ROLE OF THE NORMAL MICROFLORA IN TREATMENT OF THE VIBRO CARRIER STATE IN GERMFREE RATS

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Relations between Vibrio cholerae and certain representatives of the normal human intestinal microflora which, in experiments in vitro were found to be active antagonists, were studied. Experiments were carried out on germfree rats of the Fisher strain. When different combinations and orders of administration of the microorganisms were used, no antagonistic relations were found between V. cholerae El-Tor and Escherichia coli M-17, Lactobacillus fermenti, and Lactobacillus plantarum. Rats infected with El-Tor vibrio were completely cured of the vibrio carrier state through population of their intestinal tract with the fetal microflora of healthy rats.

KEY WORDS: germfree animals; Vibrio cholerae carrier state; normal microflora; vibrio; antagonism.

The problem of the resistance of the human body to Vibrio cholerae has for a long time attracted the attention of epidemiologists and clinicians. Many workers [1, 2, 4-6] have demonstrated the role of the gastric juice as a factor in resistance to this infection, but in certain situations V. cholerae can overcome this barrier and penetrate into the intestine, where the principal pathological process develops and where the vibrio is exposed to the action of the normal intestinal microflora. Many workers attach great importance to the intestinal microflora as a factor in resistance against enteric infections [7, 11-13]. There is evidence to show that successful infection takes place when the intestinal microflora is artifically depressed or has not been formed (for example, during infection of unweaned animals). Germfree animals are easily infected with V. cholerae and remain vibrio carriers for a long time without apparent harm, whereas ordinary animals, under the same conditions, are resistant to infection per os [3, 15].

The object of the present investigation was to study relations between V. cholerae and certain representatives of the normal intestinal microflora (Escherichia coli M-17, Lactobacillus plantarum 8RA3, and Lactobacillus fermenti 90-TS4), which are antagonists of V. cholerae and some agents of intestinal infections.

Antagonism between the chosen strains of lactic acid bacteria and V. cholerae also was demonstrated by preliminary experiments [8, 10] in vitro. However, during combined culture of E. coli M-17 and the strain of V. cholerae used in these experiments, growth of the latter was inhibited only by half compared with the control, and on solid medium no zones of inhibition of growth were found.

EXPERIMENTAL METHOD AND RESULTS

Germfree albino rats of the Fisher strain, grown in isolators of the Trexler type in the Laboratory of Gnotobiology, N. F. Gamaleya Institute of Epidemiology and Microbiology, were used. The El-Tor strain of Vibrio cholerae, Ogawa serotype, isolated from the feces of rats infected with cholera on the 40th day of the carrier state [3], was used for the experiments. To infect the animals a suspension of cells in physiological saline was prepared and given in a dose of 2×10^9 cells per animal. The number of living V. cholerae, E. coli and Lactobacillus cells isolated from the feces of the experimental animals was determined by the usual serial dilution method and by counting colonies growing on alkaline agar (pH 8.0), on TCBS differential medium on Endo's medium, and also on MPC medium, which is selective for lactobacilli.

Considering that the outcome of competitive relations between microorganisms frequently depends on the order of their introduction into the intestinal tract, the animals were infected in two variants. Rats of

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TABLE 1. Scheme of Experiments to Study Competitive Relations between Microorganisms in Germfree Rats

Experiment No.	Group of animals	Number of animals	Microorganisms and order of their administration			Dose of microorganisms	Intervals between administration, days
			1	2	3		
1	1	3	V. cholerae El-Tor	E. coli Al-17	—	2×10^9	6
	2	4	E. coli M-17	V. cholerae El-Tor	—	2×10^9	6
					V. cholerae El-Tor	2×10^9	10
2	1	4	L. plantarum 8RA3 L. fermenti 90-TS4	V. cholerae El-Tor	—	2×10^9	7
	2	4	Lactobacillus	E. coli M-17	—	2×10^9	70
			V. cholerae El-Tor	L. plantarum 8RA3			
	3	4	V. cholerae El-Tor	L. fermenti 90-TS4	—	2×10^9	7

TABLE 2. Number of Microorganisms in Intestine of Rats When Observed at Different Times

Experiment No.	Group of animals	No. of animals	Species of microorganisms	Order of infection	Day of taking samples: 2	6	7	8	14	21	27	35	42
					Number of microorganisms in 1 g feces*								
1	1	3	V. cholerae	1	8,9	9,1	Not seeded	8,3	8,4	Not seeded	9,04	Not seeded	—
			E. coli M-17	2	Not infected			9,4	9,1		9,1		
	2	4	E. coli M-17	1	9,5	8,9	Not seeded	9,3	9,05	8,8	9,1	9,2	—
			V. cholerae	2	Not infected			0	0	6,3	6,9	6,9	
			Lactobacillus	1	7,8		7,7	Not seeded	7,2	6,8	7,0	6,7	7,6
2	1	4	V. cholerae	2	Not infected			Not seeded	9,0	9,0	9,0	9,1	8,9
	2	4	V. cholerae	Simultaneously	9,3	Not seeded	8,9	Not seeded	9,05	8,9	9,0	8,6	9,0
			Lactobacillus		2,7		0		7,7	7,5	7,6	6,4	6,4
	3	4	V. cholerae	1	8,97		9,2	Not seeded	9,2	8,4	9,0	9,0	9,1
			Lactobacillus	2	Not infected				7,6	7,3	6,8	7,1	6,2

*Number of microorganisms expressed in $\log_{10}$ of absolute number.

group 1 were infected with V. cholerae, those of group 2 with E. coli M-17. In the course of 6 days, a culture of V. cholerae in a dilution of 10^8 was isolated from 2 control seedings of feces from the animals of group 1, whereas E. coli was isolated both times from the animals of group 2. Thereafter group 1 was associated with E. coli and group 2 with V. cholerae (Table 1).

Analysis of the results of the experiments to study the relations between E. coli M-17 and V. cholerae in the germfree rats showed that when E. coli was injected against the background of a carrier state of V. cholerae for 7 and 30 days this did not cause the supplanting of V. cholerae from the rats' intestine (Table 2). The number of vibrios in the feces remained unchanged throughout the period of observation (35 days). The content of E. coli seeded from the feces also remained at virtually the same level (Table 2, experiment 1, group 2). When sterile rats were associated initially with E. coli and later with V. cholerae, the latter could not be found in seedings from the feces during the 7 days after the first infection, however, its number was only half of that in the animals of group 1, the infecting doses being the same (Table 2, experiment 1).

In experiments to study competitive relations between V. cholerae, Lactobacillus, and E. coli M-17 three groups of animals were used (Table 1). In all three variants a mixture of two species was used (Lactobacillus plantarum 8RA3 and Lactobacillus fermenti 90-TS4) in equal numbers (1×10^9 bacterial cells in 1 ml in each case). Animals of group 1 were associated with the mixture of lactic acid bacteria, the animals of group 2 received V. cholerae and a mixture of lactobacilli in equal proportions; the animals of group 3 were infected with a culture of V. cholerae.

Seven days after it had twice been confirmed that the bacteria introduced had survived in the intestine, a culture of V. cholerae was injected into the animals of group 1, E. coli into those of group 2, and a mixture of lactic acid bacteria into those of group 3.

During the analysis of the results, when lactic acid bacteria were taken as the normal micrflora, in no variant of the experiment were antagonistic relations found between V. cholerae and the lactobacilli during the 6 weeks of observation (Table 2, experiment 2). Similar results were obtained by infection of noninbred germfree mice with the same strains of lactobacilli and with Vibrio cholerae asiaticae strain 154.

When E. coli M-17 was introduced 10 weeks after the formation of a mixed carrier state (Lactobacillus and V. cholerae), it likewise did not displace these latter microorganisms from the intestine of the rats. For instance, the content of E. coli seeded from 1 g of feces was 8.63, of Vibrio cholerae 8.01, and of the lactic acid bacteria 8.1 logarithmic units.

These data agree with the results of other experiments [14] to study antagonistic relations between certain representatives of the intestinal microflora: Streptococcus faecalis, E. coli, and Proteus vulgaris with V. cholerae El-Tor in germfree mice and rabbits.

The experiments of series III were carried out on 9 rats, 3 of which were monofloral carriers of V. cholerae and 6 were carriers of V. cholerae and of E. coli M-17. These animals received a suspension of feces in physiological saline taken from healthy rats kept under ordinary animal house conditions. The suspensions of feces was poured into the feeding bowls and the rats drank it in the course of 2-3 h. The number of cholera vibrios grown 24 h after population of the intestine of the experimental animals with normal microflora was reduced to single figures in a dilution of 10^6. On the 5th day no V. cholerae could be found in any dilution.

The results showed that the activity of V. cholerae can be suppressed by the normal intestinal microflora of the healthy animal. These results show that among representatives of the normal intestinal microflora of rats there exist microorganisms (individual species or communities) which possess antagonistic properties against V. cholerae.

To sum up the results of the series of experiments to study relations between certain representatives of the normal intestinal microflora and V. cholerae it must again be pointed out that in the experiments in vivo no antagonistic relations could be found between V. cholerae, E. coli M-17, Lactobacillus plantarum, and Lactobacillus fermenti, whereas in experiments in vitro these microorganisms exhibited marked antagonistic relations.

Rats infected with El-Tor vibrio were completely cured of their vibrio carrier state by population of their intestinal tract with fecal microflora from healthy rats.

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