

## Comparison of Bacteria with and without Plasmid-Encoded Proteins as Antigens for Measurement of Immunoglobulin M, G, and A Antibodies to *Yersinia enterocolitica* by Enzyme-Linked Immunosorbent Assay

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***Yersinia enterocolitica* serovar O:3 bacteria with and without plasmid-encoded proteins were compared as antigens in an enzyme-linked immunosorbent assay. Good correlations between the two antigen preparations were obtained for immunoglobulin M (IgM), IgG, and IgA antibodies of patients with yersiniosis. For routine diagnostic purposes, these antigens are considered equal.**

Infections caused by *Yersinia enterocolitica* serovar O:3 are usually associated with a strong antibody response. Isolation of bacteria from feces is not always successful, and consequently the diagnosis often depends on the demonstration of specific antibodies in the patient serum (1, 7, 8). Nowadays, enzyme immunoassay and separate quantitation of immunoglobulin M (IgM), IgG, and IgA *Yersinia* antibodies are considered important (3, 5, 8, 12, 17-20, 22, 25-28) and especially valuable in the retrospective diagnosis of yersiniosis as a cause of reactive complications (6-9, 14, 19, 27).

Both whole *Yersinia* bacteria (5-9) and purified lipopolysaccharides (10-12) have been used as antigens for detecting antibodies in sera of patients, and a close correlation between the techniques using the two antigens has been found (10). Since antibodies to both chromosome- (23) and plasmid- (13, 24, 25) encoded protein antigens have also been detected in *Yersinia* infections by qualitative immunoblotting, it seemed important to study the possible influence of the plasmid-encoded antigens on the results obtained by an enzyme-linked immunosorbent assay (ELISA) routinely used for diagnostic purposes. Therefore, we did a comparison between ELISAs using as antigens a *Y. enterocolitica* serovar O:3 strain with the plasmid (pYV+) or a plasmid-cured derivative of the same strain (pYV-).

The strain of *Y. enterocolitica* serovar O:3 used was a stool isolate from a patient with reactive arthritis. This strain contains a virulence-associated type II plasmid (21). The strain and the corresponding antigen extract are here designated pYV+ (*Yersinia* plasmid associated with virulence). A plasmid-cured derivative, pYV-, was obtained from single colonies growing on magnesium-oxalate agar at 37°C (4). The presence or absence of the virulence plasmid was verified by the autoagglutination test (16) and a plasmid purification method (21). Stock cultures were maintained at -70°C in 20% (vol/vol) glycerol-Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). The bacteria were grown in conditions optimal for the expression of the plasmid-specified polypeptides (24). First, they were subcultured overnight in nutrient broth at room temperature and inoculated in Higuchi minimal medium supplemented with 1% tryptone (Difco Laboratories, Detroit, Mich.), 0.5% yeast

extract (Oxoid Ltd., Basingstoke, United Kingdom), and 0.2% glucose. The bacteria were grown on a shaker at 37°C to an optical density of 0.3 at 540 nm. After three washings with 0.9% (wt/vol) NaCl, the bacteria were treated with 0.1% (wt/vol) sodium dodecyl sulfate for 1 h at 37°C. The extracts obtained were used as antigens in the ELISA at a protein concentration of 0.5 µg/ml.

The presence or absence of plasmid-specified proteins in antigen extracts was verified. Only the pYV+ strain was autoagglutinable, indicating the presence of a plasmid-specified outer membrane protein with molecular weight 150,000 (2, 25). This polypeptide was exclusively seen in the pYV+ strain as a strong band when whole-cell lysates of *Y. enterocolitica* serovar O:3 were separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained by Coomassie brilliant blue. In immunoblotting, rabbit anti-*Y. enterocolitica* O:3 serum recognized a few additional bands in the pYV+ strain, the most prominent with molecular weights 26,000 and 32,000, as have been reported before (15).

The ELISA for IgM, IgG, and IgA *Yersinia* antibodies was described earlier (5, 10). The concentrations of antibodies in the samples were expressed as relative units (enzyme immunoassay units; EIU); 1 U was 1/100 of the corresponding antibody concentration in the reference serum. EIUs of 100 normal serum samples were measured separately, and values of more than 2 standard deviations added to the EIUs of pooled normal control sera included on each plate were taken as positive.

Serum samples of 94 persons from our diagnostic laboratory were studied for IgM, IgG, and IgA antibodies with the pYV+ ELISA and the pYV- ELISA. Of 94 samples, 15 were known to be negative and 79 were known to be positive for *Y. enterocolitica* serovar O:3 antibodies in the routine ELISA. Correlation coefficients for IgM, IgG, and IgA results between the two ELISAs (BMDP Statistical Software; Department of Biomathematics, University of California at Los Angeles, Los Angeles) were 0.99, 0.90, and 0.92, respectively ( $P < 0.001$  for all of them). The results for IgA antibodies are presented in Fig. 1.

In addition, 79 serial samples from 48 patients with serologically and in 24 cases also bacteriologically verified *Y. enterocolitica* serovar O:3 infection were studied. All cases presented typical clinical pictures, with fever, diarrhea,

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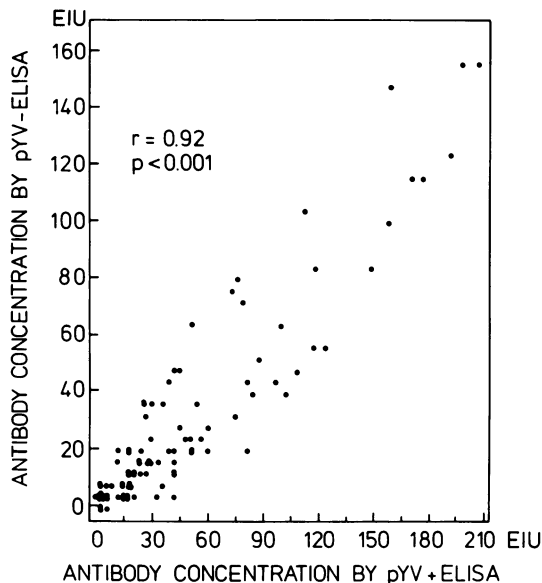


FIG. 1. Correlation between anti-*Yersinia* IgA concentrations determined by the pYV+ ELISA and pYV- ELISA; 94 human serum samples were tested.

and/or abdominal pain. The patients were divided into two groups according to their arthritic symptoms as follows. (i) Patients without arthritis included 15 females and 7 males ranging in age from 18 to 58 years (mean, 34.3 years). (ii) Patients with arthritis included 11 females and 15 males ranging in age from 20 to 66 years (mean, 44.3 years) and had clearly demonstrable swelling in at least one joint. Sera were obtained at 1 to 2 and 6 to 8 months after the onset of symptoms. All sera were stored in aliquots at  $-20^{\circ}\text{C}$  and studied at the same time.

The concentrations of *Y. enterocolitica* serovar O:3-specific IgM, IgG, and IgA antibodies of the patient groups are presented in Table 1. In the beginning of the disease,

TABLE 1. Concentrations of IgM, IgG, and IgA class antibodies to *Y. enterocolitica* serovar O:3 in sera of patients with and without reactive arthritis during follow-up

Immunoglobulin class	ELISA pYV antigen	Mean EIU $\pm$ SD at <sup>a</sup> :			
		1-2 mo		6-8 mo	
		A+ (n = 23) <sup>b</sup>	A- (n = 20)	A+ (n = 21)	A- (n = 15)
M	+	112 $\pm$ 45	128 $\pm$ 73	31 $\pm$ 19	45 $\pm$ 33
	-	118 $\pm$ 53	138 $\pm$ 78	32 $\pm$ 24	46 $\pm$ 38
G	+	75 $\pm$ 16 <sup>c</sup>	61 $\pm$ 26	53 $\pm$ 22	53 $\pm$ 29
	-	56 $\pm$ 24	62 $\pm$ 31	42 $\pm$ 25	55 $\pm$ 31
A	+	135 $\pm$ 58 <sup>d</sup>	92 $\pm$ 52	54 $\pm$ 36 <sup>e</sup>	30 $\pm$ 23
	-	131 $\pm$ 63 <sup>e</sup>	69 $\pm$ 50	44 $\pm$ 38 <sup>f</sup>	15 $\pm$ 15

<sup>a</sup> A+, Reactive arthritis; A-, no reactive arthritis.

<sup>b</sup> n, Number of patients. Samples were not available from all patients for both time intervals.

<sup>c</sup>  $P < 0.05$ , compared with the patients without arthritis.

<sup>d</sup>  $P < 0.02$ , compared with the patients without arthritis.

<sup>e</sup>  $P < 0.005$ , compared with the patients without arthritis.

<sup>f</sup>  $P < 0.01$ , compared with the patients without arthritis.

antibodies of all three classes were demonstrable in the sera of all patients with both ELISAs. When the responses of arthritic and nonarthritic patients were compared, significantly stronger IgA responses were seen in the sera of arthritic patients with both ELISAs. These patients also showed higher IgG values than nonarthritic ones in the pYV+ ELISA. At 6 to 8 months, both ELISAs also gave parallel results. The IgG antibodies were still at high levels. High concentrations of IgA and IgG antibodies in the sera of arthritic patients are in line with earlier findings (7, 9).

Technically, no great difference exists between these two antigens. Once the bacteria with and without the plasmid are available, the preparation of the antigens is identical. The antibody concentrations in patient sera observed by the two ELISA methods correlated extremely well for all three immunoglobulin classes. This may be explained by the fact that the proportional part of antibodies to plasmid-encoded proteins is small and antibodies to chromosomally encoded structures dominate. Characteristically strong IgA responses of patients with *Yersinia*-triggered reactive arthritis were evident with both techniques. In the pathogenesis of *Yersinia* infection, the plasmid-encoded structures and probably also antibodies against them are important, but methods other than those used here must be found for their quantitation. For routine diagnostic purposes, we consider the methods presented here to be equal.

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