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Antibodies in serum and secretions 1 year after salmonella gastroenteritis

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SUMMARY

The antibody response in serum and intestinal fluid in eight patients 1 year after their recovery from salmonella gastroenteritis was measured by solid phase radioimmunoassay and compared to the immune response within a few weeks of infection, reported previously in these and other patients. High concentrations of intestinal antibody were found in six patients compared to the concentrations found in 10 control subjects. By contrast the serum antibody concentration in the patients was only marginally higher than in the controls. The use of IgA and IgG specific antisera in the assay confirmed the presence of IgA antibody in the absence of IgG antibody in the gastrointestinal secretions, and the predominance of IgG antibody in the serum. The prolonged immune response in the gut after acute bacterial gastroenteritis supports the possibility of effective immunization against diseases entering via the gut.

INTRODUCTION

Antibodies in the intestine are thought to play a major role in protection against microbes which enter the body through this portal to cause local or distant disease (Tomasi, 1976). Efforts to generate resistance to these diseases in humans by stimulating immunity via the enteral route have been disappointing with the signal exception of poliomyelitis. The reasons for this failure have been difficult to determine largely due to inability to measure intestinal immunity in man.

We have previously reported both the development of an assay that effectively measures antibacterial antibody in intestinal secretions and other fluids (La Brooy, Rowley & Shearman, 1980a) and a study of the antibody response in serum and secretions after bacterial gastroenteritis measured by this assay in adults and children (La Brooy *et al.*, 1980b). In this paper we report the results of follow-up studies at a year in the adults with bacterial gastroenteritis and discuss their implications for future work in relation to gut immunity.

MATERIALS AND METHODS

All 16 adults, who were studied in the 6 weeks following an attack of salmonella or shigella gastroenteritis, were approached for permission to study them again, 1 year after their illness. Eight patients consented. In all who consented, *Salmonella spp* had been responsible for their disease. Ten subjects matched for age and sex were used as controls; eight of these control subjects were healthy, while two had been admitted to hospital with transient attacks of acute gastroenteritis but from

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whose stools no bacterial pathogen was isolated on repeated culture. Sampling and storage of intestinal fluid and serum were as described before (La Brooy *et al.*, 1980b).

The radioimmunoassay used for measuring antibody in this study was that of Zollinger, Dalrymple & Artenstein (1976) with minor modifications. In our previous studies (La Brooy *et al.*, 1980a) glutaraldehyde-fixed bacteria were used as the solid phase antigen for absorbing the antibody; in the present study, LPS coated wells were the solid phase antigen. LPS extracted from the bacteria against which antibodies were being measured (La Brooy *et al.*, 1980b) was used (200 μl of LPS at 10 μg/ml) to coat the wells of flexible microtitre trays. After repeated washing with phosphate-buffered saline (PBS), 1% bovine serum albumin in phosphate-buffered saline (PBS-BSA) was used as a non-specific filler. Trays coated with bovine serum albumin without prior exposure to LPS were used to determine background binding. The coated plates were stored at $-20°C$ and used within 2 months of preparation. In the assay, a duplicate series of serial dilutions (100 μl volumes) of the sample were incubated in the wells at 4°C for 24 hr. After repeated washing with PBS-BSA, radiolabelled purified anti-human immunoglobulin serum (La Brooy *et al.*, 1980b) was used to determine the amount of antibody bound to the wells. Twenty nanograms of 'active' antibody that retained its binding capacity after the preparatory steps of affinity chromatography and radiolabelling were added to each well. Allowance was made for background binding at each dilution by subtracting the c.p.m. bound to BSA-coated plates from the mean c.p.m. bound to the antigen-coated plates. Anti-light chain, anti-alpha and anti-gamma radiolabelled antibodies were used to measure total, IgA and IgG antibodies respectively, as described previously. The LPS used in assaying samples from the patients was derived from the Salmonella with which they had been infected. *Salmonella typhimurium* was the causative organism in six of the eight and *S. typhimurium* LPS was used to measure antibody in the control subjects.

Single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) was used to measure immunoglobulin concentrations in serum and intestinal fluid. A secretory IgA standard was used for the measurement of IgA in the intestinal fluid.

RESULTS

In the secretions of six of the eight patients studied 1 year after an attack of salmonella gastroenteritis, antibody against the responsible bacterial pathogen was present. In the control group, intestinal antibody was not found in seven of the 10 subjects, and the concentration of intestinal antibody in the other three was very low (Fig. 1). This difference between the patient and control groups remains apparent on expressing the antibody response in terms of units of antibody per milligramme of immunoglobulin (Fig. 2), indicating that the higher concentration of antibody in the intestinal fluid is not merely a reflection of higher concentrations of immunoglobulin.

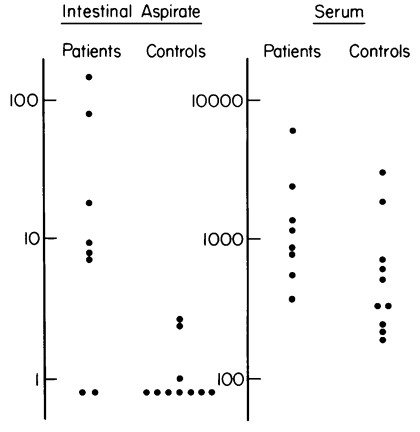

Fig. 1. Antibody responses in units/0·1 ml

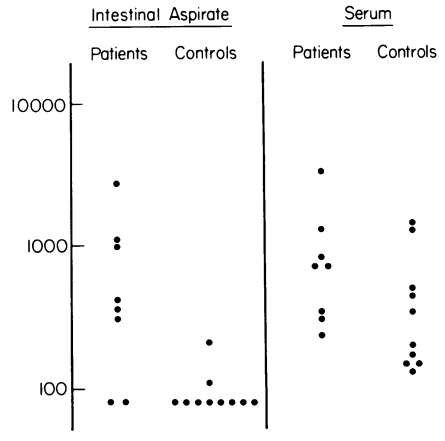

Fig. 2. Antibody responses in units/mg immunoglobulin.

Table 1. Titres of antibody using heavy chain specific antisera (units/0·1 ml)

	Intestinal fluid		Serum	
Subject	IgA	IgG	IgA	IgG
T.G.	10·5	<1	62	290
P.F.	110	<1	140	430
S.R.	14	<1	110	2200
T.H.	7·6	<1	400	2900
J.D.	34	<10	100	300
A.M.	170	<10	90	620
J.H.	<1	<1	72	570
D.S.	<1	<1	13	<10

The concentration of antibody found in the sera of patients was similar to that in controls (Figs 1 & 2).

Using the heavy chain specific antisera, only IgA antibody was detectable in the intestinal fluid while in serum, IgG antibody was predominant (Table 1).

DISCUSSION

Previous reports of the intestinal antibody response to bacterial pathogens have been limited to a few weeks after infection (Reed & Williams, 1971; McNeish *et al.*, 1975; Freter *et al.*, 1965; Waldman *et al.*, 1972). The present study confirms that there is measurable immunity in the intestine 1 year after salmonella gastroenteritis. Though challenge studies have demonstrated immunity over a similar period of time after cholera (Cash *et al.*, 1974) no *in vitro* measurements of intestinal immunity were carried out at the time. The use of suitably sensitive and reproducible assays now allow these measurements to be made, and will provide a means of studying local immunity where challenge studies are not possible for ethical or other reasons.

The duration of the intestinal immune response demonstrated in this study provides support for the concept of oral immunization against infections originating in the gut. Clearly, the conditions

for generating a potent antibacterial immune response are close to optimal during an attack of gastroenteritis in comparison to oral vaccination. The effectiveness of the adherence factors on the bacteria, their presentation to the immune apparatus of the gut and the persistence of replicating antigen within the intestinal lumen are all certain during the course of the disease. In one of the subjects studied, the pathogen persisted in the gut in spite of the antibody response and was detected 1 year after the patient had recovered clinically from the disease. The other patients had eliminated the infecting organism, as judged by standard bacteriological culture techniques, within a few weeks of the infection, but it remains possible that the bacteria continue to occupy a niche in the ecosystem of the gut in numbers that do not allow their detection in the stool by standard techniques. The persistence of the immune response however, for a prolonged period after a brief clinical illness, and the results with polio vaccine (Ogra *et al.*, 1968) provide support for the hypothesis that live avirulent bacteria possessing the relevant antigens and colonizing the gut may generate long lasting immunity after one or two vaccinations.

The fall in serum antibody concentration in patients to a concentration found in control subjects at 1 year contrasts with the findings in the first few weeks after infection when serum and intestinal antibody in patients were elevated to a similar degree (La Brooy *et al.*, 1980b). A possible explanation lies in the direct stimulation of systemic immunity by bacteria in the initial phase of the disease when mucosal ulceration is present and bacteraemia may occur. With clinical recovery, however, the bacteria may continue to stimulate the intestinal immune apparatus via antigen receptor sites such as Peyer's patches whilst heightened local immunity effectively inhibits their access to more distant parts of the immune system. An alternative explanation suggests itself from studies in which a secretory immune response and systemic tolerance has been demonstrated following the administration of antigens by the enteral route (Challacombe & Tomasi, 1980). The continuing secretory IgA response in the gut and the decline of the predominantly IgG response in the serum may be a manifestation of some inhibition of the early systemic response by suppressor T cells generated in extra-intestinal tissues, while helper T cell activity in the intestine maintains the local IgA response (Elson, Heck & Strober, 1979).

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