

Association of persisting IgA response with yersinia triggered reactive arthritis: a study on 104 patients

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SUMMARY Twelve to 16 months after *Yersinia enterocolitica* O:3 enteritis 33 (85%) of the 39 patients who developed reactive arthritis as a postinfection complication had IgA class and 28 (72%) had IgG class anti-yersinia antibodies. In contrast, 7 (32%) of the 22 patients who did not develop arthritis were positive in the IgA test and 11 (50%) positive in the IgG test. The results were about the same when the material was divided into cases with diagnosis of yersiniosis verified by stool culture or by serology. These results confirm the value of enzyme linked immunosorbent assay (ELISA) in the diagnosis of yersiniosis, particularly in cases with postinfection complications when the stool isolations remain negative.

Enteric infections caused by *Yersinia enterocolitica* are often associated with postinfection complications, of which reactive arthritis is the most prominent. Isolation of bacteria from faeces is not always successful, and, consequently, the diagnosis usually depends on the demonstration of specific antibodies in the patient's serum.¹ When agglutination techniques are used the diagnosis is based on a rise of antibody titres. Low titres are of little significance as they are also seen in healthy persons,² and if the sample contains IgG and IgA class antibodies only, the agglutination may remain totally negative.¹ We and others have found the enzyme linked immunosorbent assay (ELISA) useful in the diagnosis of acute yersiniosis and especially valuable in the retrospective diagnosis of yersiniosis as a cause of reactive complications.^{1 3-7} As certain doubts about the value of ELISA still seem to exist⁸ we have extended our earlier studies to determine the occurrence of IgM, IgG, and IgA class yersinia antibodies during a follow up study of the sera of 104 patients with *Yersinia enterocolitica* O:3 infection, 62 of whom developed reactive arthritis as a postinfection complication. The findings obtained confirm the value of ELISA in the diagnosis of yersiniosis and particularly the association of persisting IgA response in patients with yersinia triggered reactive arthritis.

Patients and methods

PATIENTS

One hundred and four patients were diagnosed as having an acute *Yersinia enterocolitica* O:3 infection on the basis of bacteriological or serological findings, or both, and the clinical picture; 37 of them had been included in a previous study.³ All 104 patients had IgM, IgG, and/or IgA class antibodies to *Yersinia enterocolitica* O:3 at a level of more than mean + six standard deviations of the values obtained for 50 Finnish blood donors. *Yersinia enterocolitica* O:3 was isolated from the faeces of 32 patients. All cases presented with clinical symptoms of acute infection. The clinical characteristics of the patients are summarised in Table 1. They were divided into three groups according to the arthritic symptoms as follows: (a) patients without arthritis, (b) patients with grade + arthritis who had joint symptoms which were reported as subjective pain without objective findings, (c) patients with grade ++ arthritis who had in addition to subjective pain clearly demonstrable swelling in at least one joint; in many cases several joints were involved (Table 1).

ANTIBODY ASSAYS

The ELISA for the detection of yersinia antibodies has been described earlier in detail.⁹ Briefly, *Y. enterocolitica* O:3 antigens (whole bacteria) were adsorbed onto polystyrene microtitre plates (Linbro, Titertek, Flow Laboratories, Irvine, Scotland). Aliquots of serum (75 µl), dilution 1:250,

Accepted for publication 31 May 1987.
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Table 1 Clinical characteristics of the 104 patients*

Grade of arthritis	Age; years Mean (range)	Total (male/female)	HLA-B27 +/-	Positive stool culture	Fever (°C) >38.0	Diarrhoea	Abdominal pain	Joint symptoms >1/2 year	Iritis	Erythema nodosum	Carditis
-	31.8 (10-66)	41 (16/25)	7/34	13	13	31	29	0	2	6	1†
+	38.9 (19-58)	20 (7/13)	3/17	7	5	16	14	6	2	4	0
++	38.9 (16-62)	43 (23/20)	33/10	12	14	37	28	14	4	4	1

*Figures given refer to the numbers of patients.

†Also glomerulonephritis.

were added and incubated at 37°C for two hours. The plates were washed three times with phosphate buffered saline containing 0.05% Tween 20, and 75 µl of alkaline phosphatase conjugated swine anti-serum to human IgM, IgG, and IgA (Orion Diagnostica, Espoo, Finland) was added. After incubation overnight at room temperature the plates were washed as previously, and fresh substrate (75 µl) (1 mg of the disodium salt of *p*-nitrophenyl phosphate/ml of diethanolamine-MgCl₂ buffer solution; Orion Diagnostica) was added to the plates, which were then incubated for 30 min at 37°C; 125 µl of 1 N NaOH was added to stop the reaction. The optical absorbance was measured with the use of a specially designed photometer (Titertek/Multiskan, Eflab, Helsinki, Finland) at a wavelength of 405 nm. A positive reference serum with high levels of yersinia antibodies of IgM, IgG, and IgA classes, a buffer control, and a pool consisting of 50 normal sera were included on each plate. The concentration of antibodies in the sample was expressed as relative units (EIU), where 1 unit was 1/100 of the corresponding antibody concentration in the reference serum. EIUs of 50 normal sera were measured separately, and values more than two standard deviations greater than the EIUs of pooled normal control sera included on each plate were taken as positive. The results obtained with the whole bacteria ELISA were confirmed by using the lipopolysaccharide (LPS) of *Y enterocolitica* O:3 as the antigen.¹⁰ Potential cross reactions and specificity of ELISA in the diagnosis of *Y enterocolitica* O:3 infections have been described previously.^{10 11}

STATISTICS

Persistence of IgM, IgG, and IgA class yersinia antibodies in different patient groups was compared with the χ^2 test.

Results

Patients who developed reactive arthritis showed a clear tendency to maintain their IgA-anti-yersinia antibodies at a considerable level (Table 2). This was observed during both follow up periods, at 6-8 months and at 12-16 months, after the initial infection. At 12-16 months 33 (85%) of 39 patients with reactive arthritis had IgA and 28 (72%) had IgG class anti-yersinia antibodies. In contrast, only seven (32%) of 22 patients with no arthritis were positive in the IgA test and 11 (50%) positive in the IgG test. The results for IgA differed significantly from those of the non-arthritic patients (Table 2).

We also analysed cases in which the diagnosis was proved bacteriologically (by stool culture)

Table 2 Persistence of IgM, IgG, and IgA class antibodies against *Yersinia enterocolitica* O:3 in sera of 104 patients after an acute *Y enterocolitica* O:3 infection

Grade of arthritis	No of patients with antibodies to yersinia/No studied								
	At 1-2 months			At 6-8 months			At 12-16 months		
	IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA
-	35/41	39/41	37/41	15/36	25/36	14/36	5/22	11/22	7/22
+	13/20	17/20	20/20	2/18	13/18	13/18	0/14	8/14	12/14*
++	36/40	38/40	40/40	20/38	34/38	32/38**	10/25	20/25	21/25**

Samples were not available from all patients for each of the three study periods.

* $p < 0.01$, ** $p < 0.001$ when compared with patients without arthritis.

Table 3 Persistence of IgA class antibodies against *Yersinia enterocolitica* O:3 in the sera of 104 patients after acute *Y enterocolitica* O:3 infection; data according to isolation of *Y enterocolitica* O:3 from the stool at the time of the initial infection

Grade of arthritis	No of patients with IgA class antibodies against yersinia/No studied (%)					
	At 1-2 months		At 6-8 months		At 12-16 months	
	Stool+	Stool-	Stool+	Stool-	Stool+	Stool-
-	11/13 (85)	26/28 (93)	7/12 (58)	7/29 (24)	0/4 (0)	7/18 (39)
+ / ++	19/19 (100)	41/41 (100)	10/14 (71)	35/42*** (83)	9/10* (90)	24/29** (83)

* $p < 0.02$, ** $p < 0.01$, *** $p < 0.0001$ when compared with patients without arthritis.

and those with a diagnosis based solely on serological and clinical evidence to discover whether the persistence of IgA class antibodies in these cases was different (Table 3). At 12-16 months after onset of the infection a difference was observed between the arthritic and non-arthritic patients in the antibody persistence both in the culture negative and in the culture positive group. At six to eight months a significant difference existed between culture positive/arthritic positive and culture negative/arthritic negative groups ($p < 0.05$).

One hundred and five serum samples (taken from 39 patients) were measured also by ELISA using *Yersinia enterocolitica* O:3 LPS as antigen. The correlation coefficients compared with whole bacteria ELISA were 0.96 for IgM antibodies, 0.98 for IgA, and 0.81 for IgG antibodies; the results obtained (data not shown) confirm those obtained with the whole bacteria ELISA.

At one to two months after the onset of infection all 101 patients studied had antibodies of IgM, IgG, and/or IgA class against *Yersinia enterocolitica* O:3. After six to eight months 40.2, 78.2, and 64.1% of the patients had IgM, IgG, and IgA class antibodies, respectively. At 12-16 months the corresponding figures were 24.6, 63.9, and 65.6%. One should note that IgM class antibodies also persist in a quarter of the patients. During the follow up the antibody levels remained even or declined, and no signs of reinfection were observed.

Discussion

Few other reports on the occurrence of yersinia antibodies of IgM, IgG, and IgA classes in reactive arthritis after *Yersinia enterocolitica* O:3 infection have been published. Larsen *et al* have emphasised the prognostic value of IgA-anti-yersinia antibodies and agree with our conclusion about their diagnostic importance.¹² Likewise, high concentrations and long persistence of IgA class antibodies in 26 arthritic patients was observed by Gripenberg using ELISA with *Yersinia enterocolitica* O:3 LPS as antigen.⁵ In addition to the long persistence of IgA class yersinia antibodies their concentration has been found to be higher in arthritic patients.^{4, 13} Recently we have also shown that a considerable part of IgA antibodies in the sera of arthritic patients is in the secretory form.¹³

A statistically significant difference in the IgA response between arthritic and non-arthritic patients was not seen by Mattila *et al*.¹⁴ This is due to the fact that they applied end point titre calculation, indicating antibodies over a wide range of avidity; the difference is best observed in the high avidity antibodies.¹⁵ Mattila *et al* also, according to their own statement, studied patient groups which were not directly comparable.

The present results emphasise the importance of antibody determination in the diagnosis of yersinia infections. Isolation of the bacteria is often not

achieved, particularly in cases with postinfection complications. Our clinical impression is that gastrointestinal symptoms in patients developing reactive arthritis are often mild and may pass unnoticed, and these patients contact the doctor because of their arthritic complications. Obviously this stage is often too late for isolation of the bacteria, but strong antibody response especially at IgA class, is even then usually observed. The response may remain unnoticed, however, if only bacterial agglutination is used for antibody determination.¹ Finally, one may recall that our observations with ELISA on the magnitude and persistence of the IgA response are in line with those indicating high levels of IgA-anti-*Klebsiella* antibodies in ankylosing spondylitis¹⁶ and of IgA-anti-*Shigella* antibodies in *Shigella* triggered reactive arthritis.¹⁷

This study was supported by grants from the Sigrid Jusélius Foundation, the Finnish Medical Foundation, the Turku University Foundation, and the US Public Health Service (No 1 RO1 AM 33311, awarded by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases).

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