The Relationship of Lysozyme to the Nephropathy in Chloroleukemic Rats and the Effects of Lysozyme Loading on Normal Rat Kidneys

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SUMMARY

The Shay chloroleukemia in Wistar/Furth rats has been shown to produce large amounts of lysozyme (LZM) which accumulates in the kidneys and is eventually excreted in the urine. The proximal tubule cells of the tumor-bearing animals develop large complex cytoplasmic droplets containing the residua of degenerated membranes. Using specific anti-rat LZM and the immunoperoxidase method, increased amounts of LZM can be demonstrated in these tubules. Intraaortic injections of rat LZM into normal rats failed to fully reproduce these structural abnormalities, indicating the probability of other contributory factors to the nephropathy in the chloroleukemic animals.

INTRODUCTION

The purpose of this paper is to describe studies of the renal abnormalities associated with the LZM-producing transplantable CL in the rat and attempts to reproduce this renal lesion by injecting homologous (rat) LZM into normal rats. Our interest in the renal consequences of excessive LZM loading [so-called “LZM nephropathy” (26)] stemmed from our observations of apparent renal tubular dysfunction in patients with MoL and MoML associated with markedly elevated serum LZM levels and lysozymuria (22, 29). These tubular dysfunctions, reflected primarily in hyperkaluria and hypokalemia (22), have been confirmed by other investigators (34, 46). As described in this report, the availability of an experimental animal model for this condition, i.e., the rat CL, provided an opportunity for elucidation of some of the underlying pathogenic mechanisms.

MATERIALS AND METHODS

Rat CL. This tumor was derived from the serial passaging of the Shay CL (39) in Wistar and Sprague-Dawley rats. The tumor originated in 1952 in a Wistar rat previously treated with methylcholanthrene (39) and was characterized as an acute myelocytic or myeloblastic leukemia (21). It was shown to be transplantable by i.p., s.c., or i.v. injection into Wistar or Sprague-Dawley rats, and its dynamics of growth and pattern of spread vary considerably with the volume and site of inoculation and the age of recipient animals (21). The percentage of takes approaches 100% in rat pups and decreases markedly in rats older than 4 weeks. When transplanted s.c., it grows as a solid local tumor (“chloroma”) with little dissemination. After i.p. or i.v. injection, the tumor disseminates more widely with frequent involvement of the bone marrow and peripheral blood (CL). The tumor was kindly provided to our laboratory in 1967 by Dr. William C. Moloney of Harvard Medical School, at which time it was in its 108th passage. In our laboratory it has been serially passaged in 4- to 6-week-old Wistar/Furth rats by either i.p. or s.c. injection. The age of recipient animals and the site of injection were determined by specific experimental needs. Older animals given s.c. injections were generally used for urine collections for LZM isolation, and younger animals given i.p. injections were used for studies of the effects of tumor proliferation. The animals given i.p. injections usually exhibit ascites within 7 days and die after 21 to 28 days.

Sample Collections. Blood samples were obtained in capillary tubes by tail vein nicking, and serum was separated by centrifugation in a capillary centrifuge (International Centrifuge Co., Needham Heights, Mass.). For urine collections, rats were housed singly in metabolic cages.

LZM Isolation and Assay. Urine was collected from the CL rats from the 14th to 28th day after tumor transplantation, i.e., when there was significant lysozymuria. The urine collections were pooled and the LZM was isolated by the previously described bentonite adsorption and acid pyridine elution method (29). Following elution, the pyridine:H2SO4 was removed by exhaustive dialysis against deionized water and the solution was lyophilized. LZM assays were made by...
were fixed in 10% neutral formalin, embedded in paraffin, and
then treated for 30 min in succession with rabbit
anti-rat LZM, sheep anti-rabbit 7 globulin, and rabbit
anti-horseradish peroxidase. Between each antiserum treat-
mantion, the sections were washed twice with phosphate-buffered
saline for 5 min. After the 3rd antiserum, horseradish
immunoperoxidase method for staining LZM in tissues has
been described in detail (15). For these studies, the kidneys
were cut into 6-Âm sections. After deparaffinization and rehydra-
tion, the sections were washed with phosphate-buffered saline and
applied at a concentration of 250 Mg/ml for 20 min. The
sections were again washed with phosphate-buffered saline and
then centrifuged at 8000 X g for 30 min. All procedures were carried out at 4°.LZM assays were performed on the supernatants, since it had been previously established that essentially all of the tissue LZM is released by this procedure.

Kidney LZM Assays. For assays of LZM content, kidneys were rapidly removed from animals killed by cervical dislocation, minced, and homogenized with a loosely fitting Teflon homogenizer and treated sonically for 30 sec (Sonifier, Heat Systems Co., Melville, N. Y.) in 4 volumes of M/15 phosphate buffer, pH 6.2. The sonically treated extracts were then centrifuged at 8000 X g for 30 min. All procedures were carried out at 4°. LZM assays were performed on the supernatants, since it had been previously established that essentially all of the tissue LZM is released by this procedure.

Cytological and EM Studies. In most experiments, the kidney contralateral to that assayed for LZM was used for cytological and EM studies. Sections for both EM studies and light microscopy were prepared in the following way. After rapid removal of the kidneys, tissue samples of about 1 cu mm were cut with a No. 10 scalpel, fixed in 3% glutaraldehyde at 4° for 1 to 4 hr, and washed in 0.1 M phosphate buffer, pH 6.2. After postfixation for 30 min in 1% osmium tetroxide, the specimens were embedded in Epon (16, 35) and sectioned with a Sorvall MT 2-B ultramicrotome. For light microscopy, 1-Âm sections were stained with 1% méthylène blue in 1% sodium borate solution. Then 400-Â sections were cut for EM
studies and were examined with either the Hitachi Model
HU-11E or the Zeiss Model 952 electron microscope.

LZM Localization by the Immunoperoxidase Method. The immunoperoxidase method for staining LZM in tissues has been described in detail (15). For these studies, the kidneys were fixed in 10% neutral formalin, embedded in paraffin, and cut into 6-Âm sections. After deparaffinization and rehydration, the sections were washed in 0.01 M phosphate buffer. They were then treated for 30 min in succession with rabbit anti-rat LZM, sheep anti-rabbit 7 globulin, and rabbit anti-horseradish peroxidase. Between each antiserum treatment, the sections were washed twice with phosphate-buffered saline for 5 min. After the 3rd antiserum, horseradish peroxidase (Type VI, Sigma Chemical Co., St. Louis, Mo.) was applied at a concentration of 250 Âg/ml for 20 min. The sections were again washed with phosphate-buffered saline and stained for peroxidase by the Karnovsky method (10).

Cytobacterial Demonstration of LZM. The method of Briggs et al. (3), as modified by Syren and Raeste (44), was used for the demonstration of LZM in the CL cells. A mixed suspension of the cells and heat-killed Micrococcus lysodeikticus or-
organisms (Lot R 1072, Schwarz/Mann, Orangeburg, N. Y.) was smeared on glass slides, fixed in formalin:ethanol (1:3), incubated for 15 min in 0.01 M phosphate buffer and stained with Wright-Giemsa stain.

Electrophoresis. Serum and urine electrophoretic analysis were done with the cellulose acetate method (Microzone; Beckman Instrument Co., Fullerton, Calif.) in barbital buffer, pH 8.6. Prior to electrophoresis, the urine samples were concentrated 30 to 40 times by dialysis against 25% polyvinyl-pyrollidone.

RESULTS

Characteristics of the Rat CL Tumor and LZM Production. Fig. 1 shows the cytology of CL cells in the ascites of a rat after i.p. transplantation of tumor. The cells have the appearance of myeloblasts or monoblasts with large nuclei and vacuolated cytoplasm. Fig. 2 demonstrates the elaboration and release of LZM by these cells as detected by the lysis of M. lysodeikticus organisms. The ultrastructure of the solid CL tumor (chloroma) is shown in Figs. 3 to 6. The nuclei are large with prominent nucleoli. A rim of compact chromatin is seen at the periphery of the nucleus, and there are many nuclear pores. The cytoplasm contains an abundance of ribosomes, both free and associated with rough endoplasmic reticulum. There are numerous polysomes comprised of 5 to 7 ribosomes. Membrane-bound granules are seen in most cells, and in some there are numerous complex phagolysosomes containing residua of cytoplasmic constituents, indicating autophagocytosis. The mitochondria are abundant and exhibit considerable morphological variation. No virus particles were found in any of the sections examined, although these have been reported by Weinstein and Moloney (45).

Serum and Urinary Proteins of the CL Rat. Chart 1 shows representative electrophoretic analyses of the serum and urine proteins of a normal and a CL rat. The most striking abnormality is seen in the urine protein pattern of the CL rat which demonstrates a distinctive cationic peak characteristic of LZM (29). The CL urine also contains relatively more a, 4, and 7 mobility proteins than the normal urine, consistent with renal tubular dysfunction, i.e., so-called “tubular proteinuria” (26). The overall pattern of the CL urinary proteins is remarkably similar to that of patients with Mol and MoMl with associated lysozymuria (29).

Fig. 7 shows sequential urinary protein electrophoretic patterns of the CL rat following i.p. tumor transplantation. These patterns demonstrate that the excretion of increased amounts of a, 4, and 7 mobility proteins precedes the appearance of lysozymuria. This is strongly indicative of associated tubular dysfunction.

LZM Concentrations in the Serum, Urine, Ascites, and Kidneys of CL Rats. Chart 2 shows the changes in serum, urine, ascites, and kidney LZM concentrations following i.p. transplantation of the CL tumor. Each point represents the mean of 5 animals. In the 1st 2 weeks following tumor transplantation, serum LZM increased from an average of 10 to 40 Âg/ml and then rose more rapidly to the range of 80
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Chart 1. Electrophoretic analyses (cellulose acetate, Microzone method) of the serum and urinary proteins of the normal and CL rat. Urine samples were concentrated 30-fold by osmorialysis. The CL rat urine shows a distinctive cationic peak identified as LZM (arrow). Alb, albumin.

µg/ml during the 3rd week. Kidney LZM levels increased at a more rapid rate than serum LZM, indicating progressive intrarenal accumulation of LZM. Except for 1 animal on the 11th day, there was no detectable lysozymuria prior to the 15th day, by which time there was an approximate 10-fold increase in kidney LZM. The greatest lysozymuria occurred on the 19th to 21st days when the kidney LZM was actually decreasing. The concentration of LZM in the ascites remained relatively constant from the time of its initial appearance until the death of the animals around the 21st day, indicating a relatively constant rate of LZM production by the tumor.

Kidney Cytology and Immunoperoxidase Localization of LZM. Figs. 8 and 9 show thin sections of the kidneys of a normal rat and a CL rat 15 days after i.p. tumor transplantation. The proximal tubule cells of the normal kidney contain fine, densely stained granules whereas, in the CL rat kidney, these granules are markedly increased in size and number. Figs. 10 through 13 show the distribution of LZM in the kidneys of normal and CL rats as determined by the immunoperoxidase method. In the normal kidney (Fig. 10), LZM is primarily restricted to the proximal convoluted tubules. The LZM is mainly concentrated in granules, although there is some diffuse background cytoplasmic staining. By contrast, in the CL rat kidney (Figs. 11 to 13), the LZM staining extends beyond the proximal convoluted tubules into the pars recta. The cytoplasm of the convoluted tubule cells is densely stained and shows many large droplets. In the pars recta, the LZM appears to be primarily concentrated in dense granules. There is no LZM staining in the glomeruli or collecting tubules in either the normal or the CL rats.

Electron microscopy confirmed the renal structural abnormalities in the CL rat. As shown in Fig. 14, these abnormalities are particularly evident in the proximal tubule cells that show numerous cytoplasmic droplets, many of which contain membrane residua (so-called “myelin figures”). The overall structure and configuration of the cell is markedly distorted, as are the contours of the nucleus and many mitochondria. The brush border appears relatively intact.

LZM Injection Studies. In an initial series of experiments, LZM was injected i.p. in normal rats. It became apparent, however, that this route of administration was relatively inefficient, apparently due to the rapid diffusion and widespread distribution of the enzyme into the tissues and body fluids. Accordingly, in subsequent experiments, the purified LZM was injected into the aortas of normal rats proximal to the renal arteries via polyethylene catheters. Injections of up to 10 mg were given daily for periods up to 22 days. Fig. 15 shows a thin section of the renal cortex of a rat that received 14 daily injections of 10 mg of rat LZM. The most evident abnormalities are a crenulation of the margins of both the proximal and distal tubules apparently secondary to swelling or hypertrophy of the endothelial cells of the peritubular capillaries. The glomerular capillaries also show thickening of basement membranes and endothelial hypertrophy. The proximal tubule cells show some increase in dense granules, but these are not as large or numerous as in the CL rat kidney. In rats that received injections for 22 days, the peritubular capillaries were markedly distended with erythrocytes.

Fig. 16 shows an electron micrograph of a proximal tubule of a LZM-injected rat (10 mg daily for 14 days), and the morphological features are clearly different from those in the CL rat kidney. There are some droplets, but these are considerably fewer and smaller and do not contain the dense myelin figures seen in the droplets of the CL rat proximal tubules. Thus, these results indicate that LZM per se is not the exclusive cause of the renal tubular abnormalities in the CL rat and further suggest that LZM loading may produce other renal structural changes, particularly in the glomerular and peritubular capillaries.
Chart 2. LZM concentrations in the serum, urine, ascites, and kidneys following i.p. transplantation of the CL tumor. Each point, mean of determinations on 5 animals.

**DISCUSSION**

The present studies provide additional information concerning the production, renal handling, and urinary excretion of LZM in rats bearing the Shay CL. This transplantable experimental tumor has been extensively investigated in the 21 years since its initial discovery in a methylcholanthrene-treated Wistar rat (39). It has generally been considered to be of myelogenous origin on the basis of cytological and enzymatic properties, particularly its relatively high content of myeloperoxidase and phosphatase (21). Its greenish color (chloroma), which derives from its peroxidase content, has been a variable property of the tumor over the many years in which it has been carried in different laboratories by serial transplantation in partially inbred Wistar rats. It is possible that this apparent variation in enzyme content is in some presently obscure way related to functional changes associated with passaging the tumor in partially incompatible hosts. In 1963, Goldbach et al. (9) first reported the finding of very high LZM concentrations in saline extracts of the CL tumor. In 1967, Rosenthal and Moloney (37) demonstrated increased serum and urine LZM levels in CL tumor-bearing rats, and this has been extensively confirmed in the present studies. Unfortunately, it is not known whether the amount of LZM elaborated by the tumor has changed significantly in the course of serial passaging.

The elaboration of LZM by cells of the monocytic and granulocytic series has been extensively documented (3, 6, 7, 29). Present evidence indicates that the enzyme is produced by both of these cell types but is handled differently by each. Thus, the LZM produced by both normal and leukemic monocytes is apparently rapidly released and presumably functions outside of the cell, whereas the LZM of cells of the granulocytic series remains predominantly intracellular and localized to secretion granules (3, 6). This explains the seemingly paradoxical finding of higher serum and urine LZM levels but lower intracellular concentrations in monocytic as compared with myelocytic leukemia (3, 6, 46). In this regard, the rat CL neoplasm more closely resembles human MoL or MoML than myelocytic leukemia. LZM is apparently readily released from the CL cells as shown by the present cytobacterial studies. The relatively prominent network of endoplasmic reticulum also implies an effective mechanism for the release of synthesized proteins. The presence of some (?) secretion) granules or lysosomes and complex phagolysosomes in the CL cells suggests that there may also be a functionally important intracellular LZM compartment. In this regard, Handler et al. (12) have demonstrated the phagocytosis of *Escherichia coli* by the rat CL cells and, in unpublished studies, we have observed phagocytosis of staphylococci and starch granules.

The role of the kidney in the handling of LZM under normal and pathological conditions has been extensively investigated since the initial discovery of LZM by Fleming (7) and his finding that the enzyme is not present in normal urine but is found in high concentrations in the normal kidney.
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Since LZM is a low-molecular-weight (M.W., 14,700) protein, it is widely distributed in the extracellular fluid compartments of the body and readily filtered through the glomerular capillaries. Beyond this point, the processing of endogenous LZM by the kidney is still not completely defined. Shibko and Tappel (40), in studies of subcellular fractions of normal rat kidney, have found LZM primarily in the lysosomal fraction. Sussman et al. (43), studying the intrarenal distribution of LZM in normal rats, found higher concentrations in the cortex than in the medulla. They further demonstrated that unilateral nephrectomy was associated with a rise in LZM content in the remaining kidney and that ureteral ligation caused a decrease in renal LZM, apparently as a consequence of decreased glomerular filtration (43). The renal handling of exogenous (heterologous) LZM, particularly egg-white LZM, has been extensively studied in experimental animals. In dogs, Marshall and Deutsch (19) studied the renal clearance of egg-white LZM (100 to 175 mg given by constant infusion), comparing it with ovalbumin, conalbumin, \( \beta \)-lactoglobulin, and ovomucoid. LZM was found to be cleared more rapidly than the other egg proteins, but not as rapidly as would have been predicted on the basis of its low molecular weight. Binding of LZM to serum and/or tissue proteins and tubular reabsorption were postulated as explanations for the less-than-anticipated renal clearances (19).

In rats and guinea pigs, Perri et al. (32) showed that egg-white LZM was concentrated by and accumulated within the kidneys, and Royce (38) and Maack (17) demonstrated the formation of cytoplasmic droplets in proximal tubular epithelial cells of rats following injection of relatively large quantities of egg-white LZM. The precise composition and pathogenesis of these droplets could not be determined, but they were presumed to contain LZM, as well as other constituents. More recently, Maack et al. (18) studied the intrarenal distribution of injected radioiodine-labeled hen egg-white LZM in mice using radioautography and analyses of renal cortical subcellular fractions. In confirmation of previous studies by other methods, radioautography showed LZM localization primarily in granules in the proximal tubule cells but also, to a lesser extent, in the thick portion of the epithelium of Bowman's capsule. In the subcellular fractions, LZM was localized to large phagolysosomes associated with acid hydrolases and to the cytoplasmic sap (18). At lower doses of injected LZM, there was relatively more of the enzyme in the sap than in the particulate fraction. It is noteworthy that the cell fractionations were done in the presence of high concentrations of KCl (0.2 M) and EDTA (1 mM) which probably dissociated some of the LZM from the particulate into the soluble fraction. Although these previous studies were primarily concerned with the renal handling of heterologous LZM, i.e., a "foreign" protein, the observations are probably relevant to the renal handling of homologous (and isologous) LZM because of the close structural similarities between egg-white and mammalian LZM's. The only published studies of the turnover and renal uptake of homologous LZM are those of Hansen et al. (13). These investigators found that \(^{125}\text{I}\)-labeled rat LZM disappeared from the plasma of normal rats with a half-life of 75 min and further established that almost all of the injected LZM was taken up by the kidneys.

With regard to the present studies, it is important to emphasize that proximal tubular damage per se has been shown both in man and experimental animals to be associated with lysozymuria (2, 14, 33). Thus, Prockop and Davidson (33) found a marked increase in urinary LZM in rats with renal tubular damage secondary to mercuric chloride. They further demonstrated that a nephrotoxic antiserum with primarily antiglomerular activity caused no significant lysozymuria. These studies were confirmed and extended by Balazs and Roepke (2) who found that sodium chromate, apparently by virtue of its specific toxicity for the proximal portion of the proximal convoluted tubules, caused an even greater lysozymuria in rats than either mercuric chloride or uranyl nitrate which affect the more distal segments of the proximal tubules. Following a single injection of sodium chromate (20 mg/kg body weight), lysozymuria was detected within 3 hr and rose to a maximum at 3 days associated with a marked decrease in kidney LZM levels. Still another form of renal injury, i.e., that associated with the homograft rejection reaction, has been shown to result in lysozymuria in both man and experimental animals (24), and this has also been related primarily to damage of the proximal convoluted tubules.

Increases in kidney and urinary LZM have been previously reported in certain experimental rat tumors other than the Shay CL. Thus, in Sprague-Dawley rats bearing the Jensen sarcoma, Perri et al. (30, 31) found a 70- to 100-fold increase in kidney LZM levels associated with lysozymuria on the 10th day following transplantation. They further showed that surgical removal of the tumor resulted in a decrease in kidney LZM concentrations to normal. Because of the absence of a comparable increase in kidney LZM in rats with chemically-induced (methylcholanthrene) autochthonous tumors, Perri et al. (30, 31) and Cappuccino et al. (4) concluded that the increased LZM in the Jensen sarcoma-bearing rats was related to a nonspecific reticuloendothelial response to a transplanted neoplasm and not to LZM production by the tumor per se. This conclusion was supported by the finding of increased kidney LZM levels in mice following reticuloendothelial stimulation by Bacillus Calmette-Guérin infection, zymosan, and endotoxin (5).

With respect to the renal handling and urinary excretion of LZM in CL rats, we previously reported (27) the finding of large cytoplasmic droplets in the proximal tubule cells of these tumor-bearing animals, and these observations have been confirmed and extended by Greenberger et al. (11). These changes are apparently analogous to similar changes observed in the kidneys of patients with MoL and MoML with associated lysozymuria (E. F. Osserman et al., unpublished data). Our earlier observations of hypokalemia and hyperkaluria in cases of MoL and MoML (22, 29) were also shown to have at least a partial corollary in CL rats by the more recent finding of Rosenthal et al. (36) of hyperkaluria (albeit without significant hypokalemia) in these tumor-bearing animals. In the present studies, we have shown that the kidneys of CL rats accumulate very large amounts of LZM for the 1st 2 weeks following tumor transplantation before the enzyme is excreted in the urine. We have further shown that there is increased nonspecific tubular proteinuria during these 1st 2 weeks indicative of renal tubular dysfunction. By the immunoperoxidase method, LZM was found to be principally...
localized in droplets in the proximal tubules of normal rats, confirming previous studies by other methods (8, 18). In the CL rat kidney, the increased LZM was shown to be distributed in a greater number of proximal convoluted tubules than in the normal kidney, and was also present in the pars recta. The pattern of LZM staining of the proximal tubules in the CL rats indicated increased droplets as well as greater concentrations throughout the cytoplasm.

These EM studies confirmed the marked structural abnormalities in the CL rat proximal tubules, i.e., the development of numerous large cytoplasmic droplets with associated distortion of nuclei, mitochondria, and overall cellular contours. Myelin figures, presumably representing the residua of collapsed membranes, were found in many of these droplets. This would indicate that the droplets represent complex aggregates of protein and lipid constituents possibly formed from a combination of absorption, cellular damage, and autophagy-cytosis. The pathogenesis of these droplets is still unclear. The fact that complex droplets did not develop to the same degree in the kidneys of the normal rats that received multiple daily intra-aortic injections of up to 10 mg of LZM would indicate that they are not due to LZM per se. However, it is possible and indeed probable that the quantity of LZM elaborated in the CL rats actually exceeds that injected in these experiments. It is also probable that constituents other than LZM, i.e., other proteins, polysaccharides, and lipids, are released into the circulation in the CL tumor-bearing animals, and these may contribute to the renal damage.

The finding of marked distortion and structural abnormalities of the mitochondria of the proximal tubule cells in the CL rats and the possibility that some of the membrane residua found in the complex droplets in these cells may have been derived from degenerated mitochondria, raises the question of the possible involvement of mitochondria in droplet formation. Oliver et al. (25) concluded that mitochondria were involved in the formation of proximal tubule droplets in rats that were given injections of egg white, on the basis of supravital staining with Janus green dye. Niemi and Pearse (23), however, could not confirm the presence of either sucinic dehydrogenase or dinitrophenyl diaphorase, i.e., mitochondrial enzymes, in the proximal tubule droplets of rats given egg white conjugated with dichlorotriazine. Similarly, Miller (20) and Strauss (41, 42) found no evidence of mitochondrial involvement in the absorption droplets that developed after hemoglobin or horseradish peroxidase injections in rats. A possible explanation for these apparently disparate findings might be that LZM per se, which is a major component of egg white, is responsible for the mitochondrial-droplet interaction and that the enzyme was inactivated by the conjugation procedure used in the Niemi and Pearse studies (23). This postulate implies that LZM specifically alters mitochondria, and recent studies in this laboratory (1) have shown that isolated rat liver mitochondria are in fact agglutinated but are not apparently disrupted by the enzyme. We have also obtained evidence that LZM alters the structure and in vitro behavior of a variety of mammalian cells in tissue culture (28), but the relevance of these changes to any possible in vivo effects of the enzyme remains to be determined.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the helpful advice of Dr. Conrad L. Pirani of the Department of Pathology and the excellent technical assistance of Carmen Colon.

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and Urinary Muramidase Activity in Leukemia and Other
Fig. 1. Cytology of the rat CL cells in the ascites after i.p. transplantation. Wright-Giemsa stain, × 2,000.
Fig. 2. Bacteriolysis of _M. lysodeikticus_ organisms overlying CL cells indicating lysozyme activity. × 1,240.
Fig. 3. Electron micrograph of the solid CL tumor ("chloroma") showing 2 cells. The nuclei (N) show prominent nucleoli (nel) and nuclear pores (np). Cytoplasmic details are shown in Figs. 4, 5, and 6. × 10,000.
Fig. 4. Detail of Fig. 3 showing numerous polyribosomes and rough endoplasmic reticulum. × 18,500.
Fig. 5. Additional cytoplasmic detail of the CL tumor with a cluster of mitochondria (m) and membrane-bound granules (g). × 30,000.
Fig. 6. Cytoplasm area with numerous irregular, complex phagolysosomes, many of which contain identifiable residue of membranes and degenerated ribosomes. × 30,000.
Fig. 7. Electrophoretic patterns of serial urines of the CL rat following i.p. tumor transplantation. The pattern of the CL rat serum is shown at the top for mobility reference. LZM (arrow) is not detected in the CL urine until the 21st day. Alb, albumin.
Fig. 8. Normal rat kidney. Thin section stained with methylene blue. × 430.
Fig. 9. Kidney of the CL rat, 15 days after tumor transplantation. Note particularly the increased number and size of the densely stained granules in the proximal tubule cells as compared with the normal kidney shown in Fig. 8. Thin section stained with methylene blue; × 430.
Fig. 10. Immunoperoxidase localization of LZM in the normal rat kidney. The specific staining is seen only in the proximal tubules. The scattered staining of some nuclei in the glomeruli is nonspecific. × 100.
Fig. 11. Immunoperoxidase staining of LZM in a CL rat kidney 15 days after i.p. tumor transplantation. The staining is more extensive than in the normal kidney and involves the distal as well as the proximal tubule cells. × 100.
Fig. 12. Proximal convoluted tubules of a CL rat kidney stained for LZM by the immunoperoxidase method. The cytoplasm is diffusely stained, and there are numerous large vacuoles and dense granules. The overall structure of the tubules is distorted. × 560.
Fig. 13. Pars recta of the proximal tubules in the same section as shown in Fig. 12. Dense granular LZM staining is evident. × 560.
Fig. 14. Electron micrograph of a section of a proximal tubule in a CL rat 15 days after i.p. tumor transplantation. There are numerous cytoplasmic droplets (cd), many of which contain dense myelin figures. The nucleus (N) is grossly distorted as are many of the mitochondria (m). The brush border (bb) is grossly intact. × 10,000.
Fig. 15. Cortex of the kidney of a rat after 14 daily intra-aortic injections of 10 mg of rat LZM. Note prominence of the endothelial cells of the peritubular capillaries (arrow) and crenulation of the margins of both the proximal (pt) and distal tubules (dt). Thin section stained with methylene blue. × 560.
Fig. 16. Electron micrograph of the kidney of a LZM-treated rat (same as in Fig. 15). There are scattered cytoplasmic droplets but no significant "myelin figures" as contrasted with the CL rat kidney (Fig. 14). × 10,000.
Lysozyme and Nephropathy in CL Rats

CL Rat Serum

CL Urine

Day 7

11

14

21

γ β α Alb
Lysozyme and Nephropathy in CL Rats

[Image: Electron micrograph showing cellular structures labeled with letters: bb, cd, m, N.]

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The Relationship of Lysozyme to the Nephropathy in Chloroleukemic Rats and the Effects of Lysozyme Loading on Normal Rat Kidneys

Matti Klockars, Henry A. Azar, Riccardo Hermida, et al.

Cancer Res 1974;34:47-60.