ANTIBODY RESPONSES IN CHOLERA PATIENTS

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INTRODUCTION

In spite of extensive studies the nature of immunity to cholera is still undefined. It is known, however, that vibrio antigens elicit immune responses in convalescent cholera patients. Whether the "so-produced" antibodies play any role in recovery from the infection or protection against a subsequent attack of cholera is at present not clear owing to marked conflicts in available evidence. It was observed that the same individual was rarely attacked twice during the same "classical" cholera epidemic (Pollitzer, 1959). However, the immunity in persons who had recovered from the classical cholera infection did not seem to persist for a long time. There was sufficient example to show that many individuals who had been affected during one epidemic contracted cholera during the later outbreak. Mosley *et al.*, (1968) showed, in cholera vaccine field trials carried out in rural Bangladesh, that the average serum vibriocidal titres of persons who lived in areas where *V. cholerae* was epidemic, was inversely correlated with clinical case rate, suggesting a possible correlation between vibriocidal antibody titres and human protection, at least when these antibodies are directed against homologus antigens of the epidemic strains (Benenson *et al.*, 1968). On the other hand, Sack, *et al.*, (1966) using the vibrio agglutination, reaction (Goodner *et al.*, 1960) and complement dependent vibriocidal assay (Finkelstein, 1962) claimed that the presence of high serum antibody titres in cholera patients at the time of admission did not alter the clinical picture which was identical to that of patients without high humoral antibody at the onset of cholera. Other workers in the field also claimed that there appeared to be no evidence that immunization *per se* modified the course of clinical cholera (Azurin, 1965, 1967). In the light of these conflicting opinions and the fact that *V.cholerae* El Tor has replaced the classical one in most of the present epidemics, further work is needed to investigate the prophylactic role of the antibodies produced in the El Tor cholera patients.

The present work was designed to assess the humoral immune response and to determine the classes of specific immunoglobulins and the levels of protective antibodies in acute and convalescent El Tor cholera patients.

MATERIALS AND METHODS

The bacterial strain, the normal rabbit serum, the hyperimmune rabbit serum against live *V.cholerae* and all experimental animals used in this study were similar to those described elsewhere (Chaicumpa *et al.*, 1980). The procedures of passive hemagglutination test, vibriocidal assay, passive baby mouse protection test and detection of specific immunoglobulins in the sera were also essentially the same. Cholera patients sera were obtained from those admitted to Bamrasnaradura Infectious Diseases Hospital on the first day of arrival (1st sera), 7 days in the hospital

(2nd sera) and 3 months thereafter (3rd sera). Stool specimens of the patients examined on the same day as the first sera revealed almost entirely pure cultures of *V.cholerae* biotype El Tor. Sera of healthy individuals were obtained from the males of the Bangkok Special Prison Hospital, Department of Correction, Ministry of Interior, Thailand. All sera used in the experiments (except the guinea pig sera) were decomplemented by heating at 56°C for 30 minutes.

RESULTS

The results of various antibody responses are shown in Table 1. The hemagglutinating antibody titres in the sera of the cholera patients were highest on 7th day after admission (2nd sera) which were significantly different from those of the first and third sera. The vibriocidal antibody responses in the same group of cholera patients followed similar pattern as the HA titre. In both instances, the antibody levels of the 1st and 3rd sera were not significantly different from the levels found in normal persons. The mouse protective antibody response of the patients, although was highest at seven days after admission, was not higher than those found in normal volunteers. Table 2 shows classes of specific antibodies as detected by anti-immunoglobulin enhancement of haemagglutination. Five patients (33.3%) developed only IgG specific antibodies. Four patients (26.6%) revealed early IgM response and late IgG production. Mixture of IgM and IgG were found in all serum samples of four patients (26.6%). One patient (5.6%) had only IgM antibody throughout the study period. Specific IgM, IgG and IgA were found in all serum samples of another patient (5.6%).

DISCUSSION

The haemagglutinating and vibriocidal antibody responses in El Tor cholera patients reached the highest levels at seven days after admission. The titres declined sharply afterwards. By the end of the third month the antibody levels were not different from the normal healthy persons. Mouse protective antibody levels, although peaked at seven days after admission, they never exceed the levels found in normal persons. At the first day of hospital arrival, the protective dose 50

Table 1

Mean and standard error of reciprocal hemagglutinating (HA), vibriocidal (Vi) and mouse protective (MPT) antibody titres of cholera patients and normal persons.

Test	Sample	Patients(15)*	Normal (18)*
HA	1st sera	8.4 ± 1.3	8.9 ± 1.3
	2nd sera	44.2 ± 1.3	8.3 ± 1.3
	3rd sera	5.0 ± 1.2	7.4 ± 1.3
Vi	1st sera	213.7 ± 1.6	568.4 ± 1.3
	2nd sera	1,827.0 ± 1.7	477.9 ± 1.3
	3rd sera	192.0 ± 1.8	517.5 ± 1.3
MPT	1st sera	2.4 ± 1.2	5.7 ± 1.1
	2nd sera	5.0 ± 1.2	4.3 ± 1.1
	3rd sera	2.1 ± 1.2	5.0 ± 1.0

* Number of persons studied.

Table 2

Classes of specific immunoglobulins in the patients' sera.

Patient No.	1st sera	2nd sera	3rd sera
1	G	G	G
2	M	M	G
3	MG	MG	MG
4	G	G	G
5	G	G	G
6	M	M	M
7	M	MG	MG
8	MG	MG	MG
9	MG	MG	MG
10	MG	MG	MG
11	G	G	G
12	M	MG	MG
13	AMG	AMG	AMG
14	M	MG	MG
15	G	G	G

G = Only IgG was found. MG = mixture of IgM and IgG were found. AMG = all IgA, IgM and IgG could be detected in the serum.

Table 3

Fold increase in haemagglutinating titres of cholera patients' sera after enhancement with anti - IgA, anti - IgG and anti - IgM respectively.

Patient No.	1st sera				2nd sera				3rd sera			
	HA* titre	anti A	anti G	anti M	HA titre	anti A	anti G	anti M	HA titre	anti A	anti G	anti M
1	32	2x	4x**	2x	256	2x	8x	2x	4	1x	32x	2x
2	2	1x	1x	4x	16	1x	1x	4x	2	1x	4x	1x
3	16	2x	4x	4x	128	2x	4x	4x	8	1x	8x	4x
4	8	1x	4x	1x	16	1x	4x	1x	8	1x	4x	1x
5	4	1x	16x	2x	16	1x	8x	2x	4	1x	8x	2x
6	4	2x	2x	4x	64	2x	2x	4x	4	2x	2x	4x
7	4	1x	2x	4x	16	1x	4x	4x	4	1x	16x	4x
8	2	1x	8x	8x	16	2x	4x	4x	4	2x	8x	4x
9	88	1x	8x	4x	16	1x	4x	4x	4	2x	16x	8x
10	16	2x	16x	4x	16	1x	8x	4x	4	2x	16x	8x
11	16	1x	4x	1x	64	1x	4x	1x	2	2x	16x	2x
12	4	1x	1x	8x	128	2x	4x	4x	16	1x	4x	4x
13	8	4x	4x	4x	64	4x	4x	4x	16	4x	4x	4x
14	8	1x	1x	4x	16	2x	8x	4x	8	1x	4x	4x
15	4	1x	8x	1x	16	1x	4x	1x	2	1x	8x	1x

* reciprocal HA titre.

** At least four fold rise in HA titre was taken as significant enhancement.

(PD_{50}) of the antibodies in these patients were significantly lower than the level of the corresponding antibodies found in the controls. The same situation was observed for the third serum samples obtained 3 months after the onset of the cholera symptoms. Many reasons can be given to explain the low level of mouse protective antibody titres in the first serum samples of these patients. One is that the feature observed might be due to the fact that during the acute onset of disease, the vibrios and their products especially the toxin caused submucosal oedema and hence facilitated the flow of IgG type circulating antibodies into the intestinal lumen. This pathotopic potentiation might result in the unusually low level of IgG circulating antibodies (thus lowered the PD_{50} of the sera) while leaving the IgM antibodies (as measured by passive haemagglutination and complement dependent vibriocidal assays) at their usual levels. It was also found that 66% of the specific immunoglobulins in the first sera were IgG (either IgG alone or mixture of IgM and IgG). Large amounts of these IgG might become coproantibodies so as to fight against the multiplying organisms in the patients' intestines during acute cholera. However, the above reasons can not be applied to explain the low PD_{50} of the third sera. Another possible explaination would be, therefore, perhaps due to the abnormality in response to T cell dependent *V.cholerae* antigens in the cholera patients while the T cell independent *V.cholerae* antigenic response (e.g. to lipopolysaccharide) is normal.

SUMMARY

Haemagglutinating, vibriocidal and mouse protective antibody responses in cholera patients were found to be maximum on the 7th day of admission. The mouse protective antibody on the first day at the hospital was lower than those of human volunteers. The circulating antibodies in the patients declined to normal levels or lower than normal before 3 months after the acute onset.

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