INFECTION AND IMMUNITY, Feb. 1995, p. 714–719 Vol. 63, No. 2
0019-9567/95/$04.00+0

Arthritogenicity of Genetically Manipulated *Yersinia enterocolitica* Serotype O8 for Lewis Rats

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Received 26 September 1994/Returned for modification 20 October 1994/Accepted 19 November 1994

Yersinia enterocolitica **strains of serotype O8 but not strains of other human pathogenic serotypes (e.g., O3 or O9) are able to induce a reactive arthritis-like disease in Lewis rats after intravenous inoculation (J. L. Hill and D. T. Yu, Infect. Immun. 55:721–726, 1987). To assess which bacterial components or pathogenic factors are crucial for arthritis induction, six genetically manipulated *Y. enterocolitica* O8 derivatives have been compared with the parental strain in Lewis rats. Neither differences in the length of the lipopolysaccharide side chain (smooth to semirough) of *Y. enterocolitica* O8 nor replacement of the virulence plasmid (pYVO8) of *Y. enterocolitica* O8 with that of the nonarthritogenic *Y. enterocolitica* O9 (pYVO9) had a significant influence on arthritogenic potential or virulence in rats. Transposon insertional inactivation of the plasmid gene *yadA* encoding the *Yersinia* adhesin and the collagen-binding protein or of the secretion of YopH resulted in decreased arthritogenicity (increase of the arthritogenic infectious dose) and pathogenicity (decreased persistence of the pathogen in spleens and livers of rats and increase of the 50% lethal dose for mice). However, mutants impaired in yersiniabactin production or uptake proved to be nonarthritogenic for rats, probably because of pronounced attenuation in virulence. From these results, we conclude that the arthritogenic potential of *Y. enterocolitica* serotype O8 is closely related to the virulence potential determined as the 50% lethal dose in mice and the ability to persist in lymphatic tissue of Lewis rats. A specific arthritogenic determinant of *Y. enterocolitica* could not be identified.**

Enteropathogenic bacteria such as *Salmonella*, *Shigella*, *Yersinia*, and *Campylobacter* spp. are invasive pathogens which cause acute inflammatory bowel disease. In some patients, mainly HLA-B27-positive ones, the intestinal infection may be complicated by sterile joint inflammation, i.e., so-called reactive arthritis (1, 2, 19, 29, 31, 33, 36, 38). The clinical course varies between acute self-limiting forms and chronic recurrent manifestations. The pathogenesis of this sequela is unclear.

Recently, *Yersinia* antigens (bacterial debris and whole microorganisms) have been detected in the synovial fluid and tissue of patients (6, 8, 22). However, cultivation of the microorganisms from this tissue was unsuccessful (8, 22, 34). This does not exclude the possibility that replicative yersiniae are present in synovial tissue during the early phase of arthritis induction. For deposition in the synovial tissue, the microorganism should be endowed with specific properties, e.g., it should (i) be transportable by synovial tissue-invading phagocytes, (ii) demonstrate synovial tissue tropism, or (iii) have the ability to multiply in synovial tissue. From this, one may conclude that specific features of the host as well as of the arthritis-triggering pathogen must coincide. To systematically study the pathogenesis of *Yersinia*-associated arthritis, an appropriate animal model for *Yersinia*-triggered arthritis is required. Two research groups succeeded in inducing aseptic arthritis in rats by experimental infection (intravenous challenge) with highly pathogenic (mouse-virulent) *Y. enterocolitica* serotype O8. It was shown that Lewis and SHR inbred rats were arthritis susceptible, whereas Fischer, DA, LDA, and Buffalo rats were arthritis resistant (17, 30). Moreover, Lewis rats developed a significantly higher serum antibody response against *Yersinia* antigen than Fisher rats did (4). The pathogen was able to persist for several months in the lymphatic tissue of Lewis and SHR rats, whereas Fischer rats were able to eliminate the bacteria within 4 weeks (4, 7, 17, 20). Thus, arthritis susceptibility is rat strain specific and correlates presumably with a strong antibody response and weak bactericidal capacity of the host. Surprisingly, the most frequently occurring human arthritogenic *Y. enterocolitica* serotype, O3, which has low virulence (50% lethal dose, 10^6 CFU) for BALB/c mice, was found to be nonarthritogenic for rats, whereas the mouse-virulent *Y. enterocolitica* serotype O8 (50% lethal dose, 7×10^2 to 7×10^2 CFU), which is rarely described as being arthritogenic in humans, proved to be arthritogenic in Lewis rats (17, 30). It should be emphasized that this does not imply that *Y. enterocolitica* serotype O3 is less pathogenic for humans than serotype O8 strains, because *Yersinia* susceptibility depends on the host (10). Recently, we have demonstrated that the phenotype of mouse virulence is closely associated with the ferric yersiniabactin uptake determinant *fyu* of the rat arthritogenic *Y. enterocolitica* serotype O8 (13, 24). This determinant is absent in the rat nonarthritogenic *Y. enterocolitica* of serotype O3 and *Yersinia pseudotuberculosis* of serotype 3.

In summary, a high antibody response, a low bactericidal capacity of the host, and appropriate virulence potential of the pathogen can be suggested to be prerequisites for arthritis development. In this study, we examined the arthritogenic potential of wild-type strains and of defined attenuated *Y. enterocolitica* O8 mutants with respect to putative arthritogenic factors such as (i) length of the O-polysaccharide chain of lipopolysaccharide (LPS; with or without repeated units), (ii) the *Yersinia* adhesin YadA (collagen-binding factor), (iii) the protein tyrosine phosphatase, YopH, and (iv) ferric yersiniabactin utilization.

Bacterial strains and their relevant characteristics are shown in Table 1. Strain WA harboring the typical virulence plasmid pYVO8 is the original *Y. enterocolitica* strain of serotype O8 which was used for arthritis induction in Lewis rats by Hill and

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TABLE 1. Bacterial strains and plasmids used

Strain	Plasmid	Genotype or relevant phenotype	LD_{50}[a]	Reference(s)
WA	pYVO8[b]	Smooth LPS	$\sim 10^2$	17
WA-314	pYVO8	Semirough LPS	7×10^2	16, 35
WA-C		Plasmidless derivative of WA-314	7×10^6	35
WA-C(pYVO9)	pYVO9[c]	Strain WA-C harboring the virulence plasmid of *Y. enterocolitica* O9	7×10^2	9, 35
WA-C(pYVO9::Tn*5*)	pYVO9::Tn*5*	Tn*5* insertional inactivation of *yadA*	7×10^5	26, 35
WA-C(pYVO8::Tn*7*)	pYVO8::Tn*7*	Tn*7* insertional mutant, inactivation of YopH transport	6×10^4	This study
WA-*fyuA*(pYVO8)	pYVO8	*fyuA* mutant of WA-C, pesticin resistant, yersiniabactin positive	$>5 \times 10^6$	24
WA-1(pYVO8)	pYVO8	Tn*5* insertional mutant, pesticin sensitive ($FyuA^+$), yersiniabactin negative	7×10^6	13, 35

[a] Fifty percent lethal dose for BALB/c mice by intravenous administration.
[b] pYVO8, virulence plasmid of *Y. enterocolitica* serotype O8.
[c] pYVO9, virulence plasmid of *Y. enterocolitica* serotype O9.

Yu (17). *Y. enterocolitica* WA-314 (serotype O8; clinical isolate) was obtained from Canada (kind gift of C. Pai, Calgary, Canada). This strain agglutinated spontaneously in 1 M NaCl solution but still reacted with a serotype O8-specific mouse monoclonal antibody (Progen, Heidelberg, Germany [25]). Thus, WA-314 is likely a semirough strain. WA-314 was cured of the virulence plasmid, resulting in strain WA-C. A spontaneous pesticin-resistant mutant WA-*fyuA* (carrying an amber stop codon by substitution of cytosine [base pair 763] for thymine within the gene *fyuA* encoding for the pesticin receptor) was obtained by pesticin treatment of WA-C (24). Because of the impaired uptake of the endogenously produced siderophore yersiniabactin, WA-*fyuA*(pYVO:8) has reduced mouse virulence (24). The yersiniabactin-deficient mutant WA-1 could be isolated by Tn*5* transposon mutagenesis. The pYVO8-harboring mutant WA-1(pYVO8) also has reduced mouse virulence (13). For WA-C(pYVO9::Tn*5*), the virulence plasmid pYVO9 (derived from *Y. enterocolitica* serotype O9) was tagged with Tn*5* transposon to inactivate expression of *yadA* (*Yersinia* adhesin). The Tn*5* insertion was localized within the *Pst*I-*Pst*I fragment (this study) of the *yadA* gene, whose related sequence has been described recently (27). For WA-C (pYVO9::Tn*7*), the virulence plasmid (pYVO8) derived from *Y. enterocolitica* O8 (strain WA-314) was tagged with Tn*7* transposon to inhibit secretion of YopH (*Yersinia* outer protein H, the protein tyrosine phosphatase; the Tn*7* insertion was localized within the intergenic region between the *yopH* and *yerA* genes [this study]).

Plasmid transfer was performed by mobilization via cointegrate formation with the vector pRK290 as described previously (16). The obtained exconjugants were passaged through mice and reisolated from the spleen 2 days after infection. By this procedure, cointegrate resolution occurred, resulting in tetracycline-sensitive microorganisms harboring the transposon-tagged virulence plasmid (16).

All strains were checked for the presence of virulence plasmids and for production of yersiniabactin, pesticin or yersiniabactin receptor (FyuA), YadA, Yops, and, in particular, YopH as described previously (11–13, 35).

LPS was extracted from strains WA and WA-314 grown at 25°C on Mueller-Hinton agar by the phenol-water method and characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent silver staining as described previously (18, 37).

Outer membrane proteins (Omps) and secreted outer proteins (Yops) of *Yersinia* spp. were prepared and characterized by SDS-PAGE (11–13).

Yersinia-specific rat serum immunoglobulin G antibodies against Yops were detected by an enzyme-linked immunosorbent assay (ELISA) (4).

Specific-pathogen-free male Lewis rats, weighing between 280 and 320 g, and female BALB/c mice were purchased from Charles River Wiga, Sulzfeld, Germany. Animals were infected by an intravenous application with given infectious doses (CFU) as described previously (4, 35). All experiments were repeated at least two times and revealed reproducible results. All strains reisolated from rat tissue were rechecked for their expression of their characteristic phenotypes (YadA, YopH, FyuA, yersiniabactin, and semirough LPS in the case of strain WA-314).

To estimate the development of clinical arthritis, the circumference of each hind paw was measured (in millimeters) daily with a measuring tape at two fixed points. The obtained four values per rat were summarized, resulting in the fourfold circumference (C). The clinical arthritis score (AS) was obtained individually for every rat from ΔC, which is the difference between the maximal C (C_{max}) during the experiment and the original C (C_0) before the experiment ($\Delta C = C_{max} - C_0$). In normal rats, the individual value of C_0 fluctuated less than 2 mm during daily measurements (mean ΔC of 10 daily measurements of 30 normal rats plus 3 standard deviations, ≤ 4 mm). Therefore, the AS was graded as follows: 0 for ΔC of 0 to 4 mm (arthritis negative); I for ΔC of 5 to 10 mm; II for ΔC of 11 to 15 mm; III for ΔC of 16 to 20 mm; IV for ΔC of >20 mm. An AS of $\geq$I was considered arthritis positive. This scoring of clinical arthritis was checked by histological examination of hematoxylin-and-eosin-stained cryosections obtained from amputated ankles of the hind paws (3).

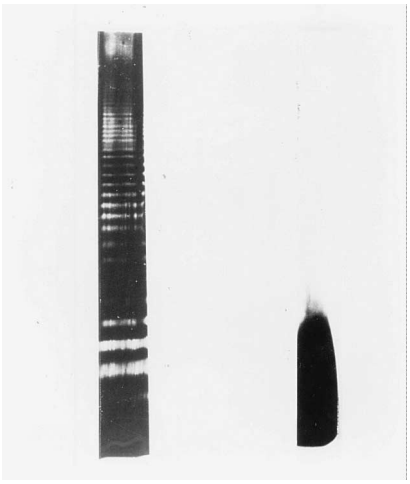

FIG. 1. LPS profile of *Y. enterocolitica* serotype O8 strain WA (left) and WA-314 (right) with a silver-stained SDS–12% polyacrylamide gel.

TABLE 2. Arthritis incidence and detection of *Yersinia* spp. after *Yersinia* inoculation

Strain	Infectious dose (CFU)	No. of rats arthritis positive/total no.[a]	No. of rats with AS of[a]:					No. of dead rats/ total no. (days)[b]	Yersinia culture		
			0	I	II	III	IV		No. of rats positive/total no.[c]		Day p.i.[d]
									Spleen	Liver	
WA	1×10^6	0/4	4					2/6 (14, 16)	2/4	2/4	20
	5×10^6	4/4			1	2	1	0/4	4/4	4/4	16
	7×10^6	7/12	5	5	1	1		0/12	4/4	0/4	20
	1×10^7	8/19	11	5	1	1	1	1/20 (14)	0/7	0/7	20
	2×10^7	4/4		2	2			1/5 (7)	2/4	1/4	17
	4×10^7	10/17	7	2	3	3	2	0/17	3/4	3/4	20
	6×10^7	4/5	1			1	3	0/5	2/2	2/2	21
	3×10^8	2/3	1		1		1	2/5 (3, 3)	2/3	2/3	20
	1×10^9	0/2	2					3/5 (12, 12, 12)	ND	ND	
WA-314	4×10^6	1/4	3		1			0/4	1/4	0/4	150
	4×10^7	3/7	4	2			1	0/7	2/7	0/7	25
	6×10^7	3/5	2	1	1	1		0/5	3/5	4/5	20
	5×10^8	0/5	5					0/5	0/5	0/5	80
WA-C(pYVO9)	4×10^6	3/4	1	2	1			1/5 (15)	2/4	0/4	20
	1×10^7	1/4	3				1	0/4	4/4	1/4	20
WA-C(pYVO9::Tn*5*)	6×10^6	1/5	4	1				0/5	0/5	0/5	22
	7×10^7	1/8	7	1				2/10 (6, 9)	1/5	1/5	20
	3×10^8	1/5	4	1				0/5	2/5	2/5	17
	1×10^9	1/1				1		4/5 (1, 2, 6, 7)	1/1	0/1	20
	3×10^9	2/2		1		1		3/5 (1, 1, 1)	0/2	0/2	20
	1×10^{10}							4/4 (1, 1, 1, 1)			
WA-C(pYVO8::Tn*7*)	1×10^8	4/7	3	4				1/8 (6)	4/7	3/7	20
	4×10^8	1/5	4	1				0/5	5/5	4/5	17
	4×10^9	0/1	1					4/5 (2, 2, 2, 2)	1/1	0/1	17
WA-1(pYVO8)	1×10^8	0/4	4					1/5 (2)	1/4	0/4	20
	3×10^8	0/7	7					3/10 (3, 3, 3)	5/7	5/7	20
	4×10^9							5/5 (2, 2, 2, 2, 2)			
WA-*fyuA*(pYVO8)	2×10^7	0/5	5					0/5	5/5	5/5	18
	3×10^7	0/5	5					0/5	0/5	0/5	20
	3×10^8	0/1	1					4/5 (3, 3, 3, 3)	0/1	0/1	19
	2×10^9	0/1	1					4/5 (2, 2, 2, 2)	1/1	0/1	19
WA-C	2×10^8	0/5	5					0/5	1/5	0/5	20

[a] Number of surviving rats with the given AS.
[b] Number of rats dead before the end of the experiment/number of rats studied. days, days of death of each rat after infection.
[c] Number of rats with positive *Yersinia* culture/number of rats studied.
[d] p.i., postinfection.

For this reason, we examined randomly sampled joints with ASs of 0 to IV for histopathological changes (in five samples of uninfected rats and in each WA-infected rat with an AS). If the histopathological parameters such as (i) proliferation of synovial lining cells (SLC) and/or (ii) mononuclear infiltration of the synovial tissue and/or cellular exudate within the periarticular cavity were positive for one tarsal joint, the corresponding rat was histologically classified as arthritis positive. It was found that rats with a clinical AS of ≥I were histologically arthritis positive. Uninfected rats never showed histopathological changes of tarsal joints. In contrast, 10 to 20% of infected rats with a clinical AS of 0 proved to be histologically arthritis positive. Thus, the AS of ≥I is a reliable value for classifying rats as clinically arthritis positive. According to this scoring, strains WA and WA-314 (clinical isolates) and the mutant strains derived from WA-314 were compared for their arthritis-causing potential (Table 1).

As shown in Fig. 1 (left lane), the SDS-PAGE profile of LPS from strain WA corresponds to the ladder pattern typical for the presence of long-chain LPS with various repeats. In contrast, strain WA-314 produced LPS lacking long-chain LPS, which is typical for semirough strains (Fig. 1, right lane). Both strains persisted for weeks in the spleen, liver, and lymph nodes and caused arthritis in Lewis rats after intravenous challenge with 4×10^6 to 4×10^7 CFU in approximately 25 to 100% of the tested animals (Table 2). Obviously, the chain length of serotype O8 LPS does not play a crucial role in arthritis induction.

It has been demonstrated that induction of *Yersinia*-induced arthritis in Lewis rats is restricted to *Y. enterocolitica* of serotype O8 (17, 30). We wondered whether this feature was associated with DNA sequence differences of the closely related virulence plasmids (75 to 90% homology) and with antigenic differences of YadA (formerly P1) of the various enteropathogenic serotypes of *Y. enterocolitica* (15, 28). Therefore, the virulence plasmid pYVO9 of the nonarthritogenic *Y. enteroco-*

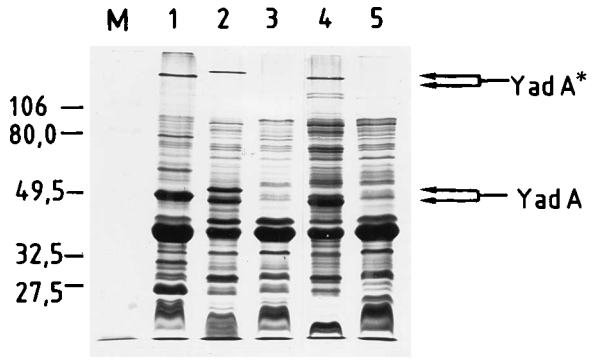

FIG. 2. Outer membrane protein profile of *Y. enterocolitica* WA-314 (lane 1), WA-C(pYVO9) (lane 2), WA-C(pYVO9::Tn*5*) (lane 3), WA-C(pYVO8::Tn*7*) (lane 4), and WA-C (lane 5) with a Coomassie-stained SDS–11% polyacrylamide gel. M, prestained molecular marker proteins in kilodaltons.

litica serotype O9 strain was mobilized into *Y. enterocolitica* WA-C (plasmid-cured derivative of WA-314). As shown in Fig. 2 and 3, this transconjugant expressed the typical Yop pattern and the YadA of serotype O9 strain. The 200-kDa YadA aggregate (Fig. 2, lane 2) and the 47-kDa YopM (Fig. 3, lane 2) are typical for serotype O9 strains, whereas the 180-kDa YadA aggregate (Fig. 2, lane 1) and the 57-kDa YopM (Fig. 3, lane 1) are characteristic for strain WA-314 serotype O8 (14, 35). As shown in Table 2, the serotype O8-O9 hybrid strain WA-C(pYVO9) also proved to be arthritogenic for Lewis rats. Thus, differences in these serotype-specific plasmid determinants are unlikely to play an essential role in arthritogenicity.

The plasmid-encoded proteins YadA and YopH are required for full virulence expression of *Y. enterocolitica*. The outer membrane protein YadA mediates binding to extracellular matrix proteins, and the secreted basic cationic protein YopH has protein tyrosine phosphatase activity. Because of these features, it is reasonable that both or one of these proteins could also be directly involved in arthritis induction. Therefore, transposon insertional mutants have been constructed. As shown in Fig. 2 (lane 3), strain WA-C (pYVO9::Tn*5*) (Tn*5* insertion within the *yadA* gene) was not able to express YadA but showed normal expression of the secreted Yop pattern (Fig. 3, lane 3). In contrast, strain WA-C(pYVO8::Tn*7*) (Tn*7* insertion within the upstream region adjacent to the *yopH* gene resulted in a YopH transport mutant) expressed the YadA outer membrane protein (Fig. 2, lane 4) but showed altered expression of the Yop pattern (Fig. 3, lane 4) (the faint band at 46 kDa is likely an isoform of the 57-kDa YopM, as has been demonstrated by anti-YopM immunoblotting; data not shown). Both mutant strains were attenuated in mouse virulence (Table 1) but were still able to cause arthritis (Table 2). However, ASs never reached values of >III. Interestingly, three of four hind paws of four rats with ASs of 0 showed histopathological changes similar to those of joints of rats with ASs of I to II. These data indicated that YadA and YopH were more or less dispensable factors for synovitis induction and suggested a link between arthritogenicity and virulence.

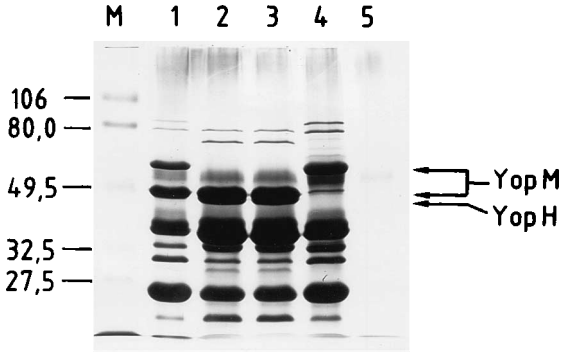

FIG. 3. Yop profile of *Y. enterocolitica* WA-314 (lane 1), WA-C(pYVO9) (lane 2), WA-C(pYVO9::Tn*5*) (lane 3), WA-C(pYVO8::Tn*7*) (lane 4), and WA-C (lane 5) with a Coomassie-stained SDS–11% polyacrylamide gel. M, prestained molecular marker proteins in kilodaltons.

We extended our study by testing attenuated strains with chromosomal mutations. Recently, we demonstrated that *Y. enterocolitica* serotype O8 produces the iron-chelating compound yersiniabactin, which can support iron utilization via the yersiniabactin receptor FyuA (13). This chromosomal iron uptake determinant is required for mouse virulence. The yersiniabactin producer mutant [WA-1(pYVO8)] and the yersiniabactin receptor mutant [WA-*fyuA*(pYVO8)] turned out to be attenuated in virulence for mice (Table 1) and failed to induce arthritis in rats (AS, 0; by chi-square test, $P < 0.05$; this is statistically significant for sublethal arthritogenic doses with respect to the parental strain WA-314) (Table 2). Surprisingly, two of four hind paws of four rats showed histologically mononuclear infiltration of the synovial tissue without severe proliferation of SLC or synovial hyperplasia resembling mild synovitis. Similar results were obtained with the plasmidless strain WA-C (Table 2). Moreover, we have shown that serotype O8 strains mutated in *fyu* were unable to persist in the spleen, liver, and lymph nodes of infected mice (references 24 and 35 and unpublished results). Application of sublethal doses (10^7 to 10^8 CFU) led to the colonization of the spleen and liver; however, abscesses could not be observed in lymph nodes, in contrast to the results with arthritogenic strains. Application of lethal doses resulted in septic shock-like lethality within 2 to 3 days after infection (Table 2). Thus, these results further support the conclusion that the ability of yersiniae to survive and persist in the spleen, liver, and lymph nodes of rats appears to be closely linked to its arthritis-inducing potential.

In addition to analyzing arthritis induction, we investigated the serum antibody response of infected rats against Yops. We expected (i) that the strength of the antibody response might reflect the load, the colonization, and the persistence of yersiniae in lymphatic tissue and (ii) that the antibody titer or the strength of the antibody reactivity against Yops might correlate with the incidence of arthritis (4). In Fig. 4, data from the Yop ELISA are shown. The sera used were drawn between 17 and 21 days after infection. As we have previously shown, this time point coincided with the peak of serum immunoglobulin G response (4). It is striking that Lewis rats challenged with nonattenuated strains developed higher antibody titers against Yops than the two weakly attenuated mutant strains lacking YadA or YopH. Rats challenged with the nonarthritogenic iron uptake mutants responded weakly at best against Yop antigen (the majority of these sera were even negative by immunoblotting; results not shown).

In summary, these results allow some speculation with respect to the pathomechanism of *Yersinia*-triggered arthritis in Lewis rats. (i) It is unlikely that the putative arthritogenic polypeptides YadA and YopH are directly involved in arthritis induction, e.g., via molecular mimicry, as has been demonstrated for the HSP60-specific T-cell-mediated adjuvant arthritis in rats or as deposited antigen in the joints (reference 21 and reviewed in reference 32). (ii) The lack of arthritogenicity of *Y. enterocolitica* of serotypes O3 and O9 and *Y. pseudotu-*

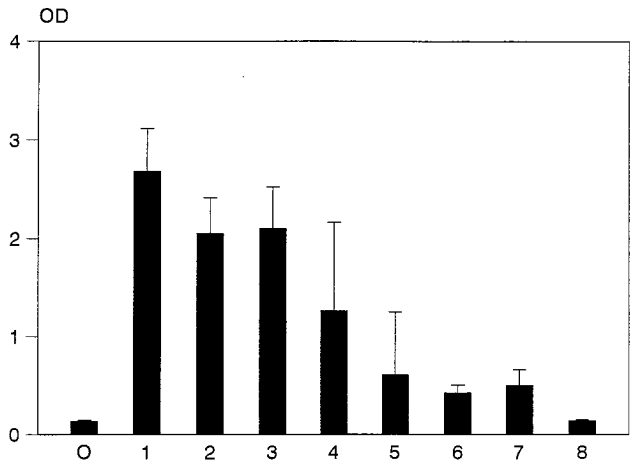

FIG. 4. Yop ELISA analysis of the serum immunoglobulin G antibody responses of normal and *Yersinia*-infected rats to Yops. Lewis rats were inoculated intravenously with *Y. enterocolitica* mutants. Serum samples were drawn on days 17 to 20 after infection and diluted 1:1,000. Columns represent mean optical density (OD) values of given representative experimental groups. The vertical bars represent standard deviations of the means (see also Table 2). Lanes: 0, normal rat serum (number of rats [n] = 10); 1, infecting strain WA (infectious dose [ID] in CFU = 4×10^7; n = 7); 2, WA-314 (ID = 4×10^7; n = 7); 3, WA-C(pYVO9) (ID = 1×10^7; n = 4); 4, WA-C(pYVO9::Tn*5*) (ID = 3×10^8; n = 5); 5, WA-C(pYVO8::Tn*7*) (ID = 1×10^8; n = 7); 6, WA-1(pYVO8) (ID = 3×10^8; n = 7); 7, WA-*fyuA*(pYVO8) (ID = 3×10^7; n = 5); 8, WA-C (ID = 2×10^8; n = 5).

berculosis 3 for Lewis rats is probably linked to the lack of the high-affinity iron uptake determinant *fyu*, which is required for yersiniae to multiply rapidly in rat tissue. (iii) Arthritogenicity of yersiniae is suggested to be closely associated with a strong specific antibody response and probably with an appropriate bacterial antigen load represented by bacterial abscesses in the spleen, liver, and lymph nodes. Therefore, it is reasonable to suggest that *Yersinia*-induced arthritis in Lewis rats is an inflammatory joint disease triggered by *Yersinia* antigen or immune complexes deposited in the synovial tissue and thus resembles human reactive arthritis caused by *Salmonella*, *Shigella*, or *Yersinia* spp. (5, 6, 8, 23).

The rat model offers the chance to analyze the mechanism of translocation of antigen–immune complex to joint tissue and to answer the question of whether live *Yersinia* bacilli disseminate during the early phase of infection into joint tissue and are able to multiply for a short time, resulting in an inflammatory synovitis. In line with this hypothesis is our finding that other so-called nonarthritogenic mutants, e.g., the iron uptake mutants or the plasmid-cured strain, were able to induce weak histopathological changes in tarsal joints with ASs of 0. Preliminary histopathological and microbiological data demonstrated an inflammatory reaction of the synovial membrane and culture-positive tarsal rat joints in the first week of infection with strains WA and WA-314, suggesting an early synovial invasion of the pathogen (unpublished data). Studies focusing on this subject are under investigation in our laboratory.

We thank Elke Baumeister for expert technical assistance. We are grateful for the competent support of Veit Krenn, Institute of Pathology, University of Würzburg, Würzburg, Germany.

This work was supported by a grant from the Bundesministerium für Forschung und Technik (BMFT 01-8607-44).

REFERENCES

1. **Aho, K., P. Ahvonen, A. Lassus, K. Sievers, and A. Tiilikainen.** 1974. HL-A 27 in reactive arthritis. A study of Yersinia arthritis and Reiter's disease. Arthritis Rheum. **17:**521–526.
2. **Ahvonen, P., K. Sievers, and K. Aho.** 1969. Arthritis associated with Yersinia enterocolitica infection. Acta Rheumatol. Scand. **15:**232–253.
3. **Böck, P.** 1989. Mikroskopische Technik, 17th ed. Urban und Schwarzenberg, Munich.
4. **Gaede, K., D. Mack, and J. Heesemann.** 1992. Experimental Yersinia enterocolitica infection in rats: analysis of the immune response to plasmid-encoded antigens of arthritis-susceptible Lewis rats and arthritis-resistant Fischer rats. Med. Microbiol. Immunol. **181:**165–172.
5. **Granfors, K., S. Jalkanen, A. A. Lindberg, O. Maki Ikola, R. von Essen, R. Lahesmaa Rantala, H. Isomaki, R. Saario, W. J. Arnold, and A. Toivanen.** 1990. Salmonella lipopolysaccharide in synovial cells from patients with reactive arthritis. Lancet **335:**685–688.
6. **Granfors, K., S. Jalkanen, R. von Essen, R. Lahesmaa Rantala, O. Isomaki, K. Pekkola Heino, R. Merilahti Palo, R. Saario, H. Isomaki, and A. Toivanen.** 1989. Yersinia antigens in synovial-fluid cells from patients with reactive arthritis. N. Engl. J. Med. **320:**216–221.
7. **Gripenberg, C., and P. Toivanen.** 1993. Yersinia associated arthritis in SHR rats: effect the microbial status of the host. Ann. Rheum. **52:**223–228.
8. **Hammer, M., H. Zeidler, S. Klimsa, and J. Heesemann.** 1990. Yersinia enterocolitica in the synovial membrane of patients with Yersinia-induced arthritis. Arthritis Rheum. **33:**1795–1800.
9. **Heesemann, J., B. Algermissen, and R. Laufs.** 1984. Genetically manipulated virulence of *Yersinia enterocolitica*. Infect. Immun. **46:**105–110.
10. **Heesemann, J., K. Gaede, and I. B. Autenrieth.** 1993. Experimental Yersinia enterocolitica infection in rodents: a model for human yersiniosis. APMIS **101:**417–429.
11. **Heesemann, J., U. Gross, N. Schmidt, and R. Laufs.** 1986. Immunochemical analysis of plasmid-encoded proteins released by enteropathogenic *Yersinia* sp. grown in calcium-deficient media. Infect. Immun. **54:**561–567.
12. **Heesemann, J., and L. Grueter.** 1987. Genetic evidence that outer membrane protein YOP1 of Yersinia enterocolitica mediates adherence and phagocytosis resistance to human epithelial cells. FEMS Microbiol. Lett. **40:**229–233.
13. **Heesemann, J., K. Hantke, T. Vocke, E. Saken, A. Rakin, I. Stojiljkovic, and R. Berner.** 1993. Virulence of Yersinia enterocolitica is closely associated with siderophore production, expression of an iron-repressible outer membrane protein of 65000 Da and pesticin sensitivity. Mol. Microbiol. **8:**397–408.
14. **Heesemann, J., H. Kalthoff, and F. Koch.** 1986. Monoclonal antibodies directed against plasmid-encoded released proteins of enteropathogenic Yersinia. FEMS Microbiol. Lett. **36:**15–19.
15. **Heesemann, J., C. Keller, R. Morawa, N. Schmidt, H. J. Siemens, and R. Laufs.** 1983. Plasmids of human strains of Yersinia enterocolitica: molecular relatedness and possible importance for pathogenesis. J. Infect. Dis. **147:**107–115.
16. **Heesemann, J., and R. Laufs.** 1983. Construction of a mobilizable *Yersinia enterocolitica* virulence plasmid. J. Bacteriol. **155:**761–767.
17. **Hill, J. L., and D. T. Yu.** 1987. Development of an experimental animal model for reactive arthritis induced by *Yersinia enterocolitica* infection. Infect. Immun. **55:**721–726.
18. **Karch, H., H. Leying, and W. Opferkuch.** 1984. Analysis of electrophoretically heterogeneous lipopolysaccharides of Escherichia coli by immunoblotting. FEMS Microbiol. Lett. **22:**193–196.
19. **Lauhio, A., J. Lahdevirta, R. Janes, S. Kontiainen, and H. Repo.** 1988. Reactive arthritis associated with Shigella sonnei infection. Arthritis Rheum. **31:**1190–1193.
20. **Merilahti Palo, R., C. Gripenberg Lerche, K. O. Soderstrom, and P. Toivanen.** 1992. Long term follow up of SHR rats with experimental yersinia associated arthritis. Ann. Rheum. Dis. **51:**91–96.
21. **Mertz, A. K., S. R. Batsford, E. Curschellas, M. J. Kist, and K. B. Gondolf.** 1991. Cationic Yersinia antigen-induced chronic allergic arthritis in rats. A model for reactive arthritis in humans. J. Clin. Invest. **88:**632–642.
22. **Nikkari, S., R. Merilahti-Palo, R. Saario, K. Söderström, K. Granfors, M. Skurnik, and P. Toivanen.** 1992. Yersinia-triggered reactive arthritis. Arthritis Rheum. **35:**682–687.
23. **Probst, P., E. Hermann, and B. Fleischer.** 1994. Role of bacteria-specific T cells in the immunopathogenesis of reactive arthritis. Trends Microbiol. **2:**329–332.
24. **Rakin, A., E. Saken, D. Harmsen, and J. Heesemann.** 1994. The pesticin receptor of Yersinia enterocolitica: a novel virulence factor with dual function. Mol. Microbiol. **13:**253–263.
25. **Schmidt, M., and K. K. Sethi.** 1987. Production and characterization of monoclonal antibodies specific for pathogenic serogroups O3, O8, and O9 of Yersinia enterocolitica. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. Orig. A **265:**113–123.
26. **Schulze-Koops, H., H. Burkhardt, J. Heesemann, T. Kirsch, B. Swoboda, C. Bull, S. Goodman, and F. Emmrich.** 1993. Outer membrane protein YadA of enteropathogenic yersiniae mediates specific binding to cellular but not plasma fibronectin. Infect. Immun. **61:**2513–2519.
27. **Skurnik, M., and H. Wolf Watz.** 1989. Analysis of the yopA gene encoding the Yop1 virulence determinants of Yersinia spp. Mol. Microbiol. **3:**517–529.
28. **Sory, M. P., J. Tollenaere, C. Laszlo, T. Biot, G. R. Cornelis, and G. Wauters.**

1990. Detection of pYV$^+$ *Yersinia enterocolitica* isolates by P1 slide agglutination. J. Clin. Microbiol. **28:**2403–2408.
29. **Toivanen, A., K. Granfors, R. Lahesmaa Rantala, R. Leino, T. Stahlberg, and R. Vuento.** 1985. Pathogenesis of Yersinia-triggered reactive arthritis: immunological, microbiological and clinical aspects. Immunol. Rev. **86:**47–70.
30. **Toivanen, A., R. Merilahti Palo, C. Gripenberg, R. Lahesmaa Rantala, K. O. Soderstrom, and U. M. Jaakkola.** 1986. Yersinia-associated arthritis in the rat: experimental model for human reactive arthritis? Acta Pathol. Microbiol. Immunol. Scand. Sect. C **94:**261–269.
31. **Urman, J. D., R. B. Zurier, and N. F. Rothfield.** 1977. Reiter's syndrome associated with Campylobacter fetus infection. Ann. Intern. Med. **86:**444–445.
32. **van Eden, W., E. J. Hogervorst, E. J. Hensen, R. Van der Zee, J. D. Van Embden, and I. R. Cohen.** 1989. A cartilage-mimicking T-cell epitope on a 65K mycobacterial heat-shock protein: adjuvant arthritis as a model for human rheumatoid arthritis. Curr. Top. Microbiol. Immunol. **145:**27–43.
33. **Vartiainen, N., and L. Hurri.** 1964. Arthritis due to Salmonella typhimurium: report of 12 cases of migratory arthritis in association with Salmonella typhimurium infection. Acta Med. Scand. **175:**771–776.
34. **Viitanen, A. M., T. P. Arstila, R. Lahesmaa, K. Granfors, M. Skurnik, and P. Toivanen.** 1991. Application of the polymerase chain reaction and immunofluorescence techniques to the detection of bacteria in Yersinia-triggered reactive arthritis. Arthritis Rheum. **34:**89–96.
35. **Vogel, U., I. B. Autenrieth, R. Berner, and J. Heesemann.** 1993. Role of plasmid-encoded antigens of Yersinia enterocolitica in humoral immunity against secondary Y. enterocolitica infection in mice. Microb. Pathog. **15:** 23–36.
36. **Vuento, R., K. Granfors, R. Leino, H. Tenkku, and A. Toivanen.** 1984. Immunological features in reactive arthritis after Salmonella infection. Scand. J. Rheumatol. Suppl. **52:**54–57.
37. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides. Extraction with phenol/water and further applications of the procedure, p. 83–91. *In* R. J. Whister (ed.), Methods in carbohydrate chemistry 5. Academic Press Ltd., London.
38. **Yu, D. T., M. Ogasawara, J. L. Hill, and D. H. Kono.** 1985. Study of Reiter's syndrome, with special emphasis on Yersinia enterocolitica. Immunol. Rev. **86:**27–45.