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Urinary antibody response after immunisation with a vaccine against urinary tract infection

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Summary. Immunisation of mice with SolcoUrovac® vaccine induced an approximately 10-fold increase of the total amount of IgG and a 2-fold increase of IgA immunoglobulins in urine. IgG antibodies to SolcoUrovac antigens appeared in urine after the first injection, and booster injections caused a further increase of the titer. IgA antibodies appeared in the urine after the second injection, and the third injection doubled the titer. IgM immunoglobulins and specific IgM class antibodies to SolcoUrovac were not found in any urine tested. The exact origin of the immunoglobulins in the urine as well as the spcificity of immune response is discussed.

Key words: Immunisation – Protection against urinary infection – Urine antibodies

Introduction

Urinary tract bacterial infection (UTI) is one of the commonest infections of man and, in spite of the availability of effective antibiotics and antimicrobial agents, it remains a cause of considerable morbidity. A special problem is posed by patients prone to recurrent UTI. The incidence of recurrence is very high and the risk of suffering a subsequent infection is 10–15 times higher than for the initial infection [9]. Other patients especially vulnerable to UTI are those with indwelling urinary catheters. Once a urethral catheter is in place, the probability of bacteriuria increases by 5 to 10%/day. Although it is usually asymptomatic such patients may get fever, acute pyelonephritis and bacteremia, associated with mortality [27, 28].

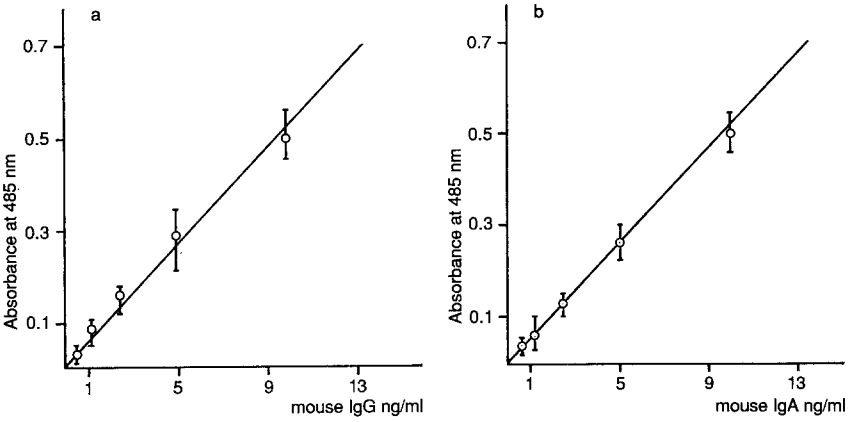
Experiments in animals have shown that immunisation against UTI can have a protective effect [1, 6, 7]. This was the rationale for introducing a polyvalent intramuscularly applied vaccine consisting of the bacteria most frequently causing UTI in humans[1].

Parenteral immunisation with a vaccine, which causes an increase of specific serum antibodies, is protective mainly in hematogenous disease. Since the majority of UTI in humans, especially infection of the lower urinary tract, is believed to be of the ascending type, antibodies in the urine could have greater importance for protection. The significance of urinary antibodies and elevation of urinary IgG and IgA as well as secretory IgA in patients with acute phyelonephritis or cystitis has been underlined in several reports [10, 18, 23, 26]. Antibodies present in the urine may bind to the infecting bacteria and can be detected in the urine sediment of patients with UTI. This phenomenon was utilised in the antibody-coated bacteria tests which have proved useful in distinguishing kidney infection from lower urinary tract infection [14, 19, 25].

Investigations in rats showed that intraperitoneal and intravesical immunisation with killed bacteria stimulated local immune response in the urinary tract and protected against ascending UTI [8]. This effect was correlated with the appearance of antibodies in the urine. The passive transfer of mouse urine containing these antibodies to susceptible mice also protected against UTI. It was interesting that the authors did not find a correlation between the presence of antibodies in the urine and the serum.

Production of urine immunoglobulins takes place in the urinary bladder. Although the rabbit's normal bladder produces relatively few immunoglobulins, bacterial infection causes not only a great increase of the total amount of immunoglobulins, in particular of the IgG class (about 25 times more), but also of the synthesis of specific antibodies [5]. The capability of the bladder mucosal system to produce

[1] SolcoUrovac®, Solco Basle Ltd., Switzerland



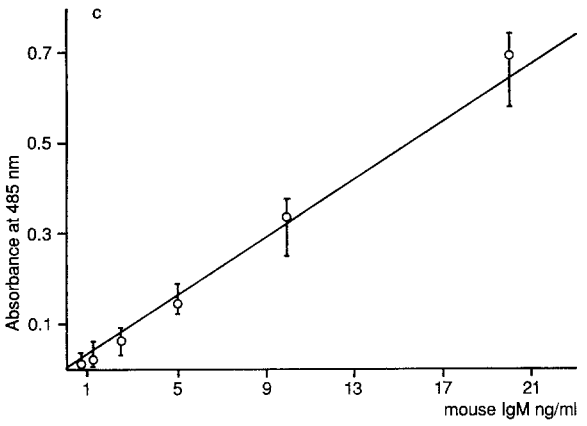


Fig. 1a–c. Dependence of the concentration of IgG (**a**), IgA (**b**) and IgM (**c**) on the readings with ELISA test

specific IgG antibodies was confirmed in experiments in rats [13].

Recently a considerable interest has been focused on the role of pilation of E. coli bacteria in the pathogenesis of UTI. Pili of these bacteria mediate adherence to uroepithelial cells which facilitates colonisation. Immunisation with E. coli pili protected against ascending pyelonephritis in laboratory animals [2, 20, 21] as well as in nonhuman primates [17]. Intrarenal antipili antibodies were produced after experimental pyelonephritis in rabbits [22].

In this study we investigated systemic immunisation with SolcoUrovac for any influence on antibodies in urine. Parallel determination of antibodies in the serum was carried out to obtain evidence about the origin of the antibodies in the urine.

Material and methods

Immunisation and obtaining samples

NMRI white female mice weighing 22–24 g were injected intraperitoneally with 0.5 ml (1 ampoule) of the liquid form of SolcoUrovac (Lot 400407). Booster injections containing the same amount of vaccine were performed twice at an interval of two weeks. Urine samples were obtained by gentle massage of the mouse bladders just before immunisation and 10 days after each injection. In this way it was possible to obtain about 0.6 ml urine from each mouse during two consecutive days. Blood samples were taken before immunisation and 20 days after the last injection. After clotting, the sera were separated by centrifugation and stored at −20°C.

Antigen

Bulk of SolcoUrovac vaccine ($A_{600} = 14.12$) in PBS buffer after the addition of 0.5% SDS was boiled for 30 min and dialysed (cut off 1,000) for 3 days against the same buffer. The lightly opaque solution was centrifuged (1 h at 10,000 × g) and the clear supernatant was used for ELISA determination (stock antigen). Antigen prepared in this way gave the highest reading in the ELISA test in comparison with sonication or multiple freezing and thawing of SolcoUrovac bulk vaccine.

ELISA procedure – was done according to standard procedure. Technical details: Dynatex plates, dilution of prepared SolcoUrovac stock antigen 1:500, dilutions of anti-mouse IgG, IgA and IgM conjugated with peroxidase were 1:500, 1:200 and 1:200, respectively. Time of reaction – 15. min. The absorbance was measured at 485 nm by a Microelisa Automatic Reader (Kontron SLT-210).

Titrations were performed in twofold dilutions and determinations done in duplicate for all samples (in preimune urine only once). The end point of titration was the highest dilution of tested sample still yielding an absorbance of at least 0.100 above the corresponding background. The backgrounds ranged between 0.110–0.250 ELISA for various immunoglobulins.

For determination of the total amount of each of the immunoglobulin classes, the microtiter plate wells were coated with goat anti-mouse IgG, IgA or IgM, in all cases at a concentration of 2 μg/ml.

Creatinine determination

The creatinine content in urine was determined by Dr. H. Ackermann, Clinical Chemical Laboratory of Kantonsspital Basel, using the kinetic assays of the enzymatic PAP method (kit from Wako Pure Chemical Industries Ltd., Osaka, Japan). Measurements were performed using the Cobas Bio apparatus (Roche Diagnostics).

Reagents

Affinity purified goat anti-mouse IgG (whole molecule), IgA (α-chain specific) and IgM (μ-chain specific) were purchased from Sigma Chemical Company. Peroxydase-conjugated goat antisera: anti-mouse IgA (α-chain specific), anti-mouse IgG (whole molecule) anti-mouse IgM (μ-chain specific) were also products of Sigma. From the same firm IgM (kappa), IgA (kappa) and purified mouse IgG were obtained. Other reagents were purchased from Merck.

Table 1. Reciprocal titer values of total amount of IgG in urine of immunised mice

Mouse No	Before immunisation	After 1st immunisation 10 days	After 2nd immunisation 10 days	After 3rd immunisation	
				10 days	20 days
1	600	1,200	3,200	1,600	1,200
2	400	1,300	4,800	3,200	2,400
3	200	3,200	6,400	6,400	3,200
4	600	2,000	3,200	4,000	1,200
5	200	4,800	5,600	6,000	n.d.
6	800	4,800	9,600	6,400	3,200
7	300	1,200	6,400	5,600	3,200
8	800	1,600	2,800	2,800	2,400
9	800	2,400	4,800	6,400	3,200
10	800	2,400	4,800	2,800	3,200
Mean	550	2,010	5,160	4,520	2,578

n.d. = not determined

Results

To check the sensitivity of the determination of the total amount of IgG, IgA and IgM classes, the relation between the measured concentration and its absorbance in the ELISA test was determined (Fig. 1). For all three immunoglobulins there was a linear relationship of the measured parameters. The smallest concentration of IgG and IgA which could be measured amounted to 1 ng/ml, and to 1.5 ng/ml for IgM.

The results of the titration of the total amount of IgG and IgA in the urine of immunised rats are presented in Tables 1 and 2. Before immunisation, there were considerable amounts of both immunoglobulin classes present in all 10 mouse urines. Ten days after the first vaccination there was an average 4-fold rise of the total IgG which reached the highest titer 10 days after the second immunisation. After the third immunisation, there was no further increase of the titer, rather a tendency to a decrease, and after 20 days the mean titer amounted to only half of the highest value.

The mean values of the total amount of IgA immunoglobulins were continuously increasing during the whole experiment and were highest 20 days after the third vaccination. There were, however, three non-responders (out of ten) which did not show any change of IgA titer after immunisation.

No immunoglobulin of IgM class was found in any of the tested urine samples.

There were no antibodies of IgG, IgA and IgM classes to SolcoUrovac® antigens in urine before immunisation (Tables 3 and 4). The first immunisation, however, caused appearance of considerable amounts of IgG antibodies in the urine of all mice, and after the second and third booster injections there was a further, approximately 4- to 5-fold rise of mean titers. IgA antibodies to SolcoUrovac first appeared after the second immunisation and were present in 60% of urines. The third immunisation caused a further, approximately 2-fold increase of this class of antibodies in the responding animals.

The titers of antibodies in the serum of mice before and after immunisation are presented in Table 5. In the serum of unvaccinated mice there were small but distinct titers of antibodies of IgG and IgA classes. After the third vaccination there was a considerable increase of all classes of antibodies, similar in each tested mouse serum.

Discussion

The human urinary tract is, in most instances, infected via the ascending route. A vaccine with protective effect against UTI should induce specific antibodies in urine.

Systemic immunisation with SolcoUrovac regularly raised specific antibodies in the urine of all mice. Before immunisation no specific antibodies were found in any case. Because the standard curves for IgG and IgA determination had the same slope and were nearly identical, the comparison of titers of these two classes of antibodies gives quantitative differences. Specific IgG antibodies predominated in the urine and were about 10 times more prevalent than IgA antibodies (Table 6). No IgM antibodies were found in any of the urines tested. The predominance of IgG and a lack of antibodies of IgM class is in agreement with earlier studies [4, 8, 11].

Table 2. Reciprocal titer values of total amount of IgA in urine of immunised mice

Mouse No	Before immunisation	After 1st immunisation 10 days	After 2nd immunisation 10 days	After 3rd immunisation	
				10 days	20 days
1	100	150	200	200	300
2	200	400	600	800	800
3	200	400	600	600	600
4	100	100	100	100	100
5	100	100	100	100	n.d.
6	100	150	150	150	150
7	100	100	100	100	100
8	100	150	200	300	300
9	150	200	200	200	200
10	50	100	150	200	150
Mean	120	185	240	255	270

n.d. = not determined

Table 3. Reciprocal titer values of IgG antibodies to SolcoUrovac in urine of immunised mice. In brackets there are the titers per one μmole of kreatinine

Mouse No	Before immunisation	After 1st immunisation 10 days	After 2nd immunisation 10 days	After 3rd immunisation	
				10 days	20 days
1	<2	20 (5)	100 (31)	60 (22)	60 (20)
2	<2	10 (2)	120 (30)	120 (29)	200 (41)
3	<2	60 (11)	100 (26)	160 (38)	100 (22)
4	<2	60 (15)	80 (20)	100 (21)	60 (13)
5	<2	20 (5)	80 (17)	120 (30)	n.d.
6	<2	30 (7)	200 (53)	240 (60)	120 (33)
7	2	10 (3)	160 (38)	60 (12)	60 (13)
8	<2	10 (3)	100 (28)	160 (40)	160 (29)
9	<2	15 (4)	60 (19)	120 (30)	60 (15)
10	<2	20 (3)	110 (26)	80 (18)	120 (22)
Mean	<2	26 (5.8)	112 (29)	122 (30)	104 (23)

n.d. = not determined

It was interesting that immunisation by SolcoUrovac® evoked in the urine a strong and rapid increase of the total amount of immunoglobulins, particularly IgG class. This increase was only partially and probably to a very low degree caused by the specific antibodies, as otherwise the titers of total immunoglobulins would have been much lower, and close parallels would have occured between the titer changes of total immunoglobulins and corresponding specific antibodies. The mechanisms of production of such a high amount of immunoglobulin in the urine is unclear, but one can suppose that it could be an effect of nonspecific stimulation of the humoral immune system.

Determination of creatinine in the urine was done in order to make the results of titration independent of urine concentration. However, calculation of immunoglobulins and antibody titers in the urine per μmole of creatinine did not change the ratios between respective data (Table 3).

As expected, after immunisation there was a strong response to vaccine antigens demonstrated by the appearance of antibodies of all three classes of immunoglobulins in the serum. But even in serum of unvaccinated mice there were distinct titers of IgG and IgM antibodies. This could suggest that the immune system of these mice had an earlier contact with some

Table 4. Reciprocal titer values of IgA antibodies to SolcoUrovac in urine of immunised mice

Mouse No	Before immunisation	After 1st immunisation 10 days	After 2nd immunisation 10 days	After 3rd immunisation	
				10 days	20 days
1	< 4	< 4	< 4	< 4	< 4
2	< 4	< 4	16	30	40
3	< 4	< 4	10	10	10
4	< 4	< 4	< 4	< 4	< 4
5	< 4	< 4	10	20	n.d.
6	< 4	< 4	10	20	20
7	< 4	< 4	< 4	10	10
8	< 4	< 4	10	30	20
9	< 4	< 4	4	4	4
10	< 4	< 4	< 4	< 4	4
Mean	< 4	< 4	6[a]–10[b]	12[a]–18[b]	12[a]–15[b]

[a] Values below 4 were assumed to be zero
[b] Values below 4 were not considered

Table 5. Reciprocal titer values of antibodies to SolcoUrovac in serum of immunised mice (n.d. = not determined)

Mouse No	Before immunisation			20 days after 3rd immunisation		
	IgG	IgM	IgA	IgG $\times 10^{-3}$	IgM $\times 10^{-3}$	IgA $\times 10^{-3}$
1	400	40	< 10	1,000	240	8
2	400	60	< 10	800	120	8
3	200	80	< 10	1,600	180	8
4	200	160	< 10	800	160	8
5	300	120	< 10	n.d.	n.d.	n.d.
6	400	160	10	2,400	120	8
7	400	60	< 10	1,600	80	8
8	200	80	< 10	1,600	120	8
9	400	120	< 10	800	320	6
10	300	160	10	800	320	6

Table 6. Increase of titer of immunoglobulins and antibodies to SolcoUrovac in urine and serum of immunised mice

Class of immunoglobulins or antibodies	Before immunisation	20 days after 3rd immunisation	Increase
Urine			
IgG total	550	2,578	4.7 ×
IgG specific	< 2	104	> 52
IgA total	120	270	2.3 ×
IgA specific	< 4	12–15	> 3–3.8 ×
Serum			
IgG specific	320	1,267,000	3,960 ×
IgM specific	140	182,000	1,750 ×
IgA specific	< 10	7,556	> 756 ×

enterobacteriaceae. The possibility that immunoglobulins nonspecifically bind to constituents of the vaccine exists but is less likely.

In the present ELISA determination, the whole mixture of dissolved and dialysed bacteria of the vaccine and not the isolated and purified antigens were used. This approach was prefered because up to now it is not known which antibodies to the known and identified antigens are involved in decisive protection. Additionally, other antibodies than against O, K or pili antigens might also be of great importance.

It is generally believed that the bladder and the urethra are sites of antibody secretion and that transudation from the serum is another source of the urinary antibodies [24]. Systemic immunisation with SolcoUrovac undoubtedly stimulated the urogenital mucosal immune system. On the other hand, which of the

antibodies in the urine originated from the serum and which were produced locally was not explained in the study. It can only be speculated that transudation depends on the concentration of antibodies in the serum. When a threshold is exceeded, then the antibodies appear in the urine, as was seen with specific antibodies of the IgG class after immunisation.

This study confirmed the assumption that the protective and curative effect of vaccination with SolcoUrovac of patients with UTI was achieved [3, 12, 15, 16], at least partially, owing to secretion and/or transudation into the urinary tract of specific antibodies to several types of E. coli and other commonly found bacteria. Additionally, a nonspecific stimulation resulting in a protective effect was possible.

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