Serum Lysozyme Concentration in Normal Adults, in Various Kidney Diseases, and after Transplantation

<table>
<thead>
<tr>
<th>Lysozyme, mg/liter (mean ± SD)</th>
<th>Ref. no</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal adults</td>
<td></td>
</tr>
<tr>
<td>5.6 ± 2.0</td>
<td>3</td>
</tr>
<tr>
<td>6.4 ± 3.6</td>
<td>16</td>
</tr>
<tr>
<td>8.5 ± 1.4</td>
<td>1</td>
</tr>
<tr>
<td>9.4 ± 2.7</td>
<td>2</td>
</tr>
<tr>
<td>5.0 ± 2.0</td>
<td>18</td>
</tr>
<tr>
<td>Patients with azotemia</td>
<td></td>
</tr>
<tr>
<td>16.0</td>
<td>16</td>
</tr>
<tr>
<td>&gt;30.0</td>
<td>17</td>
</tr>
<tr>
<td>&gt;20.0</td>
<td>1</td>
</tr>
<tr>
<td>Bilaterally nephrectomized patients</td>
<td>1</td>
</tr>
<tr>
<td>25.6 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>Chronic hemodialysis patients</td>
<td></td>
</tr>
<tr>
<td>21.9 ± 13.4</td>
<td>22</td>
</tr>
<tr>
<td>After kidney transplantation</td>
<td></td>
</tr>
<tr>
<td>initial: &gt;45.0</td>
<td>21</td>
</tr>
<tr>
<td>decreased rapidly with the kidney function</td>
<td>22</td>
</tr>
<tr>
<td>initial: 25–30</td>
<td></td>
</tr>
<tr>
<td>decreased to normal within 44 days</td>
<td></td>
</tr>
</tbody>
</table>

the dose of prednisolone given intravenously in slow infusions of 300 mg/day in three divided doses.

Tissue typing and cross matching for cytotoxic antibodies were regularly done.

Acute rejection episodes were diagnosed by increase in serum creatinine associated with diminished urine volume and sodium concentration, and by clinical signs of rejection (fever, hypertension, increase of graft size).

Resumption of kidney function was assessed by determining the last day that hemodialysis was necessary; recovery of resorptive function of tubules was assessed by noting the first day on which urinary lysozyme reached the 1 mg/liter limit; and physiological renal function was assessed by ascertaining the first day on which the serum creatinine concentration was less than 20 mg/liter. According to the speed with which kidney function was resumed, the patients were divided into six groups:

Group 1: immediate renal function
Group 2: function resumed in 1 to 10 days
Group 3: function resumed in 11 to 20 days
Group 4: function resumed in 21 to 30 days
Group 5: function resumed in more than 30 days
Group 6: nonfunctioning kidneys

The activity of lysozyme was measured daily in urine and three times weekly in the serum according to the modified method of Parry et al. (24). Reagents used in this assay are as follows:

(a) Micrococcus lysodeikticus cells (Serva Feinbiochemica, Heidelberg, W. Germany), 50 mg suspended in 190 ml of phosphate buffer (61 mmol/liter, pH 6.2). Add 10 ml of 0.3 mol/liter NaCl.

(b) Standard lysozyme solution, prepared by dissolving crystalline egg-white lysozyme (Worthington Biochemical Corp., Freehold, N.J. 07728) in the phosphate buffer or in untreated urine.

These materials were added to a standard Helma OS cuvet in the following proportions: 800 μl of cell suspension and 20 μl of enzyme solution. This mixture was briefly stirred and the rate at which it cleared was measured at 450 nm in a Viatron MPS filter photometer with an attached recorder.

Lysozyme activity was expressed in terms of concentration (mg/liter), as compared with the standard. Results from different groups were compared by means of Student’s t-test or the Mann–Whitney U-test. The relations between serum lysozyme, azathioprine dosage, and leukocyte count was calculated by linear regression.

Results
Serum Lysozyme Activity

Table 1 summarizes the serum lysozyme values we found for normal adults and for patients with renal failure who had undergone bilateral nephrectomy and kidney transplantation. The activity was high on the first day after the kidney transplantation: 38.2 ± 16.2 (SD) mg/liter. The mean value for 44 patients, measured three times per week for each during 60 days, was 16.54 ± 10.95 (SD) mg/liter. In patients with immediately functional transplants (group 1) the serum lysozyme activity decreased rapidly, reaching normal values in about nine days (mean ± SD, 9.23 ± 4.3 days). In the case of nonfunctioning grafts (group 6) serum lysozyme activity decreased more slowly, reaching the 10 mg/liter limit only after about 26 days (mean ± SD, 26.3 ± 13.9 days). The difference is statistically significant (P < .001). The mean azathioprine dosage was the same in the two groups.

The change of leukocytes and in serum lysozyme activity was compared after introduction of azathioprine therapy. Figure 1 shows the linear regression for data on 44 patients. When all data were considered together and leukocyte count was plotted vs. serum lysozyme
value, a significant direct linear correlation emerged between the two variables, indicating direct proportionality of the data ($r = 0.279, n = 708, P < .001$).

In 75 patients, we analyzed individually the linear correlation between leukocyte count and serum lysozyme; for 51 of these we found a significant direct linear correlation. Of the 24 patients for whom this was not the case, 10 had severe infections with fever >38 °C and increased leukocyte count. With azathioprine therapy the serum lysozyme activity decreased, without a corresponding decrease in the leukocyte count.

The different changes in leukocyte count and serum lysozyme activity in patients with severe infections suggested that the good correlation of the two variables in the other transplant patients might depend on the azathioprine dosage. To investigate the direct effect of azathioprine on the changes of serum lysozyme activity, we compared the average dose of this drug with the average serum lysozyme values in the group of patients with nonfunctioning kidneys (group 6). We selected this group for analysis because it is well known that the kidney takes part in the catabolism of lysozyme, and in group 6 this factor is excluded. Figure 2 shows a significant linear inverse correlation between the azathioprine doses and serum lysozyme activity. In the same group we determined the day on which the serum values of lysozyme decreased to 10 mg/liter. The rate of decrease of serum lysozyme was then compared with the average azathioprine dosage (Figure 3). We also found a significant inverse linear correlation in this case. These findings support the theory that azathioprine directly inhibits production of lysozyme. In patients with nonfunctioning grafts we could not observe any parallel changes of serum creatinine and serum lysozyme; both variables were increased. By introducing azathioprine therapy after transplantation, the serum lysozyme activity decreased simultaneously with leukocyte count and the dosage of this drug, independently of serum creatinine.

During rejection episodes, graft function was diminished and, because the catabolism rate of lysozyme was decreased, the activity of lysozyme in serum increased. But these changes were not pronounced and detectable in all cases. Intravenous therapy with a bolus dose of prednisone did not influence the changes in the activity of the enzyme in serum.

Urinary Lysozyme Activity

We investigated the changes in urinary lysozyme activity in 150 patients after cadaveric kidney transplantation, measured an average of 60 days after the operation. It was high in uremic patients with residual urine (<500 ml/day) on the day before transplantation (mean ± SD, 31.8 ± 16.5 mg/liter). It decreased in parallel with the resumption of function, and on the average by the 25th day (25.03 ± 17.2 SD days) urine lysozyme concentration was 1 mg/liter in patients with functioning graft.

Figure 4 shows the relationship between the mean values for day of kidney function resumption, recovery of tubular resorption function, and recovery of physiological renal function. Figure 5 illustrates a typ-
Table 2. Recovery of Resorption Function of the Tubuli (Urinary Lysozyme, 1.0 mg/liter) in Comparison with the Recovery of Physiological Renal Function (Serum Creatinine, 20 mg/liter) after Kidney Transplantation

| Group | 1 Immediate | 2 1–10 | 3 11–20 | 4 Days 21–30 | 5 >30 | Total
|-------|------------|--------|---------|-------------|------|-------|
| Recovery of resorption function of the tubules (mean ± SD) | 14.1 ± 10.7 | 14.8 ± 3.1 | 26.6 ± 16.7 | 35.5 ± 11.0 | 58.1 ± 5.6 | 25.03 ± 17.2
| Recovery of physiological renal function (mean ± SD) | 14.3 ± 9.5 | 23.0 ± 14.5 | 28.5 ± 14.5 | 49.1 ± 8.5 | 77.4 ± 31.0 | 33.02 ± 27.8

*Last day on which hemodialysis was necessary.

...ical case of changes in lysozymuria during rejection. Initially, the lysozyme activity is high, similar to the case of patients with chronic renal insufficiency. With the resumption of kidney function it decreases to 0 to 1 mg/liter. An appearance of lysozymuria and changes of other variables characterized rejection episodes. After successful prednisolone bolus therapy the decrease in enzyme activity reflected regeneration of tubular function. In irreversibly damaged grafts with minimal urine output, high lysozyme activity in conjunction with high sodium excretion lasting more than 25 days may be useful supporting evidence of a need for graft removal.

We investigated the sequence of occurrence of variables used for diagnosis of rejection in 72 episodes in 28 patients after transplantation. The starting point for the sequence was determined by the day on which changes of one or more variables indicated rejection. The frequency was represented as a percentage of the total of occurrences. This comparison confirmed that lysozyme in urine increased one to two days later than the other variables. We observed lysozymuria in patients with graft failure due to hypoxic damage, after gentamicin therapy, and in one patient as a sign of miliary tuberculosis.

**Discussion**

Since the investigations by Hansen et al. (1) in 1972, detailed data on lysozyme turnover have become available. They investigated the turnover rate in control persons, nephrological patients with various degrees of renal insufficiency, patients after bilateral nephrectomy, and hematological patients with disturbed turnover of neutrophil granulocytes. The importance of the hemopoietic system in the production of the enzyme and the role of the kidney in its catabolism has also been demonstrated.

In patients with renal diseases and in uremic states lysozyme catabolism is diminished and its production is decreased. Only extrarenal catabolism takes place. Thus the activity of this enzyme in serum is high (14, 16, 17). Because of the disturbed function of the proximal tubules high lysozymuria is characteristic, its degree depending on the degree of kidney damage (2). Several authors observed high lysozyme activity in serum caused by increased production in patients with hematologic disorders (5, 12, 13). Measurement of the serum lysozyme activity can be used as a method to check the effectiveness of chemotherapy in acute myelogenous leukemia. The activities decrease in accord and with the number of circulating leukocytes (14, 24). In uremic patients who are undergoing kidney transplantation, lysozyme catabolism is diminished immediately after grafting. Because of the necessary immunosuppressive therapy, there is a change in the rate at which cells are formed in the hemopoietic system, and thereby the production of lysozyme is modified. As a result, lysozyme turnover after kidney transplantation is different from that in the normal subject.

The high serum lysozyme activity—induced by renal insufficiency—decreases continuously after grafting. Our findings show that, depending on the effectiveness of the graft function, serum lysozyme activity reached the normal value between four and 17 days after kidney function was resumed, but in patients with nonfunctioning grafts this period is significantly longer. This fact demonstrates that azathioprine has a direct effect on lysozyme production.

One can conclude that two factors—regeneration of kidney function and diminished lysozyme production...
due to azathioprine—are responsible for the continuous decrease of serum lysozyme activity after kidney transplantation. Our observations support those of Keeler (6), who reported that pretreatment with the cytotoxic agent cyclophosphamide diminished the activity of lysozyme in plasma and in the total body and blocked the response to nephrectomy.

We also investigated the effect of therapy with prednisolone on the changes in serum lysozyme. Neither during regular steroid management nor after prednisolone bolus therapy did we observe any changes in lysozyme activity. According to the investigations of Dukor and Dietrich (26) steroid therapy does not prevent the accumulation of the enzyme after bilateral nephrectomy. Lysosomal membrane stabilization seems not to affect lysozyme production.

We believe that, of the immunosuppressive drugs used after transplantation, only azathioprine causes a decrease in serum lysozyme activity. Currently, the best variable for monitoring immunosuppression in patients is the leukocyte count in the peripheral blood. We compared leukocyte count and serum lysozyme activity and found a significant correlation between the two variables. In case of severe infection the leukocyte count increased despite immunosuppression, while the serum lysozyme activity decreased. This fact shows that production of lysozyme is partly independent of the turnover of granulocytes; however, the neutrophil function might also change in kidney allograft recipients (27).

Therefore it seems reasonable to assume that serum lysozyme is derived from tissue macrophages, monocytes, and probably immediately from the bone marrow (6, 28). Reticuloendothelial cells and lymphoid cells have a low lysozyme content and so they are not a considerable source of lysozyme in serum (14). Measurement of serum lysozyme might be a good index of azathioprine dosage and thereby it could be used in immunosuppressive management.

In 1964, Noble et al. (20) published the first results on changes in serum lysozyme activity after kidney transplantation in dogs. They concluded that the increased activity of the enzyme in plasma might be a sign of rejection. Since the investigations by Harrison et al. (2) and Hansen et al. (1), we know that the increasing lysozyme activity in serum is typical of the beginning of renal insufficiency. If the rejection leads to irreversible renal damage the activity of lysozyme increases, but it is not a specific sign of rejection.

More recently, Schmidt et al. (23) asserted that serum lysozyme activity changes with serum creatinine concentration. Our observations contradict their findings. The enzyme activity also decreased in patients with nonfunctioning grafts, and depended strongly on the dosage of azathioprine. The graft function, by the recovery of catabolism, and azathioprine, by the inhibition of enzyme production, together are responsible for the changes of serum lysozyme after transplantation.

Excretion of lysozyme has been widely investigated. As a protein of low molecular weight, it is filtered though the glomerulus and, depending on the effectiveness of tubular function, is resorbed. Thus degree of lysozymuria correlates very well with the degree of tubular damage (29, 30).

Lysozyme measurement as an index of tubular re- sorption function has been recommended both in chronic renal diseases and in transplant patients (2, 20–22, 31). Our observations of many patients after transplantation support the concept that measurement of the urinary excretion of lysozyme provides information about the function of the renal transplant, but often it is not useful in the management of rejection. In support of this, the initial oliguric phase is characterized by the high lysozymuria. During the recovery of function we observed a continuous decrease of enzyme excretion. A lack of lysozyme in urine pointed to the regeneration of tubular resorption function. A reduction of kidney function by rejection is reflected by the degree of lysozymuria.

In all probability, the excretion of lysozyme plays only a secondary role in lysozyme turnover. If catabolism of the enzyme is diminished in the kidney, renal excretion of it increase accordingly. The renal threshold of lysozyme in normal adults is 45 mg/liter according to Hayslett et al. (17). The activity of the enzyme in serum increases only rarely in pathological conditions. Therefore the role of the renal threshold in lysozyme turnover is unimportant.

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References