ansplant Int (1994) 7 [Suppl 1]: S311–S313
Springer-Verlag 1994

L. Paczek
J. Pazik
M. Teschner
R. M. Schaefer
W. Rowinski
J. Szmidt
M. Lao
K. Abgarowicz
L. Gradowska
M. Morzycka-Michalik
A. Heidland

Human chronic kidney allograft rejection is accompanied by increased intraglomerular cathepsin B and L activity

L. Paczek (✉)
Transplantation Institute,
Nowogrodzka 59, 02006 Warsaw, Poland

J. Pazik · W. Rowinski · J. Szmidt
M. Lao · K. Abgarowicz · L. Gradowska
M. Morzycka-Michalik
Warsaw Medical School, Warsaw, Poland

M. Teschner · R. M. Schaefer ·
A. Heidland
Department of Nephrology,
University of Würzburg

Abstract The major reason for late graft losses is chronic rejection. Recently, a large number of studies have indicated that proteolytic enzymes play an important role as mediators of glomerular injury. The cysteine proteinases cathepsins B and L degrade structural matrix proteins such as type I collagen and laminin. We investigated intraglomerular protease activities in 12 patients after kidney graftectomy because of end-stage renal disease following chronic rejection. A group of 12 patients undergoing nephrectomy because of cancer served as controls using only non-involved parts of the kidney. The activities of cathepsins B and L in homogenates of isolated glomeruli were measured fluorometrically methylcoumarylamide substrates and related to DNA content. In rejected kidney allografts we observed significantly enhanced intraglomerular cathepsin B activity and cathepsin B + L activity.

Key words Kidney allograft
Chronic rejection · Intraglomerular cathepsins B and L activities

Introduction

The major reason for late graft loss is chronic rejection. The rate of attrition of transplants after the first year has not significantly improved regardless of the type of immunosuppression [9]. In chronic rejection in the kidney there is a triad of pathogenetic findings: arteriosclerosis and nephrosclerosis (related to endothelial damage), interstitial fibrosis (related to damage and repair of elements in the interstitium) and glomerulonephritis (secondary to damage in glomeruli) [6]. In human kidney transplants the glomeruli appear to be a prime site of immunological attack in chronic rejection [1]. Recently, a large number of studies have indicated that proteolytic enzymes play important roles as mediators of glomerular injury [1, 2]. Cathepsin B and L, the cysteine proteinases, degrade matrix proteins such as type I collagen, laminin and proteoglycans.

Materials and methods

We investigated kidney tissue obtained from 12 patients after kidney graftectomy because of end-stage renal disease following chronic rejection. Of these patients, seven were women and five were men. They were between 19 and 40 years old (mean 32.3 ± 6.0 years). A group of 12 patients undergoing nephrectomy because of cancer (matched for sex and age to the study group) served as a control group using only non-involved parts of the kidneys. Isolated glomeruli were obtained by differential sieving as previously described [8]. As judged by light microscopy, highly purified (> 95%) preparations of intact glomeruli were obtained. Following ultrasound homogenization the activities of cathepsin B using 2-ARG-ARG-AMC as a substrate and cathepsin B + L using 2-Phe-Arg-AMC were measured fluorometrically. The fluorescence was measured with a Fluoroscan II using the reaction plate as a cuvette [4].

Enzyme activity was calculated as picomoles of AMC generated per microgram DNA or microgram protein per minute from a standard curve using AMC. The DNA content was measured using the Bisbenzimid H 33258 fluorometric assay [5] and the protein content was determined spectrophotometrically using the *bicinochronic acid* (BCA) method [7].

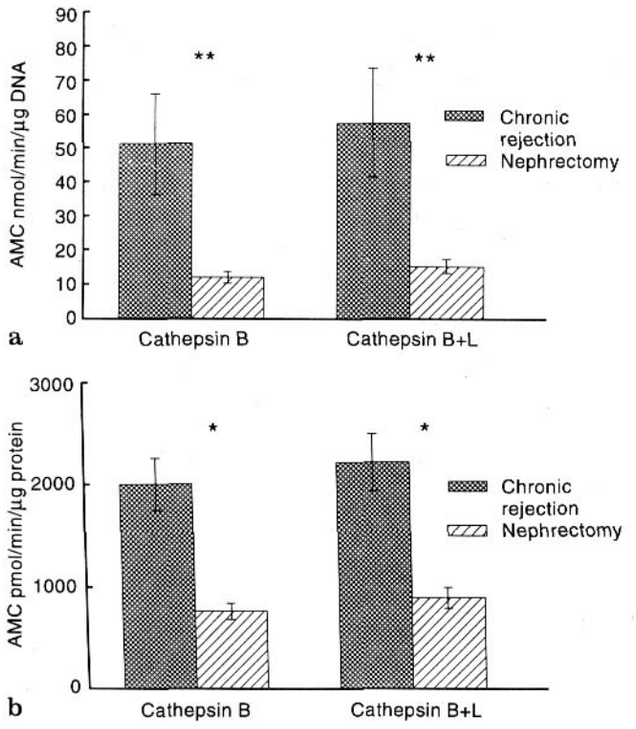

Fig. 1 a Intraglomerular cathepsin B and cathepsins B + L activities in rejected kidney allografts ($n = 12$) in comparison with controls ($n = 12$) in relation to DNA content. (▩ chronic rejection, ▨ nephrectomy; ** $P < 0.001$, Student's t-test). **b** Intraglomerular cathepsin B and cathepsins B + L activities in rejected kidney allografts ($n = 12$) in comparison with controls ($n = 12$) in relation to protein content. (▩ chronic rejection, ▨ nephrectomy; $P < 0.05$, Student's t-test)

Results

When proteinase activity was related to intraglomerular DNA content significantly enhanced intraglomerular cathepsin B activity in rejected kidney allografts (51 ± 15 nmol AMC/min per µg DNA) was found in comparison with controls (12 ± 1.7 nmol AMC/min per µg DNA). Cathepsin B + L activity was 57 ± 16 and 15 ± 2 nmol AMC/min per µg DNA in rejected allografts and controls, respectively (Fig. 1 a). Similar relationships were found between enzyme activity and protein content (Fig. 1 b).

Discussion

Chronic rejection is primarily responsible for late loss of allografted organs and remains an important clinical problem. The principal manifestations of chronic rejection in all organs are persistent inflammation and graft arteriosclerosis [10]. The main role in chronic rejection is played by the immune system, but additional non-immunological factors could also contribute. An altered activity of intraglomerular proteinases might represent an additional common pathogenetic factor.

Cathepsins B and L are effective lysosomal cysteine proteinases of broad specificity, with no substantial inhibitory potential in plasma [3]. Cysteine proteinases are present in normal glomeruli and vigorously degrade GBM and other structural proteins in vitro. In addition, the ability of proteolytic enzymes to influence the production of several key metabolites, autocrine mediators and intracellular signalling agents is well documented [1].

In chronically rejected kidney allografts a 2–3-fold increment in intraglomerular cathepsin B and L activity was observed. The relationship between enzyme activity and DNA content is an indicator of the average capacity of cells for proteolysis. The relationship between enzyme activity and protein content may express the ratio of proteolytic enzymes to substrate: the higher the enzyme activity, the higher the protein turnover. Such high enzyme activities were observed despite and glomeruli from chronically rejected allografts showing moderate protein accumulation. The protein/DNA ratio (mg/µg) was 25.7 ± 4.9 in glomeruli from rejected kidney allografts and 17.0 ± 5.3 in controls; the difference was not significant.

For analysis, only cases characterized as chronic rejection were enrolled. De novo as well as recurrent cases of glomerulopathy were excluded from the study. By light microscopy chronic rejection glomerulopathy was characterized by endothelial and mesangial cell proliferation accompanied by sclerosing lesions. In general, glomerular changes were common but they were of moderate degree. However, histological examination revealed that an acute reaction coexisted with the chronic reaction. The manifestation of the acute reaction was lymphocytic infiltration of interstitial tissue as well as profound arteriopathy.

The methodology applied in this study allowed us to isolate glomeruli only, so that interstitial changes had no impact on the results.

The significance of the high proteolytic activity of isolated glomeruli in chronic rejection is obscure. High

proteolytic potency could destroy the structure of the glomerulus and lead to irreversible damage. Excess active proteolytic enzymes indicates that in the glomeruli during chronic rejection very active metabolic process take place. Unfortunately, we cannot say whether this phenomenon is due to an increased synthesis of cathepsins or due to a glomerular infiltration of immune system cells.

The question arises as to whether treatment with inhibitors of proteolysis could modify the course of kidney damage in chronic rejection.

References

1. Baricos WH, Shah SV (1991) Proteolytic enzymes as mediators of glomerular injury. Kidney Int 40:161 (1992)
2. Davies M, Martin J, Thomas GJ, Lovett DH (1991) Proteinases and glomerular matrix turnover. Kidney Int 41:671
3. Katunuma N, Kominami E (1985) In: Weber G (ed) Advances in enzyme regulation. Pergamon Press, Oxford, p 159
4. Kirschke H, Wood L, Roisen FJ et al (1983) Biochem J 214:871
5. Labarca C, Peigen K (1979) Anal Biochem 102:344
6. Olsen ST (1992) In: Budrick JF (ed) Pathology of allograft rejection. Marcel Dekker, New York, p 333
7. Smith PK, Krohn RJ, Hermanson et al (1985) Anal Biochem 150:76
8. Spiro RG (1967) J Biol Chem 242:1915
9. Tilney NL, Whitley DW, Diamond JR et al (1991) Transplantation 52:389
10. Tullius SG, Heemann, Wagner K, Tilney NL (1993) Changes of chronic kidney allograft rejection. Transplant Proc 25:906