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NO EFFECT OF CYCLOPHOSPHAMIDE ON ASCENDING OF *CORYNEBACTERIUM RENALE* FROM URINARY BLADDER INTO KIDNEYS IN MICE

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Renal infection caused by inoculating *Corynebacterium renale* into the urinary bladder of mice was not influenced by the administration of cyclophosphamide (Cy). The results suggest that the ascending of *C. renale* from the urinary bladder into the kidneys may be independent of humoral immunity.

INTRODUCTION

Pyelonephritis due to *C. renale* in cows is known to be caused by the penetration of organisms from the lower urinary passage[2,10]. A model of pyelonephritis due to *C. renale* in mice was produced solely by the retrograde infection[11].

How *C. renale* ascends from the urinary bladder into the kindeys remains obscure. There is evidence that (1) a considerable number of healthy cows shed *C. renale* in the urine[2,6,16], and that (2) severe hemorrhagic cystitis due to *C. renale* does not always progress to pyelonephritis[5], which indicates that the ascending of *C. renale* seems to be normally repressed by unknown defense mechanisms of the host. We hypothesize that the organisms may ascend from the urinary bladder into the kidneys when the host is incompetent in immunity. The effect of the immunosuppressive drug Cy on the ascending of *C. renale* from the urinary bladder into the kidneys of mice was examined in the present study. The results are described below.

MATERIALS AND METHODS

C. renale strain *C. renale* strain 115 was used, which was used to make a model of the retrograde infection of *C. renale* in mice[11].

Mice Female, 6 to 7-week-old, ddY-F (24~30 g), NIH (16~22 g), ddN (20~26 g), were used.

Methods of infection The organisms, which were cultivated at 37°C for 1 day on nutrient agar medium, were suspended in saline and used for inoculation. The retrograde infection[11] was done as follows. The mice were anesthetized with ether and

forced to micturate, the abdominal wall was incised, and 0.02 ml of a suspension containing 6.2×10^2 to 6.1×10^7 organisms was inoculated into the urinary bladder. No foreign body was placed in the bladder. The inoculation of *C. renale* was done 2 days after the first administration of Cy.

Cy administration Endoxan (Shionogi Co., Osaka) 100 mg per vial was dissolved in 3 ml (33.3 mg/ml) or 6 ml (16.6 mg/ml) of physiological saline solution just before injection, and 0.01 ml of the solution per g of body weight was given by intraperitoneal injection 2 days before and 1 or 2 days after challenge. An additional administration of Cy was made 4 days later, in the experiment using the ddY-F mice.

Examination of mice The mice were observed for daily changes of body weight and killed at various intervals between 2 and 12 days after Cy administration.

Bacteriological examination was done as follows. The urinary bladder, kineys and other organs were examined by microbiological technique for recovery of *C. renale.* Portions of these organs were inoculated on nutrient agar plates and incubated for 2 days at 37°C. Quantitative bacterial cultures were done on the homogenates of the renale tissue. The homogenate was made with 20 volumes of saline solution, and then a serial 10-fold dilution of the homogenate was inoculated on nutrient agar plates. The colonies that developed were counted.

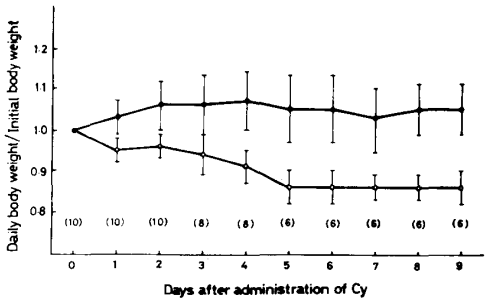
RESULTS

1 Effect of Cy on body weight and the anatomical findings of spleen of normal mice (strain ddN)

In non-infected mice injected with Cy, body weight began to decrease from 1 day after Cy administration and decreased by 15% on the 9th day after the first administration of Cy. In non-infected mice given saline, body weight increased by 6% on the same day (fig. 1).

In non-infected mice administered Cy, atrophy of the spleen was observed, and a decrease of the small lymphocyte was remarkable in the germinal center, follicular areas and the periarterial areas. In non-infected mice given saline, similar findings were not observed.

FIGURE 1 *Change in body weight of mice administered Cy*



The numbers in parentheses indicate the number of mice used per point.

○: Administered Cy, 0.166 mg/g on days 0 and 3

●: Given saline (control)

2 Effect of Cy on the anatomy of the urinary bladder and kidneys of normal mice

The bladder wall was not as soft as usual and was thickened. Hyperemia and

TABLE 1 *Effect of Cy on the recovery of C. renale*

STRAIN OF MICE	INOCULUM SIZE	ADMINISTRATION OF CY		INFECTION OF KIDNEY		AVERAGE LOG NO. OF ORGANISMS	
				Rate	Statistic significance	Per g of kidney	Statistic significance
ddN	5.8×10^7	0.166 mg*1	Once	1/2*2	ND*3	3.4	ND
		—*4		1/2		3.1	
		0.166	Twice*5	4/8	NS*7	5.4 (3.4–6.3)*6	NS
		—		6/8		5.1 (2.1–7.9)	
NIH	6.1×10^7	0.333	Twice	3/4	NS	6.1 (3.9–7.4)	NS
		—		5/5		6.2 (4.2–7.0)	
ddY-F	8.7×10^4	0.333	Twice	0/5*8	ND	0	ND
		—		2/5		4.7 (3.5–5.9)	
ddY-F	6.2×10^2	0.333	Three times	0/5	ND	0	ND
		—		0/4		0	

*1 mg per g of body weight
*2 The numerator denotes number of mice from which C. renale was recovered; the denominator indicates number of mice examined.
*3 Not determined.
*4 Saline was given in place of Cy.
*5 An additional Cy was administered 1 day after challenge.
*6 In parenthesis is the range of log number of organisms per g of infected kidney.
*7 Not significant ($P>0.05$)
*8 Of the 5 mice, 2 were received Cy three times.

a slight hemorrhage of the bladder were found in non-infected mice administered Cy once or twice. The effect of Cy decreased considerably 6 days after the second administration of Cy. In non-infected mice given saline, similar findings were not observed.

No particular histopathological changes were found in the kidneys of the mice administered Cy.

3 Effect of Cy on retrograde infection of *C. renale* in mice

The 6.2×10^2 to 6.1×10^7 organisms of *C. renale* were inoculated into the bladder of mice 2 days after the first administration of Cy. The rate of renal infection and the average number of the organisms in the kidneys were not significantly different between the mice administered Cy and those inoculated with saline, in ddN and NIH mice administered 10^7 organisms (tab. 1). ddY-F mice were inoculated with 10^4 and 10^2 organisms, which generally insufficient in number to case renal infection[11], in order to see if the mice become susceptible to such *C. renale* by pretreatment with Cy. Contrary to expectation, the Cy-treated mice were not infected.

DISCUSSION

Cy is known to affect selectively B-cell-mediated immunity in mice without markedly affecting their ability to mount a cell-mediated immune response[8,13,15]. This immune suppression resulted in an increase in susceptibility to those infectious diseases, such as in mice infected with influenza virus[7], *Rickettsia sennetsu*[14], *Leptospira interrogans* serovar *pomona*[1], *Pseudomonas aeruginosa*[9], *Mycoplasma pulmonis*[12] and *Histoplasma capsulatum*[3], but a decrease in susceptibility to *Plasmodium berghei* subsp. *yoelii*[4].

In the present experiment, the rate of renal infection in the mice inoculated retrograde with *C. renale* and the growth of *C. renale* in the kidneys of these mice were not influenced by the administration of Cy. The fact that the ascending of *C. renale* from the urinary bladder to the kidneys was neither accelerated nor depressed by pretreatment of the mice with Cy may suggest that the ascending of *C. renale* is independent of humoral immunity.

The mice vaccinated with the killed organisms of *C. renale* were not protected against the pyelonephritis due to the retrograde infection of *C. renale*, despite the fact that a sufficient amount of humoral antibody was present in the mice before the infection (authors' unpublished data). This may also suggest the independence of *C. renale* pyelonephritis from humoral immunity.

The role of humoral immunity in the defense mechanisms against *C. renale* infection was thus eliminated. The next experiment should try to examine whether the organisms can ascend from the urinary bladder to the kidneys in the host, which is incompetent of cellular immunity.

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