

Antibodies to Tamm-Horsfall protein associated with renal damage and urinary tract infections in adults

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Autoantibodies to Tamm-Horsfall protein associated with renal damage and urinary tract infections in adults. Forty-seven adults with urinary tract infection (UTI), 9 with recent acute pyelonephritis and 38 with previous renal infection, were investigated for the presence of autoantibodies to Tamm-Horsfall protein (THP). All patients except 6 had or had had vesicoureteric reflux (VUR). In patients with recent acute pyelonephritis, only IgA antibodies were significantly elevated. Among the patients with previous UTI, more than 6 months before the time of testing, a graded response was found for IgG and IgM specific antibodies, with the lowest value in those with renal damage and elevated serum creatinine and the highest in those with a normal X-ray. A negative correlation was found between IgG antibodies to THP and elevated serum creatinine ($r = -0.76$, $P < 0.02$). No significant correlation was found between VUR itself and antibodies to THP. A low IgG antibody level to THP in patients with a history of previous UTI seems to be a useful indicator of renal scarring. Possible immunologic mechanisms behind the low antibody level and the renal damage are discussed.

Auto-anticorps anti-protéine de Tamm-Horsfall associés à des lésions rénales et à des infections urinaires chez l'adulte. Quarante-sept adultes atteints d'infection urinaire, 9 ayant une pyélonéphrite aiguë récente et 38 une infection rénale antérieure ont été étudiés pour la présence d'auto-anticorps anti-protéine de Tamm-Horsfall (THP). Tous les malades sauf six avaient ou avaient eu un reflux vésico-urétéral. Chez les malades ayant un antécédent récent de pyélonéphrite seul les anti-corps IgA étaient significativement élevés. Parmi les malades dont l'antécédent d'infection urinaire remontait à plus de six mois une réponse a été obtenue pour les anti-corps spécifiques IgG et IgM, avec la valeur la plus faible chez ceux qui étaient atteints de lésions rénales et avaient une créatininémie élevée et la valeur la plus élevée chez ceux qui étaient indemnes de lésions radiologiques. Une corrélation négative a été observée entre les anti-corps IgG anti THP et l'augmentation de la créatininémie ($r = -0,76$, $P < 0,02$). Il n'a pas été observé de corrélation significative entre le reflux par lui-même et les anti-corps anti THP. Un taux faible d'anti-corps IgG anti THP chez des malades ayant des antécédents d'infection urinaire peut être un indicateur utile de lésions rénales. Les mécanismes immunologiques qui peuvent sous-tendre le taux faible d'anti-corps et les lésions rénales sont discutés.

One of the major causes of kidney damage and scarring is urinary tract infection (UTI). Although UTI is a very common infection, few patients develop chronic kidney damage. In children with acute pyelonephritis, that risk has been calculated

to be about 10% [1, 2]. Also, in patients with a history of acute pyelonephritis, fairly comprehensive investigations are necessary to detect renal damage and reduction of renal function. There is thus a need for simplified techniques.

Autoantibodies to the Tamm-Horsfall kidney glycoprotein (THP) have been described in children with UTI [3-6]. During acute bacterial pyelonephritis, increased levels were noted, with the highest values found among children with vesicoureteric reflux [5]. But, in children with renal scarring but with no UTI at the time of testing, low levels of IgG specific autoantibodies were found [5, 6].

In the present communication, we report a study of determination of autoantibodies to THP in adult patients with UTI, present or previous, and vesicoureteric reflux (VUR).

Methods

Patients. The study comprised 38 women and 9 men, aged 17 to 68 years, with a median age of 33 years from the Department of Nephrology, Lund. All patients had or had had a history of symptomatic UTI consisting of attacks of acute pyelonephritis. The diagnosis of UTI was based on the presence of significant bacteriuria, that is, $\geq 10^5$ bacteria/ml. For the diagnosis of acute pyelonephritis, the additional criteria were used: fever $> 38.5^\circ$ C, back or loin pains, and erythrocyte sedimentation rate > 25 mm/hr. To be included in the UTI-free groups, it was required that there should be negative urine cultures for the 6 months previous to the study.

Blood serum samples were obtained as part of regular controls and were analyzed for creatinine and autoantibodies to THP. The sera from 9 patients with acute pyelonephritis were obtained 6 to 22 days (median, 13 days) after onset of the symptoms. Sera samples were stored at -20° C until analyzed.

All patients were radiologically examined with intravenous urography (IVU) and micturation urethrocytography. Renal damage was defined as scarring, calyceal blunting, and/or abnormally small kidney(s). The findings on micturation urethrocytography were graded as follows: (1) no reflux or reflux into the ureter but not to the pelvis, (2) reflux into the renal pelvis, and (3) previous reflux into the renal pelvis in patients who had undergone a successful antireflux operation.

The patients were divided into four groups according to the serum creatinine concentrations, roentgenologic findings, and presence of UTI at the time of testing. The results are summarized in Table 1.

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Table 1. Summary of findings in patients with present or previous acute pyelonephritis

Group	No. of patients		Age, years		UTI at time of testing	Serum creatinine ^a μmoles/liter	I.v. urography ^c	Vesico-ureteric reflux ^d		
	F	M	Median	Range				1	2	3
A	7	2	35	18 to 68	Yes ^b	80.9 ± 9.7	Normal (4) Abnormal (5)	8	1	0
B	6	0	32	17 to 59	No	74.3 ± 5.5	Normal	2	3	1
C	18	3	34	20 to 53	No	83.1 ± 13.6	Abnormal	2	8	11
D	7	4	30	17 to 61	No	226.8 ± 128.2	Abnormal	3	6	2

^a Means ± SD^b All with acute pyelonephritis^c Abnormal is kidney scarring, deformation of calyces, and/or abnormally small kidney(s)^d 1 means no reflux or reflux into the ureter(s); 2, reflux into the pelvis; 3, same as 2, but surgically corrected.

Group A had 7 women and 2 men, aged 18 to 68 years with acute pyelonephritis caused by *Escherichia coli*. All had serum creatinine concentrations below 100 μmoles/liter. One patient had VUR into the renal pelvis, and one into the ureter. Five patients had abnormal IVU.

Group B had 6 women, 17 to 59 years of age, all with normal IVU, previous UTI, but not during the preceding 6 months, and serum creatinine concentrations below 100 μmoles/liter. Three had actual VUR into the renal pelvis; another one was successfully operated for VUR, and in one case the VUR had disappeared on long-term antibiotic therapy.

Group C had 18 women and 3 men, 20 to 53 years of age, all with previous acute pyelonephritis, but not during the preceding 6 months, serum creatinine concentrations below 100 μmoles/liter, and renal damage as judged from IVU. VUR into the renal pelvis was found in 8 patients and into the ureter in one patient. Another 11 had had an antireflux operation performed.

Group D had 7 women and 4 men, aged 17 to 61 years, all with previous acute pyelonephritis, but not during the preceding 6 months, elevated serum creatinine (median, 140 μmoles/liter; range, 110 to 430 μmoles/liter), and renal damage as judged by IVU. Six of the patients had VUR into the renal pelvis and one into the ureter. Another 2 had had their VUR surgically corrected.

The *reference group* consisted of 70 children and young adults of both sexes, 1 to 38 years of age, admitted to or working in the Children's Hospital, Göteborg, and are described elsewhere [5]. Those with a history of renal disease, present infection, or other conditions that could influence the immunologic status were excluded, and all had a negative urine culture.

Tamm-Horsfall protein preparation. This was prepared by repeated precipitation with 0.58 M sodium chloride, followed by extensive dialysis against distilled water, and lyophilization [7]. The purity of the preparation was tested by polyacrylamide gel electrophoresis in sodium dodecylsulphate (SDS) carried out as described by Weber and Osborn [8] with 7.75% polyacrylamide in the gel. Concentrations of 0.125 mg of THP were applied on the gel, and one single protein band corresponding to a mol wt of about 80,000 daltons was seen.

Crossed immunoelectrophoresis [9] and line immunoelectrophoresis [10] were also used for testing the purity of the THP. In those techniques, 0.25% SDS (wt/vol) was added to the THP overnight at + 4° C for depolymerization. The THP was used in

concentrations from 25 μg/ml to 1 mg/ml. When rabbit antisera against human THP (Behringwerke AG, Marburg, FRG, and noncommercial source) and against human urinary protein (Dakopatts, Copenhagen, Denmark) were tested against the THP preparation in crossed immunoelectrophoresis and line immunoelectrophoresis, one single fused precipitation line was seen. The rabbit antisera against the THP preparation were also tested in crossed immunoelectrophoresis against THP, as well as against serum and urine obtained from one of the donors of urine for the THP preparation. The urine gave one precipitate that showed a fused precipitation line with the THP. No precipitates could be detected between the serum and the THP antisera.

Enzyme-linked immunosorbent assay (ELISA). This was used for determination of the immunoglobulin class-specific autoantibodies against the THP. The basic procedure was the same as the one described by Ahlstedt et al [11]. Briefly, plastic tubes were coated with THP (10 μg/ml) in phosphate-buffered saline (PBS), at a pH of 7.2 for 3 hours at 37° C. After washings with PBS supplemented with 0.05% Tween 20, the serum samples were added in duplicate at concentrations of 10⁻². After 5 hours' incubation time in room temperature on a roller drum and further rinsings, the enzyme-conjugated antihuman IgG, IgA, or IgM was added and allowed to react in room temperature on the roller drum. The conjugates were prepared using antisera from Dakopatts A/S, Copenhagen, Denmark, and alkaline phosphatase from Sigma Chem. Co., St. Louis, Missouri. On the next day, the tubes were again washed, and the enzyme substrate *p*-nitro-phenylphosphate (1 mg/ml; Sigma Chemical Co.) was added. The enzyme reaction was performed to appropriate color intensity or for 100 min, stopped with 3 M sodium hydroxide, and read at 405 nm. The readings were converted to a reaction time of 100 min, and expressed as percent of the mean of the reference material, that is, 100% = mean of the references. The coefficients of variation based on double determinations and day-to-day variations have been found to be 7, 13, and 8% for IgG, IgA, and IgM antibodies, respectively.

Statistical procedures. Analysis of variance was used to compare antibody levels in the three groups with no UTI at the time of testing and to relate antibody levels to the type of VUR. The Spearman coefficient (*r*) of rank correlation was calculated to compare the serum creatinine values and the IgG antibodies to THP in patients with increased serum creatinine concentrations [12]. To test the significance of the difference between the

Table 2. Determination of autoantibodies to Tamm-Horsfall protein in patients with present or previous UTI

Patient groups	N	Autoantibodies to Tamm-Horsfall protein ^a		
		IgG %	IgA %	IgM %
A	9	99.0 ± 69.5	244.6 ± 128.9 ^d	108.8 ± 37.8
B	6	82.7 ± 38.3	166.8 ± 91.4	119.5 ± 32.2
C	21	67.0 ± 21.5 ^b	135.6 ± 42.5 ^d	89.8 ± 26.1
D	11	43.9 ± 16.5 ^b	114.5 ± 43.5	77.6 ± 17.6 ^c
Reference group		100 ± 30	100 ± 40	100 ± 33

^a Values are expressed as the percent of the mean of a reference group (=100%). Values shown are the means ± 1 SD.

^b $P < 0.001$, ^c $P < 0.01$, values that are significantly low compared with reference group values.

^d $P < 0.01$, values that are significantly high compared with reference group values.

Table 3. Estimation of IgG antibodies to Tamm-Horsfall protein in patients with previous UTI vs vesico-ureteric reflux^a

Group	N	Serum creatinine $\mu\text{moles/liter}$	IgG antibodies to THP ^b
1. No reflux or reflux into ureter	7	102	77.4 ± 34.0
2. Reflux into ureter and pelvis	17	143	60.6 ± 25.8
3. Same as 2, but successfully operated	14	107	58.1 ± 22.2

^a None of the differences between the groups are significant ($P > 0.1$)

^b Values are the means ± SD.

means of the control group and those patients with no present UTI, we used Student's *t* test.

Results

The results of the estimation of autoantibodies to THP are summarized in Table 2 and 3. The results of the individual patients are presented in Fig. 1.

The mean IgG and IgM antibody levels to THP in the sera from the patients in group A with acute pyelonephritis did not differ from those of the reference group. Only one of the patients with normal IVU had an increased IgG level of 267% and IgM level of 193%. The mean level of IgA antibodies was significantly higher than the reference values. All 4 patients with normal IVU had high IgA antibody levels, whereas only one of the patients with an abnormal IVU had a similar level.

The 6 patients constituting group B with previous UTI but no infection at the time of testing, and a normal IVU, had antibody levels to THP that did not differ significantly from the reference group ($P > 0.1$). One patient had IgG antibodies below -2 SD of the reference group and 2 had high IgA levels of antibodies.

The two patient groups with renal damage but no UTI during the preceding 6 months had mean antibody levels to THP significantly differing from the reference group. Group C, those with normal serum creatinine, had high IgA levels ($P < 0.01$) and low IgG levels of antibodies to THP ($P < 0.001$), whereas group D, those with elevated serum creatinine, had low IgG and IgM antibody levels ($P < 0.001$ and $P < 0.01$, respectively).

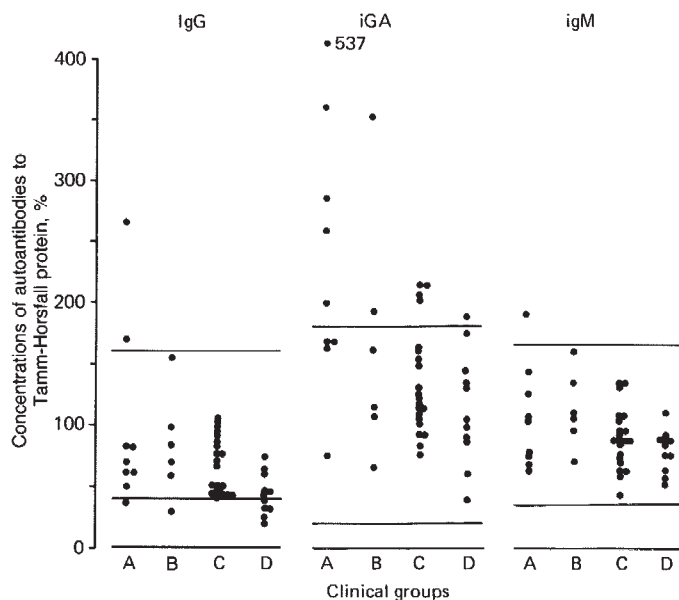


Fig. 1. Serum IgG, IgA, and IgM autoantibodies to Tamm-Horsfall protein in different patient groups with present or previous bacterial pyelonephritis: A denotes group of 9 patients with acute pyelonephritis; B, 6 patients with previous UTI and normal i.v. urography; C, 21 patients with previous UTI and renal damage, and D, 11 patients with previous UTI, renal damage as well as elevated serum creatinine. The values indicated are expressed as percent of the mean of the reference group. Indicated are also the mean ± 2 SD of the reference group.

In group D, 5 of 11 patients had IgG values below -2 SD and another 5 had values between -1 and -2 SD of the reference group.

An analysis of the variance of the antibody levels found was made for the three groups of patients with previous UTI but no UTI at the time of testing (group B, C, and D). Significant differences were found among the groups for IgG ($P < 0.001$) and IgM ($P < 0.05$) but not for IgA ($P > 0.1$) antibodies to the THP. The highest mean levels were seen in serum from patients with normal IVU, and the lowest values were among those with both abnormal IVU and elevated serum creatinine concentrations. Furthermore, a negative correlation was found between low IgG autoantibodies in serum and the corresponding elevated serum creatinine values. The Spearman coefficient of rank correlation was -0.76 ($P < 0.02$). No such correlation was found for IgM or IgA antibodies and serum creatinine values.

The males are not presented separately in the data but their levels of IgG and IgM autoantibody against THP were low ($P < 0.001$ and $P < 0.01$, respectively) in accordance with the findings in the corresponding females.

No statistical difference in autoantibody levels to THP could be revealed when the patients were grouped according to the findings of VUR: no reflux or only reflux into the ureter, reflux into the renal pelvis, and surgically corrected VUR (Table 3), although the lowest antibody levels were found among the patients with VUR into the renal pelvis.

Discussion

Significantly low levels of autoantibodies to THP, especially of the IgG class, were described in children with renal parenchymal reduction due to UTI but without present bacteriuria [5, 6]. The present study confirms this finding in adult patients with

a history of previous UTI. There were gradually diminishing levels of IgG and IgM autoantibodies with an increasing degree of damage judged by IVU and serum creatinine concentration. As in children [5, 6], the most marked depression was noted for the IgG antibodies. Furthermore, there was a negative correlation between the serum creatinine levels and IgG autoantibodies against the THP. Consequently, the highest serum creatinine corresponded to the lowest levels of IgG autoantibodies. Thus, in adults too, a low IgG antibody level to THP in patients with a history of previous UTI seems to be a useful indicator of renal scarring.

The sera samples from the patients with recent acute pyelonephritis were obtained at an interval that, judged from the children studies on autoantibodies to THP [3–6], seemed to be optimal to register increased levels. In these adult patients, only the IgA antibody levels were high. Unfortunately, the only earlier report on THP antibodies in adults with acute UTI is also hampered by the lack of sequential analysis [13]. Thus, further studies are needed to evaluate the usefulness of analysis of antibodies against THP as a tool for level diagnosis in adult UTI.

The study did not reveal any significant correlation between VUR itself and antibody levels to THP. The lowest values in the VUR-groups could probably be attributed to the renal damage. But, the difference (nonsignificant) between group 1 and 3 could hardly be explained in this way. Extended patient studies are needed to elucidate the relationships. As far as children are concerned, the highest antibody levels were found among those with VUR and acute pyelonephritis [5], which favors the hypothesis that the combination of VUR and bacteriuria is a threat to the kidney [14].

The mechanism behind the low antibody level in patients with renal damage during a UTI-free period remains unclear. Several groups have independently shown that the periodic acid-Schiff-positive interstitial deposits often found in scarred kidneys are composed of THP [15–17]. One interpretation of this is that the THP antibodies are trapped into the deposits and consumed. Another would be that the low antibody levels are due to an immunosuppressive effect directed towards the formation of autoantibodies to THP. In experimental autoimmune interstitial nephritis induced by injections of renal tubular material, there was initially evidence for an immune mediated cytotoxicity [18, 19]. About a week later, however, a strong polyclonal immunosuppression developed that was mediated by T-cells in peritoneal lymphnodes and in the spleen. Similar mechanisms may operate in man during the nephritis following renal infection. Endotoxins have profound effects on the immune system, activating both T and B cells, as well as macrophages [20–22], and leading to renal damage if the immune reactions are not modulated.

THP introduced interstitially during the infection may also release a cell-mediated immune response. Cellular immunity to THP has been noted in patients with renal tubular acidosis and chronic hepatitis [23]. The lymphocytes from the patients were cytotoxic to baby hamster kidney cells [24]. Further, THP has lectin-like stimulatory effects on lymphocytes in vitro [25]. An active immunosuppression to THP may be favorable and may stop further damage to the renal parenchyma.

Severe uremia is also an immunosuppressive factor, but in the present study only 2 patients had advanced renal failure

(serum creatinine, 400 to 430 μ moles/liter), but no one had had manifest uremia. Among children, even lower IgG antibody levels have been found, although the renal damage was not severe, and relatively low antibody levels were found among children with unilateral damage or even focal scarring [5, 6]. Thus, diminished access of TH antigen to immunocompetent cells due to scarred tissue could hardly be an explanation for low antibody levels.

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