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Antibody response to the lipopolysaccharide and protein antigens of *Salmonella typhi* during typhoid infection

II. MEASUREMENT OF INTESTINAL ANTIBODIES BY RADIOIMMUNOASSAY

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SUMMARY

Antibodies to the lipopolysaccharide (LPS) and protein antigens of *S. typhi* in secretions of small intestine obtained from 12 typhoid patients, four typhoid carriers and 16 non-typhoid control subjects were measured by a solid-phase radioimmunoassay technique. Intestinal secretions obtained from typhoid patients as a group had significantly higher anti-LPS and anti-protein antibodies than those from the control group. These antibodies were both IgM and IgA classes. There was no correlation between the IgM or IgA antibody levels in serum and those in the intestinal secretions. In the intestinal secretions obtained from typhoid carriers, on the other hand, only IgA-class antibodies to the LPS and protein antigens of *S. typhi* were present at high levels.

INTRODUCTION

In the preceding paper we have reported results concerning measurement by solid-phase radioimmunoassay (SP-RIA) of antibodies to the protein and lipopolysaccharide (LPS) antigens of *Salmonella typhi* in thyphoid patients' sera (Tsang *et al.*, 1981). A significant increase in anti-LPS and anti-protein antibodies was observed in all three major immunoglobulin classes (IgM, IgG and IgA). This is contrary to the general opinion that antibody response in typhoid is mainly of the IgM type. In this paper we have extended the antibody measurements to include the intestinal fluid obtained from typhoid patients, typhoid carriers and non-typhoid controls in an attempt to gain insight into local gut immunity during typhoid infection.

MATERIALS AND METHODS

Intestinal secretion and serum specimens were collected from 12 typhoid patients, aged 16–52 years, 16 normal adult controls matched for sex and age as far as possible and four chronic typhoid carriers. Diagnosis of typhoid fever was confirmed by blood culture in all the patients and of the typhoid carrier state by bile culture and repeated positive stool culture. The four typhoid carriers, two males and two females aged from 56–72, had known histories of the carrier state ranging from 8

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months to 4 years but no known history of previous typhoid fever. Consent was obtained from these subjects before sampling.

Samples of intestinal secretions from typhoid patients and carriers were obtained at the duodenojejunal junction through a disposable Dennis gastrointestinal sump tube (Brunswick Co., USA.). Fluoroscopy was used to position the tube. Samples of intestinal secretions from control subjects were obtained at the duodenojejunal junction through a small flexible plastic tube inserted with the aid of an endoscope. Endoscopy was done in these subjects mostly because of complaints of ulcer syndrome. Specimens were taken when no ulcer or other abnormality was seen. The aspirate was checked by ensuring that is was bile-stained and its pH was greater than 7·0, and it was then collected onto ice and stored at −70°C in aliquots until assayed. Serum specimens were collected at the same time and stored in aliquots at −20°C. Sampling was performed only once in all 16 non-typhoid controls, four typhoid carriers and nine typhoid patients and twice at 1–2-week intervals in the remaining three patients. Thus, altogether 15 specimens of intestinal secretions with 15 matched serum specimens were obtained from the 12 typhoid patients. The patients were at the 13th to 51st day of the illness and were afebrile when the specimens were taken.

Determination of immunoglobulin levels, preparation of the lipopolysaccharide and protein (Barber's protein) antigens and measurement of antibody content by a solid-phase radioimmunoassay (SP-RIA) technique were as described in detail in our previous paper (Tsang *et al.*, 1981) with the following modifications: (1) to determine IgA level in intestinal secretions, a secretory IgA standard was used instead of a serum IgA standard; (2) all assays for antibody content in the intestinal secretions were performed at 4°C. In brief, polyvinyl microtitre plates (Cooke Engineering Co., Alexandria, Virginia, USA), coated with the protein or LPS antigens of *S. typhi*, were used to absorb antibodies against them in serial dilutions of secretions and serum. The amount of firmly bound specific antibody was measured using ^{125}I-labelled anti-immunoglobulin antibody. 'Total' antibodies were measured with labelled anti-light chain antibody while antibodies of the IgA, IgG and IgM classes were measured with labelled anti-alpha, anti-gamma and anti-mu respectively. The antibody titre of the sample was that dilution at which 2 ng (5%) of the added 40 ng of labelled anti-immunoglobulin was bound. As 50-μl volumes of the dilution of samples were added in each well in the assay, the 'absolute' antibody content was expressed as antibody units/50 μl. Using the immunoglobulin levels determined by the Mancini method, antibody content was also expressed as units per mg of immunoglobulin. A 'reference' serum from a typhoid patient was used as an internal standard to check assays done on different days.

RESULTS

Levels of immunoglobulins A, G and M in the intestinal secretions from typhoid patients and non-typhoid controls

These are shown in Fig. 1. IgA was the predominant immunoglobulin in secretions obtained from the duodenojejunal junction. IgG and IgM were also detectable and, though there was slightly more IgG than IgM in the secretions, especially in samples obtained from typhoid patients, the ratio of IgG to IgM did not reflect that found in the serum. The levels of immunoglobulins A and M in the intestinal secretions of typhoid patients did not differ significantly from those of control subjects.

Antibody response to the LPS and protein antigens of S. typhi *in intestinal secretions from typhoid patients and non-typhoid controls*

The levels of the 'total' and the IgA-, IgG- and IgM-class anti-LPS and anti-protein antibodies in intestinal secretions obtained from typhoid patients and non-typhoid controls are presented in Figs 2 & 3. Like the serum antibodies, although the antibody levels in intestinal secretions obtained from typhoid patients were highly variable with a skew distribution, typhoid patients as a group were found to have significantly elevated antibodies to both the LPS and the protein antigens of *S. typhi* as compared with non-typhoid controls. But unlike serum antibodies, which involved all three major classes of immunoglobulins (Tsang *et al.*, 1981), antibodies detected in all the intestinal secretions except one from typhoid patients were of the IgM and IgA classes: either of the IgM class

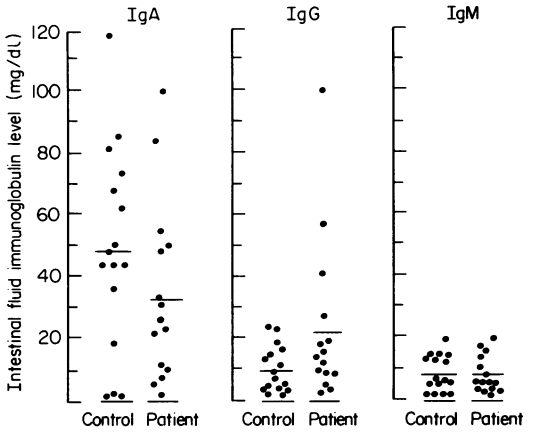

Fig. 1. IgA, IgG and IgM levels in samples of intestinal secretions obtained from typhoid patients and normal controls. (——) Mean immunoglobulin levels.

(two samples) or the IgA class (two samples) alone or of both the IgM and the IgA classes (10 samples). Only in one sample of intestinal secretions from a patient were high levels of IgG-class antibodies detected together with high levels of IgM- and IgA-class antibodies. This sample, however, also contained IgG at the highest level (100 mg/dl) as compared with all the other samples, probably due to a leakage of serum to the intestinal secretions in this particular case.

By contrast to those typhoid patients, only antibodies of the IgA class were detected at high

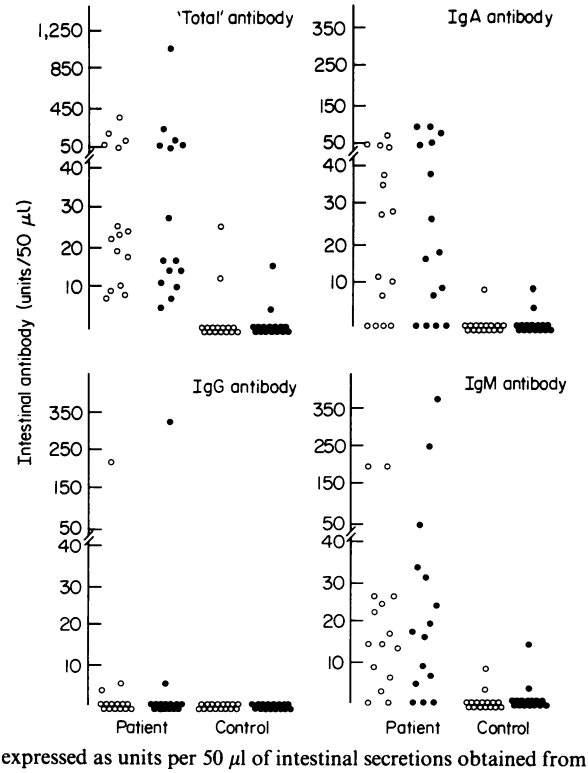

Fig. 2. Antibody levels expressed as units per 50 μl of intestinal secretions obtained from typhoid patients and normal controls. (o) Antibodies to the LPS antigens of *S. typhi*, (•) antibodies to the protein antigens of *S. typhi*.

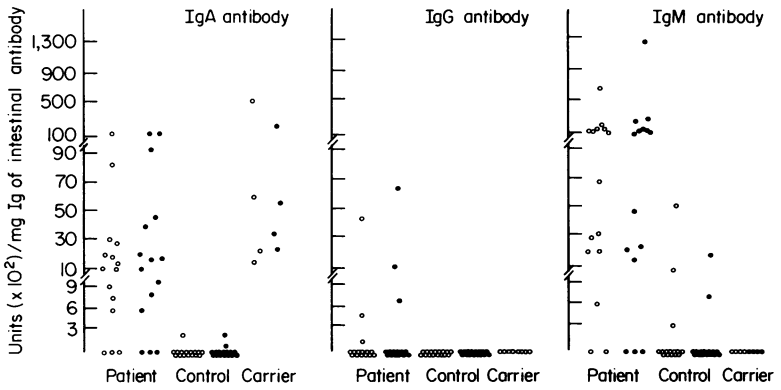

Fig. 3. Antibody levels expressed as units per mg Ig in scretions of the small intestine obtained from typhoid patients, typhoid carriers and normal controls (cf. Fig. 2). (o) Antibodies to the LPS antigens of *S. typhi*, (•) antibodies to the protein antigens of *S. typhi*.

levels in the four samples of intestinal secretions obtained from typhoid carriers, while antibodies of the IgG and the IgM classes were not detectable (Fig. 3).

Comparison of intestinal antibody levels with serum antibody levels

For comparison of the antibody levels in matched serum and intestinal fluid samples obtained from the same patient at the same time, antibody titres were adjusted to units per mg immunoglobulin to compensate for the difference in immunoglobulin levels in the two types of specimens. From the scatter diagrams presented in Fig. 4, it was clear that there was no correlation between the serum antibody levels and the intestinal antibody levels. While in most serum specimens from typhoid patients, antibody levels were higher than those in the corresponding specimens of intestinal secretions, on individual occasions intestinal antibody levels were found higher than serum antibody levels.

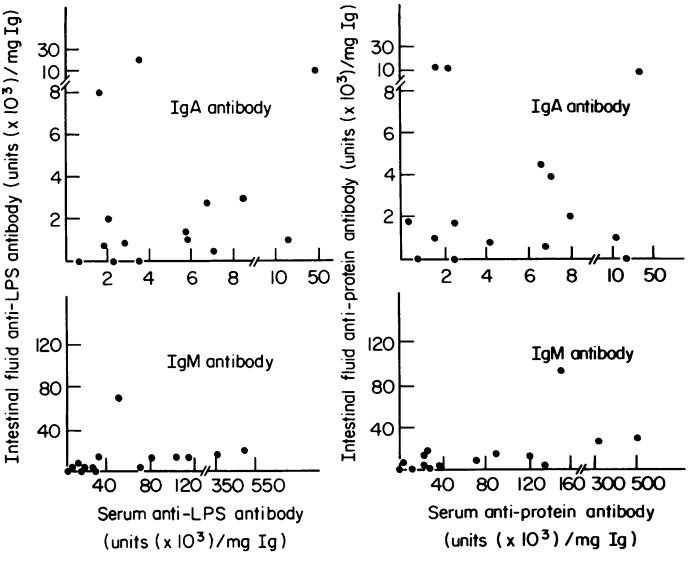

Fig. 4. Scatter diagram showing the relationship between the levels of antibodies in intestinal secretions and those in matched serum samples obtained from the same typhoid patients.

DISCUSSION

The main finding in this study was that antibodies of both the IgA and the IgM classes were demonstrated at high levels in the intestinal secretions obtained from typhoid patients. By contrast, antibodies of the IgA class only were detected in the intestinal secretions obtained from typhoid carriers. Judged from the latter finding, it is unlikely that the presence of IgM-class antibodies in the intestinal secretions from patients was due to a cross-reaction between our anti-mu and immunoglobulin A. It is also unlikely that the presence of IgM-class antibodies was due to a leakage by transudation from the serum because (1) in all except one of the samples of intestinal secretions from typhoid patients there was no concomitant increase in the IgG-class antibodies (this was true even after antibody titres had been adjusted to units per mg immunoglobulin); (2) nor was there a concurrent rise in IgM levels in the intestinal secretions as compared with non-typhoid controls; and (3) IgM antibody levels in the intestinal secretions did not correlate with those in the serum in individual patients. Therefore, these IgM-class antibodies were most likely synthesized locally independent of the serum antibody response.

The presence of secretory IgM has been well documented in persons with IgA deficiency (Brandtzaeg, Fjellanger & Gjeruldsen, 1968; Thompson, 1970). In individuals with selective IgA deficiency, the IgM antibodies present in local secretions have been shown to be capable of replacing secretory IgA antibodies both quantitatively or qualitatively as these individuals do not have an increased incidence of mucosal infections (Rockey *et al.*, 1964; Goldberg, Barnett & Fudenberg, 1968). Some authors, however, suggested that IgM antibodies assumed an important protective role in secretions only when secretory IgA is not available (Walker, 1976). As our typhoid patients had no evidence of deficiency in IgA, the presence of IgM-class antibody cannot be explained as a compensatory response for selective IgA deficiency. This prompted us to postulate that the IgM antibody response detected in the intestinal secretions of typhoid patients might represent the usual sequence of humoral antibody response to antigen stimulation: 19S antibodies followed and superseded by 7S antibodies (Carpenter, 1975). As endotoxins of Gram-negative bacteria such as *S. typhi* are known to be a potent stimulant for the production of immunoglobulin M, it is not surprising that during typhoid infection an antibody response at the intestinal mucosal surface is initially of both the IgM and IgA types (as in the case of typhoid fever) and later replaced by the IgA type alone (as in the case of typhoid carrier state).

We have previously reported that antibodies to the protein antigens of *S. typhi* were present at high levels in sera from typhoid ptients. This study further demonstrated their presence in the intestinal secretions. It is also worth mentioning that in a previous study on non-bacteraemic cases of Salmonella gastroenteritis reported by La Brooy *et al.* (1980), the mean antibody units per mg Ig were found greater in the intestinal secretions than in serum. In this study on typhoid fever, however, antibody units per mg Ig were found greater in the serum than in the intestinal secretions in most instances (Fig. 4). This might be expected since typhoid fever, unlike Salmonella gastroenteritis, is a systemic infection and presents *S. typhi* antigens in a systemic fashion.

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REFERENCES

BRANDTZAEG, P., FJELLANGER, I. & GJERULDSEN, S.T. (1968) Immunoglobulin M: local synthesis and selective secretion in patients with immunoglobulin A deficiency. *Science*, **160**, 789.

CARPENTER, P.L. (1975) *Immunology and Serology* 3rd edn, p. 93. W. S. Saunders Co., Philadelphia, London, Toronto.

GOLDBERG, L.S., BARNETT, E.V. & FUDENBERG, H.H. (1968) Selective absence of IgA: a family study. *J. Lab. clin. Med.* **72**, 204.

LA BROOY, J.T., DAVIDSON, G.P., SHEARMAN, D.J.C. & ROWLEY, D. (1980) The antibody response to bacterial gastroenteritis in serum and secretions. *Clin. exp. Immunol.* **41**, 290.

ROCKEY, J.H., HANSON, L.A., HEREMANS, J.F. & KUNKEL, H.G. (1964) Beta-2A aglobulinemia in two healthy men. *J. Lab. clin. Med.* **63**, 205.

THOMPSON, R.A. (1970) Secretory piece linked to IgM in individuals deficient in IgA. *Nature*, **226**, 946.

TSANG, R.S.W., CHAU, P.Y., LAM, S.K., LA BROOY,

J.T. & ROWLEY, D. (1981) Antibody response to the lipopolysaccharide and protein antigens of *Salmonella typhi* during typhoid infection. I. Measurement of serum antibodies by radioimmunoassay. *Clin. exp. Immunol.* **46,** 508.

WALKER, W.A. (1976) Host defense mechanisms in the gastrointestinal tract. *Pediatrics*, **57,** 901.