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**SOME EXPERIMENTS ON ASCENDING OF
CORYNEBACTERIUM RENALE IN
MICE FROM URINARY BLADDER
INTO KIDNEYS**

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Immunization of mice with killed organisms of *Corynebacterium renale* did not prevent the ascending of organisms of *C. renale* from the urinary bladder into the kidneys, despite the fact that the antibodies against the organisms were produced in mice. The rate of the ascending of organisms into the kidneys in athymic nude (nu/nu) mice and their heterozygous littermates (nu/+) with *C. renale* inoculated into the urinary bladder did not significantly differ. BCG-pretreatment did not significantly influence the ascending of *C. renale* from the urinary bladder into the kidneys. The results may indicate that the ascending of *C. renale* from the urinary bladder into the kidneys is not seriously affected by systemic humoral and cell-mediated immunity.

INTRODUCTION

The ascending mechanisms of *Corynebacterium renale* from the urinary bladder into the kidneys of cows are unknown⁸⁾. An experimental model of *C. renale* pyelonephritis was produced in mice by inoculating the organisms into the urinary bladder¹⁰⁾. With the aid of the mouse model, it was previously found that cyclophosphamide, a suppressant of humoral immunity, did not affect the ascending of *C. renale* from the urinary bladder into the kidneys¹⁰⁾.

In the present study, experiments were carried out on ascending of *C. renale* in mice from the urinary bladder into the kidneys, using the mice systemically immunized with killed organisms, athymic nude (nu/nu) mice, and the mice pretreated with BCG.

MATERIALS AND METHODS

Mice: Female, 5 to 7 weeks-old, ddY-F (24-30 g), ddN (20-26 g), BALB/c athymic nude (nu/nu, 11-18 g) and BALB/c mice heterozygous for the nude gene (nu/+, 16-20 g)

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were used. BALB/c athymic nude mice and their heterozygous littermates were housed in a vinyl isolator or an isolated room in filter-top cages and fed commercially gamma-irradiated mouse feed pellets or autoclaved commercial mouse feed pellets and autoclaved water.

Bacterial strain: *Corynebacterium renale* strain 115 was solely used.

Methods of *C. renale* inoculation: The inoculation of *C. renale* into the urinary bladder was carried out following the method previously described¹¹. Mice were inoculated with 0.02 ml of a suspension of a fresh culture of strain 115 containing 1.8×10^5 to 5×10^8 organisms.

Immunization of mice: Mice were actively immunized with heat-and formalin-killed organisms of *C. renale*. Heat-killed organisms were prepared by suspending the fresh bacterial culture in saline and heating the suspension at 56°C for 30 minutes in a water bath. 5×10^7 heat-killed organisms per 0.1 ml dose were injected intravenously three times with 2 day intervals between injections. Formalin-killed organisms were prepared by the addition of formalin to a suspension of *C. renale* in saline at a rate of 0.1 %, and keeping it at 37°C for 2 days. The 5×10^7 and 1×10^9 formalin-killed organisms per 0.1 ml dose were injected intravenously or subcutaneously 5 days apart. The control mice in each group were given a similar volume of saline in the same manner.

BCG-pretreatment: BCG vaccine containing 2×10^8 viable organisms per 0.1 ml dose was injected intravenously via a lateral tail vein. The control mice were given a similar volume of saline in the same manner.

Tuberculin reaction: Tuberculin reaction tests were performed 3 weeks after BCG-pretreatment as follows. Five μg of tuberculin (purified protein derivative, PPD) in a volume of 0.02 ml was injected into the right rear footpads of mice; a similar volume of saline was injected into the left rear footpads as a control. The degree of footpad swelling was measured with a micrometer after 24 hours. The degree of tuberculin reaction = [the thickness of footpad swelling after injecting PPD (mm)] - [the thickness of footpad swelling after injecting saline (mm)].

Bacteriological examination: Seven days after inoculation, the mice were examined for recovery of *C. renale* as previously described¹¹. In case of death, the mice were examined before the 7th postinfection day.

Serological examination: The procedure employed was carried out following the method previously described⁶. The agglutinating anti-body against *C. renale* was titrated a week after the last vaccination. One to 4 mice of each group were examined for the antibody. The serum antibody titer was determined by the agglutination test⁶. Antibody response was judged positive when the mice showed definite agglutination at 1:20 or more serum dilution.

Statistics: Probability values for differences in frequency were determined by Fisher's exact test. A probability value (P) of ≤ 0.05 was considered significant.

RESULTS

Effect of immunization with killed organisms on the ascending of *C. renale* from the urinary bladder into the kidneys: The antibodies against *C. renale* were produced before challenge in the mice immunized with heat- or formalin-killed organisms (tab. 1). A week after the last immunization, 3.2×10^7 to 5×10^8 organisms of *C. renale* were inoculated into the urinary bladder of the vaccinated mice. The immunization did not inhibit the ascending of *C. renale* from the urinary bladder into the kidneys of the vaccinated mice.

TABLE 1 *Effect of immunization with killed organisms on the recovery of Corynebacterium renale from the kidneys*

STRAIN OF MICE	IMMUNIZATION		AGGLUTININ TITER BEFORE CHALLENGE	INOCULUM SIZE	RECOVERY RATE OF <i>C. RENALE</i> FROM THE KIDNEYS
	With	Route			
ddY-F	Heat-killed organisms	iv	1:320-1:640(2)*1	3.2×10^7	4/5*2
	—		<1:20	"	4/5
ddN	Formalin-killed organisms	iv	1:320-1:640(4)	5×10^8	10/10
		sc	1:160-1:640(4)	"	10/10
	—		<1:10	"	10/10

iv = intravenous, sc = subcutaneous

*1 In parenthesis is No. of mice examined.

*2 Numerator indicates No. of mice from which *C. renale* was recovered; denominator denotes No. of mice examined.

Experiments using athymic nude (nu/nu) and normal (nu/+) BALB/c mice: Athymic nude and normal BALB/c mice were injected with 1.8×10^7 and 8.3×10^7 organisms of *C. renale* into the urinary bladder. The rate of the ascending of *C. renale* into the kidneys was not significantly different between the athymic nude and the normal BALB/c mice (tab. 2). The rate of recovery of *C. renale* from the kidneys was, however, slightly higher in athymic nude mice than in normal BALB/c mice. A smaller number of organisms, 10^6 organisms, did not cause renal infection in either athymic nude or normal BALB/c mice.

Recovery of *C. renale* from tissue and organs other than the urinary organs was completely negative in athymic nude mice as in normal BALB/c mice.

Effect of pretreatment with BCG on the ascending of *C. renale* from the urinary bladder into the kidneys: The mice treated with BCG had a positive footpad swelling response to PPD, tuberculin reaction. Twenty-four hours after PPD injection, the difference between the right (injected with tuberculin) and the left (control) footpads

TABLE 2 *Recovery of Corynebacterium renale from the kidneys of athymic nude (nu/nu) and normal (nu/+) BALB/c mice*

MICE	INOCULUM SIZE	RECOVERY OF <i>C. RENALE</i> FROM THE KIDNEYS	
		Rate	P
nu/nu	8.3×10^7	5/8*	NS
nu/+	"	2/10	
nu/nu	1.8×10^7	3/10	NS
nu/+	"	1/10	

* Numerator indicates No. of mice from which *C. renale* was recovered; denominator denotes No. of mice examined. NS=Not significant ($P > 0.05$)

TABLE 3 *Effect of pretreatment with BCG on the recovery of Corynebacterium renale from the kidneys of mice*¹*

BCG-PRE-TREATMENT	INOCULUM SIZE	RECOVERY OF <i>C. RENALE</i> FROM THE KIDNEYS	
		Rate	P
+	2×10^7	6/11* ²	NS
-	"	9/10	

*¹ Strain of mice was ddN.

*² Numerator indicates No. of mice from which *C. renale* was recovered; denominator denotes No. of mice examined. NS=Not significant ($P > 0.05$)

TABLE 4 *Recovery of Corynebacterium renale from the kidneys of mice which were positive in antibody response and positive in recovery of the organisms from the urinary bladder of mice*¹*

PRETREATMENT OF MICE	RECOVERY OF <i>C. RENALE</i> FROM THE KIDNEYS* ²			POSITIVE RATE (%)	P
	Total	No. positive	No. negative		
BCG	18* ³	13	5	72	NS
—	18	17	1	94	

*¹ Strain of mice was ddN.

*² The recovery of *C. renale* from the kidneys was done on the day 7 postinfection.

*³ No. of mice which were positive in recovery of *C. renale* from the urinary bladder and positive in antibody response.

was 0.34 ± 0.079 mm in the BCG-pretreated mice, whereas it was almost zero in the control mice.

Three days after the tuberculin reaction test, these mice were inoculated by injecting 2×10^7 organisms of *C. renale* into the urinary bladder. The rate of renal infection was not significantly different between the mice pretreated with BCG and the control mice (tab. 3). However, the rate was slightly higher in the control mice than in those pretreated with BCG.

In the mice which were positive in recovery of *C. renale* from the urinary bladder and positive in antibody response, the rate of recovery of *C. renale* from the kidneys was not different between the mice pretreated with BCG and those not treated (tab. 4). These results may suggest that the clearance of *C. renale* was not particularly increased in the kidneys of the mice pretreated with BCG.

DISCUSSION

Immunization of mice with killed organisms did not inhibit the ascending of *C. renale* from the urinary bladder into the kidneys. Ascending of *C. renale* from the urinary bladder into the kidneys seems to be not influenced by the systemic immunization. The results support the previous findings that the ascending of *C. renale* was not affected by the treatment of mice with cyclophosphamide, a suppressant of humoral immunity¹⁰. No effect of immunization with killed organisms of *Escherichia coli* was reported against ascending pyelonephritis with *E. coli* in mice⁹. On the contrary, ascending pyelonephritis with *Proteus mirabilis* was prevented by immunizing rats with killed organisms⁹ and immunizing rabbits with common antigen derived from *Salmonella typhimurium*⁴. The difference of the results may be due to the difference of animals and bacterial species.

The rate of the ascending of *C. renale* from the urinary bladder into the kidneys was not significantly different between athymic nude (nu/nu) and normal (nu/+) BALB/c mice, and between the mice treated with BCG and those not treated. It seems that the ascending of *C. renale* from the urinary bladder into the kidneys is not influenced by cell-mediated immunity. Using neonatally thymectomized or antilymphocyte globulin-treated rats, COLES et al.³⁹ found that ascending pyelonephritis with *E. coli* was not affected by either the presence or the absence of T cells. On the other hand, ARAKI et al.¹¹ were able to prevent retrograde pyelonephritis with *P. mirabilis* infection in the rat by transfer of spleen and lymph node cells from the rats infected with the organisms.

The results of the present study may indicate that neither humoral nor cell-mediated systemic immunity are primarily concerned with the ascending of *C. renale* from the urinary bladder into the kidneys. Mechanisms of the ascending of *C. renale* should be studied from other aspects such as vesicoureteral reflux¹⁾ urinary obstruction,²⁾ non-specific immunity and local immunity.

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