Study of Antibody Production to Tamm-Horsfall Protein in Renal Transplant Donors and Recipients

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The content of Tamm-Horsfall protein was measured in the urine of humans without renal diseases, pregnant women, and donors and recipients of renal transplant using a new test system for measuring Tamm-Horsfall protein including antigenic diagnostic agent and immune serum. Production of antibodies to Tamm-Horsfall protein was characterized using antigen diagnostic system.

Key Words: *Tamm-Horsfall protein; immune serum; erythrocytic diagnostic agent; antibodies*

Tamm-Horsfall protein (THP) is produced in the ascending portion of the thin limb and convoluted tubules of Henle loop. Fixed to the cell surface facing inside the loop with a glycosyl-phosphatidylinositol anchor, it belongs to a category of sequestrated tissue antigens (STAg). THP is characterized by unique structure and properties; it is expressed only in the kidneys on the apical cell membrane of epitheliocytes in the thin ascending limb of Henle's loop [3] and is regarded as the main protein component of normal human urine. The molecular weight of THP is 90 kDa, up to 30% of it are carbohydrate components [4].

Measurement of THP concentration in biological fluids is very difficult, because its excretion in clinically healthy people varies from 4 to 244 mg/day [1] and depends on many factors, including total volume of daily urine, diets, and motor activity [4].

The excretion of THP is reduced *vs.* the norm in renal diseases involving the glomeruli [8]. THP excretion is higher in patients with tubular proteinuria than in those with selective (glomerular) proteinuria [1]. An approximately 40% increase of THP excretion was observed in renal transplant donors after nephrectomy; the level of THP excretion in transplanted kidneys was the same before and after transplantation [12]. According to other authors, daily excretion of THP decreases in kidney recipients immediately after surgery and gradually increased later (after 2-3 weeks) [13].

Destruction of the Henle's loop epitheliocytes in renal diseases can lead to THP release into the blood, which leads to development of autoimmune response [9]. The content of IgA autoantibodies (AAb) to THP significantly increases in acute pyelonephritis or cystic ureteral reflux. The production of AAb to THP can be arrested in cicatricial changes of the kidneys [6]. The titer of AAb to THP in patients with cystitis is the same as in the controls. It is assumed that the involvement of Henle's loop structures into pathological processes in inflammatory diseases of the urinary tract can be differentiated by measuring the level of AAb to THP [5]. The titers of anti-THP AAb are elevated in some autoimmune diseases and in kidney recipients within 7 days after surgery [10].

Immune response to THP can be induced by increased load to the kidneys during pregnancy, which is associated with an increase in anti-THP IgG and IgA AAb titers in the blood of pregnant women [11].

We measured AAb to THP in renal transplant donors and recipients. Urinary secretion of THP in transplant recipients, production of AAb to THP

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after kidney extirpation in donors and in transplant recipients were studied.

MATERIALS AND METHODS

A test system consisting of a monospecific serum to THP and antigenic erythrocytic diagnostic system was used.

Chromatographically purified THP and rabbit immune serum (Biomedical Technologies Inc.) served as the reference preparations.

THP was detected and measured in urine samples from patients without renal diseases, pregnant women, and kidney recipients in antibody neutralization test.

Serum AAb to THP in clinically healthy subjects, pregnant women, kidney donors and recipients were evaluated in passive hemagglutination test [2].

Diagnostic systems used in the study worked with reference sera up to the titer of 1:10,240.

Excretion of THP was studied in 39 kidney transplant recipients and 6 donors (men and women aged 18-50 years). The reference groups were formed by analyzing the material from 100 clinically healthy subjects of both sexes and 15 pregnant women during the third trimester (10 of these with late gestosis without nephropathy).

RESULTS

The mean concentrations of THP in the morning urine of clinically healthy subjects was 574.5±86.0 μg/ml. Serum AAb to THP were detected by passive hemagglutination test in different groups of patients. The mean AAb titer in clinically healthy subjects (if seropositive) was lg 1.6±0.1 (Table 1).

The diagnostic titer (titer of AAb to THP in passive hemagglutination test) was determined by the analysis of serological findings in the control group; normally THP is absent in 95% subjects. This titer was determined as lg 2.3.

The level of THP excretion in pregnant women was higher than in controls (912±112 μg/ml), while serum titer of AAb to THP did not surpass the diagnostic titer, being lg 1.7.

Hence, physiological load to the kidney during pregnancy is associated with increased secretion of THP, but not with increased production of AAb.

The level of THP secretion in kidney recipients during month 1 after transplantation virtually did not differ from the level in the control group (506.7±94.0 μg/ml). The titer of AAb to THP in seropositive recipients was significantly surpassed the diagnostic titer (Table 1), though kidney recipients received standard immunosuppressive therapy (azathioprine, cyclosporin, prednisolone).

For studies of the production of AAb to THP seropositive recipients of the kidney had to be divided into several groups, depending on the type of surgery because of significant difference in the titers between controls and seropositive recipients. The maximum titer of AAb to THP was observed in recipients after transplantation of cadaveric kidney no earlier than 6 months before examination (lg 2.7±0.4). The lowest titer was detected in recipients after transplantation of cadaveric kidney performed more than 6 months before the study (lg 2.4±0.3). The difference between the titers in these two groups was statistically negligible. The titer in recipients after transplantation of the kidney from relatives less than 6 months before examination was lg 2.5±0.3, which was less than in a similar group after transplantation of cadaveric kidney, the difference being (again) statistically negligible. Hence, the type of surgery is not essential for the level of AAb to THP.

The dynamics of AAb in kidney recipients varied depending on the period after transplantation. The highest number of seropositive patients was

TABLE 1. Mean Urinary THP Level and Serum Level of Anti-THP in Kidney Recipients Less than 6 Months after Transplantation and in Normal Subjects

Parameter	Clinically healthy subjects	Recipients (less than 6 months after transplantation)
Urinary THP level	574.5±86.0 μg/ml (n=52)	506.7±94.0 μg/ml (n=34)
Production of serum antibodies to THP		
seropositive	Mean titer=lg 1.6±0.1 min=lg 1.3, max=lg 2.5 (n=68)	Mean titer=lg 2.7±0.2 min=lg 2.2, max=lg 3.7 (n=34)
seronegative	Mean titer=0 (n=32)	Mean titer=0 (n=5)

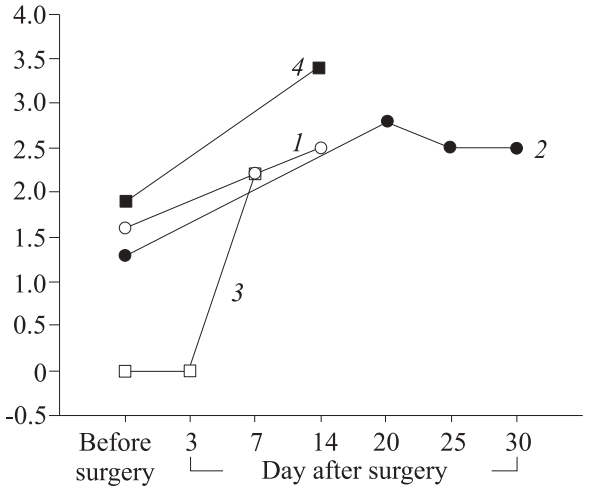

Fig. 1. Dynamics of autoantibodys titers to Tamm-Horsfall protein in the blood of donors before and after nephrectomy. *1-4*: donors.

observed 7-21 days after surgery (Table 2). The highest titers of anti-THP AAb were detected during the first and second months after transplantation; starting from month 3 the titer decreased significantly, though remained higher than in clinically healthy subjects.

The intervention stimulated the production of AAb to THP; the peak was observed by months 1-2, after which the titer slowly decreased.

The next stage of our study was evaluation of AAb in kidney donors. The mean titer of AAb to THP in this group (n=6) was lg 2.9±0.5, 5 donors were seropositive.

Individual monitoring of anti-THP AAb production was carried out in kidney donors. Dynamics of the titers was followed up in a group of 4 donors with the highest levels of AAb (Fig. 1). The sera were tested before and after nephrectomy. Before the intervention AAb titers did not surpass the diagnostic one and one patient had no AAb at all. On day 7 after surgery the titer increased and remained high during 1 month after the intervention, after which they slightly decreased, though remained above the diagnostic titer. As hospitalization of donors was short, we could not carry out a retrospective study.

Hence, the number of seropositive subjects in the group of clinically healthy humans was significantly lower than in other groups. All sera from pregnant women were positive, the levels of AAb to THP not surpassing the diagnostically significant titer. The mean titer lg in seropositive controls was 1.6±0.1, virtually the same as in pregnant women (1.7±0.6). On the other hand, in kidney donors and recipients these values were 2.9±0.5 and 2.7±0.2, respectively. Despite the presence of anti-THP AAb in virtually all pregnant women, their titers were no higher than in the control group, while in kidney recipients and donors this parameter was significantly surpassed lg 2.3.

Studies of THP secretion showed no appreciable differences in the patients and controls. This can be due to specific changes in this parameter during functional adaptation of a transplanted organ. Previously just a short-term decrease in THP excretion after the intervention and subsequent leveling of the parameter to the normal level were observed [7]. On the other hand, THP secretion increased during pregnancy in women without signs of nephropathy. Presumably, increased THP secretion in the studied cases was caused by kidney overload, which can be confirmed by correlation between THP secretion and glomerular filtration, increased after nephrectomy, but unchanged in recipients [12].

It was previously shown that AAb to THP are produced in pyelonephritis [9]. Reaction of these anti-THP AAb with THP is believed to lead to the formation of AAb-STAg complex and favor the development of interstitial nephritis with subsequent kidney sclerosis. One more opinion is that the production of anti-THP AAb can be due to ascending infection of the urinary tract. We have shown that not only these factors, but also surgical intervention, cause production of AAb to STAg (THP in our case).

Anti-THP AAb in high titers are detected in kidney recipients and donors. The increase of anti-THP AAb titer in pregnant women was not paralleled by their accumulation in titers surpassing the

TABLE 2. Changes in AAb Titer in Kidney Recipients at Different Terms after Transplantation

Titer	Period after transplantation		
	day 7-21 (n=17)	month 1-2 (n=17)	more than 3 months (n=17)
Seropositive	16	13	12
Seronegative	1	4	5
Mean titer for seropositive group, lg	2.7±0.3	2.8±0.2	2.4±0.1

diagnostic. These data confirm the concept of THP as a STAg inducing the development of an autoimmune process after alteration of the renal tissue. The pathogenetic significance of increased level of AAb to this STAg after the intervention deserves further investigation.

REFERENCES

1. V. G. Drannik and G. N. Maidannik, *Urol. Nefrol.*, No. 5, 69-74 (1990).
2. V. M. Nikitin, *Handbook of Serological Tests* [in Russian], Kishinev (1977).
3. D. Cavallone, N. Malagolini, and F. Serafini-Cessi, *Biochem. Biophys. Res. Commun.*, **280**, No. 1, 110-114 (2001).
4. O. Devuyst, K. Dahan, and Y. Pirson, *Nephrol. Dial. Transplant.*, **20**, 1290-1294 (2005).
5. A. Fasth, J. Bjure, M. Hellstrom, *et al.*, *Acta Paediatr. Scand.*, **69**, No. 6, 709-715 (1980).
6. A. Fasth, U. Bengtsson, B. Kaijser, and J. Wieslander, *Kidney Int.*, **20**, No. 4, 500-504 (1981).
7. J. Kaden, J. Groth, May G., and B. Liedvogel, *Urol. Res.*, **22**, No. 3, 131-136 (1994).
8. K. L. Lynn and R. D. Marshall, *Clin. Nephrol.*, **22**, No. 5, 253-257 (1984).
9. R. Marier, E. Fong, M. Jansen, *et al.*, *J. Infect. Dis.*, **138**, No. 6, 781-790 (1978).
10. D. Pennica, W. J. Kohr, W. J. Kuang, *et al.*, *Science*, **236**, No. 4797, 83-88 (1987).
11. T. Sandberg and A. Fasth, *Scand. J. Urol. Nephrol.*, **21**, No. 4, 297-300 (1987).
12. O. Torffvit, A. L. Kamper, and S. Strandgaard, *Ibid.*, **31**, No. 6, 555-559 (1997).
13. L. B. Zimmerhackl, *Eur. J. Clin. Pharmacol.*, **44**, Suppl. 1, 39-42 (1993).