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The humoral antibody response to *Shigella dysenteriae* type 1 infection, as determined by ELISA

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An enzyme-linked immunosorbent assay (ELISA) for determining the class-specific humoral antibody response to the lipopolysaccharide antigen from Shigella dysenteriae *serotype 1 bacteria has been tested. Two or more serum samples from each of 60 persons infected with this organism during a dysentery outbreak in a boarding school for young men near Haiphong, Viet Nam, and single serum samples from 39 healthy Vietnamese and from 20 healthy Swedes were included in the study. Comparison of the titres in the sera from the patients and the Vietnamese controls showed that the patients had significantly elevated IgA titres in sera collected 10, 30 and 45 days after onset of infection, and significantly elevated IgG titres in sera collected 30, 45 and 180 days after the onset. The titres in the patients' sera, compared with those in the Swedish controls, were significantly elevated for IgA and IgM as well as IgG in the samples collected after 10, 30, 45 and 180 days. The use of rabbit antisera, specific for enteropathogenic bacteria, and absorption experiments with human sera indicated that the* S. dysenteriae *type 1 lipopolysaccharide antigen is specific with respect to the O-antigenic polysaccharide chain.*

Shigella organisms cause bacillary dysentery which typically presents as fever and diarrhoea (*1*). The infection has a global distribution, countries with poor hygiene having the highest incidence. Infection is by the faecal-oral route and the most common mode of spread is by person-to-person transmission owing to the low infectious dose. In developing countries, food- and waterborne transmission is also common. Flies may be an important vector in areas with inadequate excreta disposal.

Epidemics with *Shigella dysenteriae* serotype 1 (Shiga's bacillus) were reported from Central America and Mexico in 1969-70, Bangladesh in 1972, and Sri Lanka in 1976 (*1*). In Viet Nam, the health authorities estimate that *S. dysenteriae* type 1 causes approximately 20% of all cases of bacillary dysentery (unpublished data, 1978).

Diagnosis of shigellosis is most often based on the isolation and identification of the organisms in faecal samples (*1*, *2*). Detection of the humoral antibody response is little used for diagnosis because whole killed bacteria, which had been used as antigens in agglutination tests, are now considered to be insensitive and unreliable (*2*). Using a crude O-polysaccharide antigen preparation in a passive haemagglutination assay, Cáceres & Mata (*3*) demonstrated that more than 80% of the patients studied during a shigellosis epidemic responded with antibody production. This assay could therefore be considered as more sensitive and specific than previously used tests, but there was still a drawback in that the agglutination reactions preferentially measured the IgM antibodies. As a result of increasing knowledge of the chemical structure of the *Shigella* O-antigens (*4*) and with methods for their isolation in pure form (*5*), and with the development of sensitive immunoassays (*6*), it is now possible to make a more reliable serodiagnosis of shigellosis.

In the present paper we report on the development of an enzyme-linked immunosorbent assay (ELISA) for studies of the class-specific humoral antibody response after *S. dysenteriae* type 1 infection.

MATERIALS AND METHODS

Shigella dysenteriae *type 1 epidemic*

An outbreak caused by *S. dysenteriae* type 1 occurred in a boarding school for young men in the Xuan Nguyen community, Haiphong, in early May

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1979. Approximately 100 boys aged 14-20 years (median, 17 years) fell ill during the epidemic. *S. dysenteriae* type 1 bacteria were isolated and typed (*7*, *8*) from 40 of the 60 patients included in this study.

Serum specimens

Two or more serum samples were drawn from each of the 60 patients, during the above-mentioned dysentery outbreak, on the 10th, 30th, 45th and 180th day after onset of the disease.

As control material, single serum samples were collected from healthy Vietnamese and Swedish persons. The Vietnamese control group consisted of 39 persons (22 females and 17 males) living in the Tu Liem district, Hanoi; their ages varied from 14 to 68 years with a median age of 26 years. The Swedish control group consisted of 20 persons (17 females and 3 males), all healthy blood donors, aged 18-26 years with a median age of 21 years.

Rabbit antisera

Type-specific and pooled serum preparations of rabbit antisera for serological identification of the following enteropathogenic organisms were used: *S. dysenteriae* type 1; *S. flexneri* pool B (serotypes 1-6); *S. flexneri* serotypes 2, 5, 6 and Y; *S. boydii* pool C1 (serotypes 1-7) and pool C2 (serotypes 8-15); *S. sonnei*; *Escherichia coli* pool I (serotypes O26:B6, O55:B5, O111:B4, O127:B8), pool II (serotypes O86:B7, O119:B5, O125:B15, O126:B16, O128:B12), and pool III (serotypes O25:11L, O44:74L, O78, O114, O124); and *E. coli* var. Alcalescens O1.

Preparation of antigens for ELISA

The *S. dysenteriae* type 1 strain 3818-0 was provided by Dr P. Gemski Jr, Division of Biochemistry, Walter Reed Army Institute of Research, Washington, DC, USA. The *S. flexneri* serotype 1b, *S. sonnei*, *E. coli* strain HF 4704, and the rough *S. flexneri* strain 4bR came from the strain collection in the Department of Bacteriology, National Bacteriological Laboratory (NBL), Stockholm.

The bacteria were grown in batch cultures and the lipopolysaccharides extracted as described earlier (*5*).

Immunoglobulin-enzyme conjugate for ELISA

The following alkaline phosphatase conjugates were used: rabbit anti-human IgA (specific for α-chains and secretory piece, NBL preparation C2), swine anti-human IgM (specific for μ-chains, Orion Diagnostica Oy, Helsinki, Finland), swine anti-human IgG (specific for γ-chains, Orion Diagnostica Oy), and sheep anti-rabbit Ig (polyspecific, NBL preparation A51).

ELISA procedure

ELISA was performed according to the method of Engvall & Perlmann (*9*), as previously described (*10*). Disposable polystyrene tubes (Nunc Inter Med, Denmark), 11×55 mm, were each coated with 1 ml of antigen in coating buffer, 0.05 mol/l carbonate buffer, pH 9.6, at 20-25 °C for 18 hours. Control tubes were treated with coating buffer only. Before assay, the tubes were washed four times with washing buffer, 0.15 mol/l NaCl containing 0.05% (v/v) of Tween 20. The sera to be tested were diluted in incubation buffer, phosphate-buffered saline, pH 7.4, containing 0.05% of Tween 20. 1 ml aliquots of the diluted test sera, one positive and one negative control serum, and incubation buffer only were added to the washed antigen-coated and control tubes. The tubes were incubated at 20-25 °C for 4 hours, and then washed as before. 1 ml of alkaline phosphatase conjugated antibodies, diluted in incubation buffer, was added to each tube. The tubes were incubated at 20-25 °C for 18 hours. After having been washed again, the tubes were filled with 1 ml of enzyme substrate solution, paranitrophenylphosphate (1 mg/ml) in 1 mol/l diethanolamine-HCl buffer, pH 9.8, containing 0.5 mmol/l $MgCl_2$, and incubated at 37 °C in a water bath. When the colour through a spectrophotometer corresponded to an absorbance of 0.7-1.0 at 405 nm, or after 100 min at the latest, the enzyme reaction was stopped by adding 0.1 ml of 5 mol/l NaOH. The absorbance at 405 nm was measured in a spectrophotometer using a 1 cm cuvette.

The results were expressed either as end-point titres or as relative titres, the *end-point titre* being the reciprocal of the serum dilution that gives an absorbance of 0.1 at 405 nm per enzyme reaction of 100 minutes (tenfold dilutions from 10^{-2} to 10^{-6} were tested in duplicates), and the *relative titre* being the absorbance value (mean of duplicates) of the 10^{-3} dilution of the serum multiplied by the dilution factor (1000). The amount of unspecific reaction of the sera seen in the control tubes when the sera were diluted 1:1000 corresponded to an absorbance of $\leqslant 0.100$ at 405 nm per 100 min and was not accounted for.

The intra-assay variation was $< \pm 10\%$ and the interassay variation $< \pm 20\%$, as estimated with the positive and negative control sera. No adjustment of the titres in individual sera was therefore considered necessary.

Absorptions of human serum samples

Serum samples, diluted 1:100, were mixed with approximately 5×10^8 bacteria from an overnight cul-

ture (washed and resuspended in phosphate-buffered physiological saline, pH 7.4) in a total volume of 2.0 ml and incubated at 37 °C for 150 min, followed by overnight incubation at 4 °C. The bacteria were then pelleted by centrifugation at 3000 *g* for 15 min, after which the supernatants were collected and assayed in ELISA.

RESULTS

Coating doses of lipopolysaccharide antigens

Phenol-water extracted lipopolysaccharide from *S. dysenteriae* type 1 was suspended in coating buffer, in varying concentrations for coating of the solid phase. The coated tubes were incubated with serum collected from a patient recovering from *S. dysenteriae* type 1 infection. Serum collected from one healthy individual served as a negative control. The lowest antigen concentration to give the highest absorbance value was found to be 1.0 μg/ml, a concentration used in all subsequent experiments. After similar experiments, the coating doses were determined to be 0.5 μg/ml for *S. flexneri* serotype Y, 1.0 μg/ml for *S. sonnei* and *S. flexneri* serotype 1b, 5.0 μg/ml for *E. coli* HF4704, and 10.0 μg/ml for *S. flexneri* 4bR.

Specificity of the S. dysenteriae *type 1 lipopolysaccharide antigen*

To test its specificity, the *S. dysenteriae* type 1 lipopolysaccharide antigen was used for estimation of antibody titres in sera from rabbits after exposure to various enteropathogenic bacteria (Fig. 1). The end-point titre against the *S. dysenteriae* type 1 lipopolysaccharide was 2.3×10^6. Only 2 out of the other 12 sera gave end-point titres that were $> 4.2 \times 10^3$; these were anti-*S. flexneri* type 6 (20-fold lower than the anti-*S. dysenteriae* type 1 titre) and anti-*S. boydii* pool C1 (50-fold lower). The structure of the O-antigenic polysaccharide chain of *S. flexneri* type 6 is different from that of *S. dysenteriae* type 1, making it unlikely that the lipopolysaccharide from the latter should detect antibodies against that particular *S. flexneri* lipopolysaccharide (Table 1). The structure of the core in *S. flexneri* type 6 has not het been studied. The structure of the *S. boydii* serotype 6 O-antigenic polysaccharide chain is so far the only one known among the seven included in the *S. boydii* pool C1, and it is unrelated to the *S. dysenteriae* type 1 lipopolysaccharide structure (Table 1). The cross-reactivities observed above were still so small that we considered it likely that the latter lipopolysaccharide was specific for the detection of anti-*S. dysenteriae* type 1 antibody response.

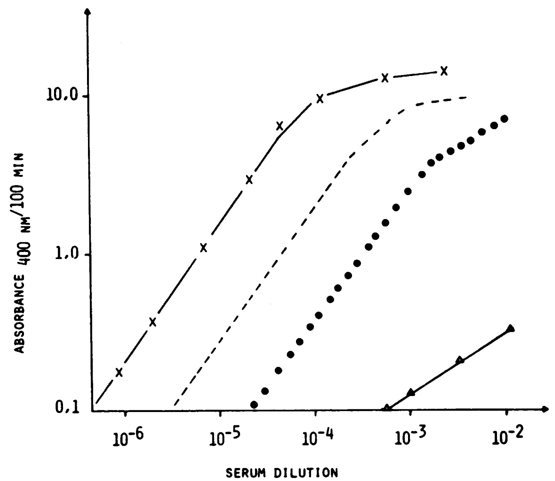

Fig. 1. ELISA titration of different hyperimmune rabbit antisera against *Shigella dysenteriae* type 1 lipopolysaccharide antigen. x-x *S. dysenteriae* type 1 serum; ----*S. flexneri* type 6 serum; ••••• *S. boydii* pool I serum; ΔΔΔ all other 10 sera (see Materials and Methods for details).

End-point versus relative ELISA titres

Sera from 29 of the *S. dysenteriae*-infected patients and from 10 healthy controls were tested in duplicate in tenfold dilution steps, from 10^{-2} to 10^{-6}, against the *S. dysenteriae* type 1 lipopolysaccharide antigen. The end-point titre was compared to the relative titre. The Spearman rank correlation between the end-point and the relative titres for IgG antibodies was determined (Fig. 2). The $r_s = 0.91$ ($P < 0.005$) means that the estimation of a relative ELISA titre using only the 10^{-3} dilution of test serum was in this assay as reliable as the estimation of the end-point titre using five serum dilutions. A similar calculation of the correlation between the end-point and relative titres for IgM gave a $r_s = 0.92$ ($P < 0.005$). Based on these results, all subsequent titres were determined on the 10^{-3} dilution of the serum samples and referred to as relative ELISA titres.

Relative ELISA titres against S. dysenteriae *type 1 lipopolysaccharide antigen*

A total of 123 samples, collected 10, 30, 45 and 180 days after onset of the epidemic, were assayed for antibody titres against *S. dysenteriae* type 1 lipopolysaccharide using class-specific conjugates (Table 2). the median IgA titre peaked already in the 10th day samples, and decreased thereafter: 400, 280, 250 and 140. The median IgG titre reached its peak in the 30th day samples. For IgM, no clear-cut tendency was seen, the median titres varying from 250 to 350. For

Table 1. Structures of O-antigenic chain and core polysaccharide in *Shigella* bacteria

Structure	Bacterial type	Reference
→3 α-L-Rha*p* 1→3 α-L-Rha*p* 1→2 α-D-Gal*p* 1→3 α-D-Glc*p*NAc 1→	*S. dysenteriae* type 1 O-antigen	(*4*)
β-D-Gal*p* ↓1 4 α-D-Gal*p* 1→2 α-D-Gal*p* 1→2 α-D-Glc*p* 1→3 α-D-Glc*p* 1→	*S. dysenteriae* core = coli R4 core	(*11*)
α-L-Rha*p* 1→2 α-L-Rhap 1→3 α-L-Rha*p* 1→3 β-D-Glc*p*NAc 1→2	*S. flexneri* type Y O-antigen	(*4*)
α-D-Glc*p* OAc ↓1 ↓2 4 α- L-Rha*p* 1→2 α-L-Rha*p* 1→3 α-L-Rha*p* 1→3 β-D-Glc*p*NAc 1→2	*S. flexneri* type 1b O-antigen	(*4*)
α-D-Glc*p*NAc ↓1 3 α-D-Glc*p* 1→2 α-D-Glc*p* 1→2 α-D-Gal*p* 1→3 α-D-Glc*p* 1→	*S. flexneri* core = coli R3 core	(*11*)
→4 β-D-Gal*p*A 1→3 β-D-Gal*p*NAc 1→2 α-L-Rhap 1→2 α-L-Rha*p* 1→	*S. flexneri* type 6 O-antigen	(*4*)
α-D-Glc*p*A ↓1 4 →3 β-D-Gal*p*NAc 1→3 α-D-Gal*p* 1→6 α-D-Man*p* 1→2 α-D-Man*p* 1→	*S. boydii* type 6 O-antigen	(*4*)
β-D-Glc*p* ↓1 3 α-D-Gal*p* 1→2 α-D-Gal*p* 1→2 α-D-Glc*p* 1→3 α-D-Glc*p* 1→	*S. sonnei, S. boydii* core = coli R1 core	(*11*)

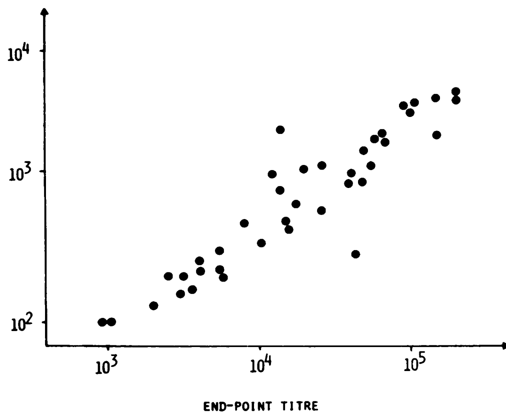

Fig. 2. Correlation between end-point and relative ELISA titres for immunoglobulin G.

all antibody classes a wide range in titres was seen. In a comparison with the two control groups there was no big difference between the median IgA and IgM titres though the range was wider in the Vietnamese control group than in the Swedish group for both IgA and IgM. The median IgG titre was three times higher in the Vietnamese control group than in the Swedish control group. Also for IgG a wider range of titres was seen in the Vietnamese control group than in the Swedish one.

A statistical analysis of the observed anti-*S. dysenteriae* type 1 lipopolysaccharide titres using the Wilcoxon test revealed some important differences in the comparison between sera from Vietnamese patients and Vietnamese controls (Table 3). In the 10th day samples, only the IgA titres were significantly higher than in the control samples. In both the 30th and 45th day samples the titres for all three classes were significantly higher. After 180 days, the IgM and IgG titres were still significantly higher. When comparing Vietnamese patients with the Swedish controls, the titres in the patients' samples were significantly higher in all instances tested. In a comparison of the titres between the Vietnamese control group and the Swedish control group, it was found that the IgG titres were significantly higher ($P \leqslant 0.001$) and the IgM titres just higher ($0.01 < P \leqslant 0.05$) in the Vietnamese control group, whereas no significant difference was found for the IgA titres.

The IgG titres reached the peak value in samples collected 30 days after the onset of infection. Grouping the sera into three classes revealed that the high IgG titres, i.e., > 1000, were not reached until 4 weeks after the onset of infection but also persisted for at least 6 months (Table 4).

Table 2. Class-specific relative ELISA titres against *Shigella dysenteriae* type 1 lipopolysaccharide

Sera collected from:	No. of sera tested	Relative ELISA titres[a]		
		IgA	IgM	IgG
S. dyseneriae-infected patients				
10 days after onset	28	400 (70–2100)	250 (20–1270)	680 (110–2300)
30 days after onset	44	280 (50–2700)	300 (100–2200)	1700 (190–3500)
45 days after onset	28	250 (70–5600)	270 (80–2400)	1350 (190–3500)
180 days after onset	23	140 (40–860)	350 (160–1000)	830 (260–3200)
Healthy Vietnamese controls	39	90 (50–680)	160 (60–1160)	420 (80–1690)
Healthy Swedish controls	20	80 (80–130)	110 (40–400)	140 (110–800)

[a] Figures are median values; those in parentheses indicate the range.

Table 3. Levels of significance in the differences in immunoglobulin titres between *S. dysenteriae*-infected patients and controls (Vietnamese and Swedish)[a]

Sera collected from:	IgA		IgM		IgG	
	Vietnamese controls	Swedish controls	Vietnamese controls	Swedish controls	Vietnamese controls	Swedish controls
S. dysenteriae-infected patients						
10 days after onset	**	**	*	***	NS	***
30 days after onset	***	***	***	***	***	***
45 days after onset	***	***	**	***	***	***
180 days after onset	NS	**	***	***	**	***

[a] Key: NS = $P > 0.05$
* = $0.01 < P \leqslant 0.05$
** = $0.001 < P \leqslant 0.01$
*** = $P \leqslant 0.001$

Table 4. Relative ELISA titres for IgG against *Shigella dysenteriae* type 1 lipopolysaccharide

Sera collected from:	No. of sera tested	No. of sera having a relative ELISA titre for IgG of:		
		< 500	500–1000	> 1000
S. dysenteriae-infected patients				
10 days after onset	28	12 (43)[a]	4 (14)	12 (43)
30 days after onset	44	2 (5)	4 (9)	37 (86)
180 days after onset	23	6 (26)	7 (30)	10 (44)
Healthy Vietnamese controls	39	21 (54)	13 (33)	5 (13)
Healthy Swedish controls	20	16 (80)	4 (20)	0 (0)

[a] Figures in parentheses are percentages.

Absorption studies on human sera

The rather high *S. dysenteriae* type 1 lipopolysaccharide titres seen in some of the sera from healthy Vietnamese controls suggest that at the time of the investigation these sera already had antibodies directed against this lipopolysaccharide. A comparison of these titres with those seen in the sera from the Swedish controls pointed in the same direction. Convalescent sera (collected 3–6 weeks after a culturally verified infection) from three of the patients with *S. dysenteriae* type 1 infection, and from three patients with a *S. flexneri* type 1b infection were subjected to absorptions with heat-killed *S. dysenteriae* type 1 and *S. flexneri* type 1b bacteria and the relative titres (IgM and IgG) were determined before and after absorption (Table 5a, b). It is evident that the absorption removed only the antibodies against the absorbing strain. Usually the titre was reduced by more than 80% by the homologous, and by less than 20% by the heterologous absorption.

In another absorption experiment, one of the *S. dysenteriae* convalescent sera was assayed for ELISA titres against the following four lipopolysaccharide antigens to see whether antibodies against the core structure are detected: *S. sonnei* lipopoly-

Table 5a. Relative ELISA titres for IgM and IgG against *S. dysenteriae* type 1 and *S. flexneri* type 1b lipopolysaccharide (LPS) antigens in sera from three *S. dysenteriae*-infected patients, before and after absorption

Serum from *S. dysenteriae*-infected patients	Relative ELISA titres for IgM and IgG against:			
	S. dysenteriae type 1 LPS		*S. flexneri* type 1b LPS	
	IgM	IgG	IgM	IgG
No. 1: unabsorbed	210	750	360	800
absorbed with *S. dysenteriae*. type 1 bacteria	80	110	380	800
absorbed with *S. flexneri* type 1b bacteria	180	810	110	150
No. 2: unabsorbed	180	1130	240	2480
absorbed with *S. dysenteriae* type 1 bacteria	40	140	180	2610
absorbed with *S. flexneri* type 1b bacteria	120	1200	70	680
No. 3: unabsorbed	1730	2710	320	1720
absorbed with *S. dysenteriae* type 1 bacteria	70	380	190	1700
absorbed with *S. flexneri* type 1b bacteria	1370	2910	70	270

Table 5b. Relative ELISA titres for IgM and IgG against *S. flexneri* type 1b and *S. dysenteriae* type 1 lipopolysaccharide (LPS) antigens in sera from three *S. flexneri*-infected patients, before and after absorption

Serum from *S. flexneri*-infected patients	Relative ELISA titres for IgM and IgG against:			
	S. flexneri type 1b LPS		*S. dysenteriae* type 1 LPS	
	IgM	IgG	IgM	IgG
No. 1 unabsorbed	440	2600	870	2600
absorbed with *S. flexneri* type 1b bacteria	160	340	740	2680
absorbed with *S. dysenteriae* type 1 bacteria	260	2370	40	510
No. 2 unabsorbed	170	1300	130	610
absorbed with *S. flexneri* type 1b bacteria	90	220	100	680
absorbed with *S. dysenteriae* type 1 bacteria	130	1340	30	350
No 3: unabsorbed	170	2100	90	1340
absorbed with *S. flexneri* type 1b bacteria	60	260	80	1400
absorbed with *S. dysenteriae* type 1 bacteria	140	2090	20	210

saccharide and *E. coli* HF4704 lipopolysaccharide (with core polysaccharides related to that of *S. dysenteriae*) and *S. flexneri* type Y lipopolysaccharide and *S. flexneri* type 4bR lipopolysaccharide (with core chains unrelated to *S. dysenteriae*). The results (Table 6) revealed the following:

(i) Absorption with *S. dysenteriae* type 1 bacteria (the homologous strain with both O- and core-polysaccharide) reduced the homologous titre by more than 90%, reduced the titre against *S. sonnei* lipopolysaccharide (with a different O chain, but similar core) by 44%, and reduced the titre against *E. coli* HF4704 (no O chain, but similar core) by 87%. The titres against the two *S. flexneri* lipopolysaccharides (with different O- and core-polysaccharides) were unchanged by the absorption with the *S. dysenteriae* type 1 bacteria.

(ii) Absorption with *S. sonnei* bacteria reduced the titre against the homologous lipopolysaccharide as well as the titre against *E. coli* HF4704 lipopolysaccharide (same core as *S. sonnei* lipopolysaccharide, but no O-polysaccharide) by more than 90%, the anti-*S. dysenteriae* type 1 lipopolysaccharide (same core, but different O-polysaccharide) titre by approximately 50%, but left the anti-*S. flexneri* lipopolysaccharides (core- and O-polysaccharide unrelated) titres unchanged.

(iii) Absorption with *E. coli* HF4704 bacteria (only core polysaccharide) reduced the homologous titre by more than 90%, the titres against *S. dysenteriae* type 1 and *S. sonnei* lipopolysaccharides by 30–40%, and left the titres against the two *S. flexneri* lipopolysaccharides unchanged.

(iv) Absorption with the *S. flexneri* type Y strain reduced the *S. flexneri* titres by approximately 70% leaving the titres against the three other lipopolysaccharides unchanged.

(v) Absorption with *S. flexneri* 4bR reduced the homologous titre by approximately 75% and the other titres by no more than 25%.

This experiment shows that antibodies were formed against both the O-and core-polysaccharide antigens of *S. dysenteriae* type 1 and were detectable by ELISA.

DISCUSSION

The use of an immunoassay, either for assessing the antibody response in individuals, or for sero-epidemiological purposes, requires well-defined antigens (preferably defined at the molecular level) and should permit immunoglobulin class determinations. The *S. dysenteriae* type 1 ELISA reported in this study fulfils these requirements to a higher extent than previously reported tests. The specificity of the O-antigenic polysaccharide of the *S. dysenteriae* type 1 lipopolysaccharide was evident both in assays with rabbit antisera against other enterobacterial strains (Fig. 1), and in absorption experiments using the patients' sera (Tables 5a, 5b and 6). The observation that rabbit serum against *S. flexneri* type 6 was bound to the *S. dysenteriae* type 1 lipopolysaccharide, although with an efficiency 20-fold less than that for the homologous serum, cannot be explained, considering the absence of chemical relatedness between the O-polysaccharide chains of the two bacterial species (Table 1). Alternatively, the antibodies formed against the core polysaccharide should be related, or identical. The core structures of the *Shigella* bacteria studied so far, all fall within the known core types R1–R4 (Table 1) (*10*). However, the structure of the *S. flexneri* type 6 core has not yet been determined. The extent of cross-reactivity between the *S. dysenteriae* type 1 lipopolysaccharide and the *S. boydii* pool C1 antiserum was even smaller (Fig. 1). The wealth of knowledge accumulated over the years also indicates that cross-reactivity between *S. dysenteriae* type 1 antiserum and antiserum from other enterobacteria is relatively rare (*7*).

The observation that the patients' sera contained antibodies reactive both with the *S. dysenteriae* type 1 lipopolysaccharide and the *E. coli* HF4704 and *S. sonnei* lipopolysaccharides, and that absorption with heat-killed *S. dysenteriae* type 1 and *E. coli* HF4704 and *S. sonnei* bacteria removed these antibodies (Table 6), strongly suggests that antibodies had been formed against both the core- and, as expected, the O-polysaccharide chain. The fact that antibodies should be elicited against core structures in *Shigella* bacteria is not surprising since structural studies of various *Shigella* lipopolysaccharides have shown that many completed cores are left uncapped by O-polysaccharide chains (*11*). Since the number of reported core types in *Shigella* is low and some appear in different species (*10*), this may lead to complications in sero-epidemiological work. It would therefore be an advantage if the antigens used for antibody detection had either just a few core chains in their lipopolysaccharide preparations, or if artificial glycoconjugates devoid of core structures could be used as antigens.

To express the antibody content determined by ELISA as a traditional end-point titre, it is necessary to analyse serial dilutions of each serum sample. This is not only laborious but also consumes large amounts of reagents. Therefore we compared the end-point titre with the relative titre of the 10^{-3} dilution of sera collected 10 and 30 days after onset of the infection and in sera collected from healthy controls (Fig. 2). A statistically significant correlation between the two measurements was obtained for IgG as well as IgM.

Table 6. Relative ELISA titres for IgG against five different lipopolysaccharide (LPS) antigens in a serum from a patient with a culturally verified *S. dysenteriae* type 1 infection, before and after absorption

Type of serum tested	Relative ELISA titres for IgG against:				
	S. dysenteriae type 1 LPS (R4 core + O-ag)	*S. sonnei* LPS (R1 core + O-ag)	*E. coli* HF4704 LPS (R1 core only)	*S. flexneri* type Y LPS (R3 core + O-ag)	*S. flexneri* type 4bR LPS (R3 core only)
Unabsorbed	1390	860	780	2350	920
Absorbed with *S. dysenteriae* type 1 bacteria (R4 core + O-ag)	50 (96)[a]	480 (44)	100 (87)	1850 (21)	780 (15)
Absorbed with *S. sonnei* bacteria (R1 core + O-ag)	760 (45)	80 (91)	50 (94)	2520 (−7)	840 (9)
Absorbed with *E. coli* HF4704 bacteria (R1 core)	950 (32)	550 (36)	30 (96)	2710 (−15)	830 (10)
Absorbed with *S. flexneri* type Y bacteria (R3 core + O-ag)	1270 (9)	910 (−6)	800 (−2)	770 (67)	400 (57)
Absorbed with *S. flexneri* type 4bR bacteria (R3 core)	1120 (20)	780 (9)	620 (21)	1770 (25)	240 (74)

[a] The figures within parentheses indicate the percentage reduction or increase (minus sign) in titres after absorption.

Consequently, the relative ELISA titre can here be considered to be as true an estimate of the antibody content as the end-point titre.

Statistical evaluations were performed to see if there were any significant differences in titres between the Vietnamese patients and Vietnamese or Swedish controls (Table 3). The patients/Vietnamese controls comparisons revealed, for diagnostic purposes, useful differences in that the IgA titres were significantly higher (P values from < 0.01 to $\leqslant 0.001$) in the patients' serum samples collected 10, 30 and 45 days after onset of shigellosis; the IgG titres were significantly higher somewhat later, i.e., in the 30, 45 and 180 day serum specimens. The IgA, IgM as well as IgG titres in the Vietnamese patients, when compared with those in the Swedish controls, were found to be significantly higher in the 10, 30, 45 and 180 day samples (Table 3). Of interest is that when the two control groups were compared, the only significant difference ($P \leqslant 0.001$) was seen in the IgG titres. The ELISA, as used in this investigation, appears to give for sero-epidemiological purposes significant differences between the patients and the control groups.

The observation that serum samples from Vietnamese controls had high *S. dysenteriae* type 1 titres, whereas those seen in sera from Swedish controls were low (Tables 2 and 4), and the simultaneous appearance of high *S. dysenteriae* type 1 and *S. flexneri* type 1b lipopolysaccharide titres in sera from Vietnamese patients (Tables 5a and b) point to a high prevalence of shigellosis in Viet Nam. This only emphasizes again the importance of using structurally defined antigens, e.g., glycoconjugates without core structures should be preferred.

The results of this study also emphasize the importance of Ig class-specific antibody determination. Furthermore, paired serum samples, one collected during the acute phase of the disease and the other during the convalescent phase, should be analysed whenever possible.

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RÉSUMÉ

DÉTERMINATION DE LA RÉPONSE EN ANTICORPS HUMORAUX À L'INFECTION PAR *SHIGELLA DYSENTERIAE* DE TYPE 1 AU MOYEN DE LA TECHNIQUE ELISA

Un titrage avec immunoadsorbant lié à une enzyme (ELISA) a été essayé en vue de la détermination de la réponse en anticorps humoraux spécifiques de classe à l'antigène lipopolyosidique de *Shigella dysenteriae*, sérotype 1. Des sérums ont été recueillis chez 60 malades de sexe masculin âgés de 14 à 20 ans (médiane: 17 ans) qui avaient été infectés par ce micro-organisme au cours d'une épidémie de dysenterie dans un pensionnat près de Haiphong, Viet Nam, en mai 1979. Un ou plusieurs échantillons de sérum ont été prélevés chez chaque sujet entre le 10e jour et le 180e jour après le début de la maladie. Comme matériel témoin, on a recueilli des échantillons de sérum chez 39 Vietnamiens en bonne santé, âgés de 14 à 68 ans (médiane: 26 ans) vivant à Hanoï, ainsi qu'à partir de 20 sujets suédois en bonne santé, âgés de 18 à 26 ans (médiane: 21 ans).

Les épreuves effectuées par la technique ELISA avec 13 sérums hyperimmuns de lapins obtenus au moyen de diverses entérobactéries, ont montré que l'antigène lipopolyosidique de *S. dysenteriae* de type 1 était spécifique pour la détection de la réponse en anticorps au lipopolyoside de ce micro-organisme.

Les titrages par cette technique de sérums provenant de malades dysentériques ont montré une élévation des titres d'IgA: les titres relatifs médians étaient de 400, 280, 250 et 140 dans les sérums recueillis aux jours 10, 30, 45 et 180, respectivement, après le début de la maladie. La médiane des titres d'IgA dans les sérums provenant des témoins vietnamiens n'était que de 90. En ce qui concerne les IgG, les titres correspondants étaient de 680, 1700, 1350 et 830 chez les malades et de 420 dans le groupe témoin. Les titres des IgM étaient de 250, 300, 270 et 350 chez les malades et de 160 dans le groupe témoin. Parmi les témoins suédois, les titres médians étaient de 80, 110 et 140 pour les IgA, IgM et IgG, respectivement. Ainsi, les titres d'IgA étaient élevés au début de l'infection et revenaient aux niveaux observés chez les personnes bien portantes dans les 6 mois. La réponse en IgG était quelque peu retardée, atteignant son titre maximal 1 mois après le début de la maladie et restant élevée au bout d'encore 5 mois environ. Parmi les sérums recueillis 10 et 30 jours après le début de l'infection, 43% et 86% respectivement présentaient des titres supérieurs à 1000, alors que les proportions correspondantes étaient seulement de 13% parmi les sérums prélevés chez les témoins vietnamiens et de 0% dans ceux des témoins suédois. La réponse en IgM était plus variable et aucune tendance nette ne pouvait être discernée.

Le fait que chez les témoins vietnamiens en bonne santé les titres des anticorps anti-lipopolyoside de *S. dysenteriae* de type 1 étaient relativement élevés donne à penser que les infections dues à ce micro-organisme ne sont pas rares au Viet Nam.

Des expériences d'absorption portant sur des sérums humains montrent que l'antigène lipopolyosidique de *S. dysenteriae*, type 1, est spécifique pour la recherche de la réponse en anticorps à la dysenterie due à ce micro-organisme et qu'il se forme des anticorps non seulement contre la chaîne polyosidique antigène O, mais également contre le noyau polyosidique du lipopolyoside.

REFERENCES

1. WHO Scientific Working Group. Enteric infections due to *Campylobacter, Yersinia, Salmonella,* and *Shigella. Bulletin of the World Health Organization*, **58**: 519-537 (1980).
2. Wilson, G. S. & Miles, A. A., ed., Topley and Wilson's *Principles of bacteriology and immunity*. London, E. Arnold, 1964.
3. Cáceres, A. & Mata, L. J. Serologic response of patients with Shiga dysentery. *Journal of infectious diseases*, **129**: 439-443 (1974).
4. Kenne, L. & Lindberg, B. Bacterial polysaccharides. In: Aspinall, G. O., ed., *The polysaccharides*. London, New York, San Francisco, Academic Press, 1983, pp. 287-363.
5. Lindberg, A. A. & Holme, T. Evaluation of some extraction methods for the preparation of bacterial lipopolysaccharides for structural analysis. *Acta pathologica et microbiologica scandinavica*, Section B, **80**: 751-759 (1972).
6. Carlsson, H. E. & Lindberg, A. A. Application of enzyme immuno-assay for diagnosis of bacterial and mycotic infections. *Scandinavian journal of immunology*, **8** (suppl. 7): 97-110 (1978).
7. Edwards, P. R. & Ewing, W. H. *Identification of Enterobacteriaceae*. Minneapolis, Burgess Publishing Company, 1972.
8. Kaufmann, F. *The bacteriology of Enterobacteriaceae*. Copenhagen, Munksgaard, 1966.
9. Engvall, E. & Perlmann, P. Enzyme-linked immunosorbent assay (ELISA): quantitative assay of immunoglobulin G. *Immunochemistry*, **8**: 871-874 (1971).
10. Carlsson, H. E. et al. Enzyme-linked immunosorbent assay for immunological diagnosis of human tularemia. *Journal of clinical microbiology*, **10**: 615-621 (1979).
11. Jansson, P.-E., et al. Structural studies on the hexose region of the core in lipopolysaccharides from *Enterobacteriaceae. European journal of biochemistry*, **115**: 571-577 (1981).