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Acute Mesenteric Lymphadenitis Due to *Yersinia pseudotuberculosis* Lacking a Virulence Plasmid

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A serotype 4a strain of *Yersinia pseudotuberculosis* lacking the virulence plasmid pYV (pYV$^-$ strain) was isolated from the mesenteric lymph nodes but not from the stool or the appendix of a 10-year-old girl with a diagnosis of acute mesenteric lymphadenitis. Microscopically, reticulocytic abscess and lymphadenitis were present in the enlarged mesenteric lymph nodes. Antibody against the isolate was detected in the serum. The isolate was negative for the presence of plasmid pYV and plasmid pYV-mediated properties, including autoagglutination and calcium dependency, but was positive for chromosome-mediated properties, including invasion into HeLa cells and tissues of mice and the Serény test. Mice were orally infected with this pYV$^-$ strain, and rapid elimination from the intestine occurred 14 days later. Hence, the potential to inhibit the phagocytosis encoded by plasmid pYV was lacking. As the pYV$^-$ strain was recovered from the mesenteric lymph nodes and the spleen, the invasiveness was encoded by chromosomal genes. The count of the pYV$^-$ strain in the mesenteric lymph nodes increased to $10^{4.6}$ cells per g within 4 days. These findings suggest that pYV$^-$ *Y. pseudotuberculosis* was the causative agent of acute mesenteric lymphadenitis in the absence of gastroenteritis.

Since the first report of *Yersinia pseudotuberculosis* infection in humans by Saisawa (22) in 1909, an increasing number of reports on enteric infection with *Y. pseudotuberculosis* has been published. In patients with symptoms suggestive of subacute appendicitis, the relatively normal appearance of the appendix and the unusual appearance of the mesenteric lymph nodes at laparotomy prompted removal and investigation of the nodes. Since the isolation of *Y. pseudotuberculosis* from enlarged mesenteric lymph nodes by Knapp and Masshoff (14) in 1984, numerous cases of mesenteric lymphadenitis due to *Y. pseudotuberculosis* have been reported (5, 10). However, in some cases, *Y. pseudotuberculosis* was isolated from the excised mesenteric lymph nodes but not from the feces and excised appendix (5, 10), although *Y. pseudotuberculosis* was readily isolated from the feces in the presence of gastroenteritis. These findings raised the question of a possible difference in pathogenicity between strains of *Y. pseudotuberculosis* causing mesenteric lymphadenitis and gastroenteritis.

Recent studies (2–4, 12) showed that the virulence of *Y. pseudotuberculosis* depends on the pYV plasmid, which is responsible for inhibiting phagocytosis by mouse peritoneal macrophages (21), and chromosomal genes, which are responsible for invasion into epithelial cells in vitro and facilitation of the translocation of bacteria across the intestinal epithelium (18). Therefore, *Y. pseudotuberculosis* is similar to *Y. enterocolitica* and *Y. pestis*. The loss of plasmid pYV always correlates with the loss of pathogenicity (4). Simonet et al. (24, 25), however, reported that chromosomal genes also prompt the in vivo replication of *Y. pseudotuberculosis*, although the loss of plasmid pYV is associated with a significant decrease in the level of virulence. We report here a case of acute mesenteric lymphadenitis due to *Y. pseudotuberculosis* serotype 4a lacking plasmid pYV (pYV$^-$).

A 10-year-old Japanese girl was found to be infected with *Y. pseudotuberculosis* serotype 4a on 2 April 1990. None of the other members of her family became ill. On 3 April she was admitted to Shimane Central Hospital, Izumo, Japan, with severe pain in her right abdomen, a fever of 37.4°C, and pharyngitis. At laparotomy on 4 April, the appendix and bowel were normal but there were enlarged lymph nodes in the mesentery of the ileocecal region. The appendix and one of the lymph nodes were removed for biopsy, and the abdomen was closed. The feces were sampled at the same time. The patient recovered on conservative treatment and was discharged on 13 April. Laboratory data on 3 April included the following: erythrocyte count, 465 × 10^4/mm^3; hemoglobin, 15.5 g/dl; hematocrit, 39.0%; leukocyte count, 9,700/mm^3; C-reactive protein, 4.0 mg/dl. Macroscopically, the node was large, round, dark, and hemorrhagic. The sections of tissue samples of the excised mesenteric lymph nodes and appendix were stained with hematoxylin and eosin by the established method. Microscopically (Fig. 1), the affected node showed histocytic proliferation in the subcapsular and medullary sinus regions and a mild follicular hyperplasia. There were focal, paracortical ill-defined granulomas composed of epitheloid cells. Collections of neutrophils were present in the centers of the granulomas. Lack of multinucleated giant cells and central caseous necrosis was characteristic.

Y. pseudotuberculosis serotype 4a (melibiose fermentation) was isolated directly from the mesenteric lymph nodes at 25°C by aerobic incubation for 48 h on a plate of cefsulodin-irgasan-novobiocin agar (Difco Laboratories) but not from the appendix and feces after cold enrichment with phosphate-buffered saline at 4°C for 3 weeks. Other enteric bacterial pathogens, however, were not isolated from these specimens, despite the use of a wide range of bacterial examinations as described previously (6). The agglutinin titers of sera against the isolate were 1:20, 1:160, and 1:160 on 5, 11, and 19 April, respectively, determined by using our method (6). The isolate (Pa12983) identified as *Y. pseudotu-*

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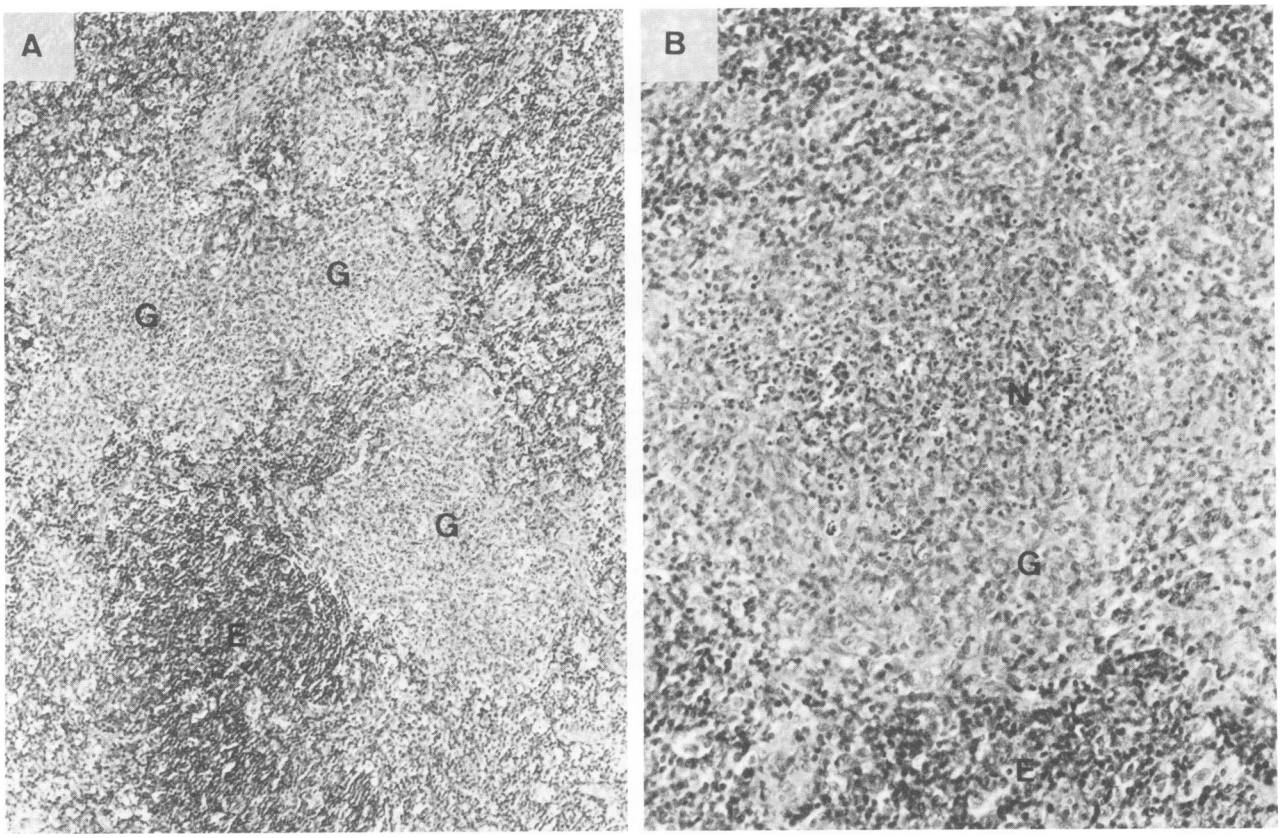

FIG. 1. Abscess-forming reticulocytic lymphadenitis of mesenteric lymph nodes in a 10-year-old girl. A section of a mesenteric lymph node showing an area of granulomas (G) surrounding an epitheloid zone (E) is shown. Collections of neutrophils (N) are present in the centers of the granulomas. Hematoxylin and eosin stain. Magnification: A, ×200; B, ×400.

berculosis serotype 4a showed a negative reaction for several plasmid pYV-mediated properties (presence of a virulence plasmid [13], autoagglutination [15], and calcium dependency at 37°C [11]) and a positive reaction for chromosome-mediated properties (invasion into HeLa cells [28] and the Serény test [23]). The presence of a virulence plasmid was determined by S. Kaneko (Tokyo Metropolitan Research Laboratory of Public Health, Tokyo, Japan). All 50 strains isolated from our patient showed a negative reaction for autoagglutination. These histopathological, bacteriological, and serological observations suggested that this strain of pYV$^-$ *Y. pseudotuberculosis* serotype 4a was the causative agent of acute mesenteric lymphadenitis in the absence of gastroenteritis. In most cases of mesenteric lymphadenitis reported in Europe and Australia since 1954 (5, 10, 14), enlarged lymph nodes were present in the ileocecal region, the appendix and bowel were normal, and the organisms were isolated only from excised mesenteric lymph nodes and not from other intestinal tissues, as was the case in our patient. However, all 33 *Y. pseudotuberculosis* isolates from stool specimens of patients with gastroenteritis in our laboratory (9) harbored plasmid pYV (data not shown). These findings strongly suggest that the cases of mesenteric lymphadenitis reported in Europe and Australia might be associated with pYV$^-$ *Y. pseudotuberculosis*, although references related to the presence of plasmid pYV in the isolates were not provided.

To test the abovementioned hypothesis, mice were then infected with three strains of *Y. pseudotuberculosis* serotype 4a, i.e., Pa12983 (pYV$^-$), 149 (pYV$^+$), and 149C (pYV$^-$). Strain 149, isolated from a wild mouse, was provided by M. Tsubokura (Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori, Japan); strain 149C was derived by growth on magnesium oxalate agar at 37°C and had been cured of the plasmid. Chromosome-mediated properties were positive in strains 149 and 149C, and plasmid-mediated properties were positive in strain 149 but not in 149C. The 50% lethal dose (19) for ddY mice (weight, 15 to 20 g; Shizuoka Agricultural Cooperation for Laboratory Animals, Shizuoka, Japan) was examined (18). The 50% lethal doses of strains Pa12983, 149C, and 149 determined after intraperitoneal injection were 6.9×10^8, 4.72×10^8, and 4.0×10^5 cells, respectively. Two groups of *Yersinia*-free ddY mice were deprived of drinking water for 24 h. To observe the capacity of the bacteria for invasion and intestinal colonization, one group of mice was then allowed to drink from a 50-ml water suspension of each strain containing about 10^9 bacteria per milliliter for 1 h (Table 1). For observation of virulence and intestinal colonization, another group was allowed to drink for 24 h (Table 2). At periodic intervals thereafter, the mice were examined for systemic disease, symptoms of diarrhea, and bacterial shedding. Two mice from each group were killed, and portions of the mesenteric lymph nodes, spleen, liver, cardiac blood, Peyer's patches, ileal tissue, and ileal, cecal, and rectal contents were removed for colony counts.

Colonization of the intestine by *Y. pseudotuberculosis* strains was assessed from 1 h after inoculation by quantitat-

TABLE 1. Recovery of *Y. pseudotuberculosis* Pa12983, 149, and 149C from the internal regions of mice after 1 h of oral ingestion of drinking water containing 10^9 viable bacteria

Internal region	No. of bacteria ($\log_{10}$ CFU/g) recovered at time (h) after administration																	
	Pa12983 (pYV$^-$) (n = 2 each)						149C (pYV$^-$) (n = 2 each)						149 (pYV$^+$) (n = 2 each)					
	1	3	6	12	24	48	1	3	6	12	24	48	1	3	6	12	24	48
Cardiac blood							1.2											1.0
Kidney							0.5											
Liver							4.2											
Lung	1.0	1.3		1.3	1.0	1.1	2.2											1.3
Spleen							2.2											3.5
Mesenteric lymph nodes			1.0	1.7	2.6	4.1	4.2		2.2	3.2	4.1	5.2		2.2	3.5	2.2	4.2	5.1
Peyer's patches	6.9	4.6	3.2	4.9	3.8	4.6	8.2	5.2	4.1	3.8	4.3	4.2	7.3	6.6	5.2	5.2	7.2	8.2
Ileal tissue	8.1	6.1	2.9	4.2	3.3	3.5	9.2	5.2	5.3	4.2	3.6	3.6	8.3	7.3	5.3	3.4	5.2	7.1
Ileal contents	9.0	7.1	3.4	4.8	3.2	3.2	9.2	6.6	6.2	4.2	3.2	2.2	8.5	8.5	5.4	4.1	6.1	6.3
Cecal contents	8.3	9.3	7.1	5.2	3.8	2.2	8.3	9.5	8.2	7.2	4.2	3.4	8.4	8.2	8.3	5.3	6.2	6.3
Rectal contents	8.1	9.3	7.1	4.9	3.6	2.2	3.2	9.4	8.3	8.2	4.5	3.4		8.0	8.2	5.4	5.3	7.2

ing both free luminal bacteria and tissue-associated bacteria (Tables 1 and 2). In mice inoculated for 1 h (Table 1), the counts of three strains gradually decreased in the Peyer's patches, ileal tissue, and ileal and rectal contents for 12 h postinfection and the counts of two pYV$^-$ strains (strains Pa12983 and 149C) continued to decrease to much lower numbers in the same sites from 24 to 48 h postinfection. In contrast, the count of a pYV$^+$ strain (strain 149) increased to much higher numbers in the same sites from 24 h postinfection. In mice inoculated for 24 h (Table 2), two pYV$^-$ strains decreased gradually to much lower numbers in the same sites and then were gradually eliminated from the intestinal contents on day 14 or 21 postinfection. In contrast, a pYV$^+$ strain produced diarrhea in mice on day 4 postinfection, and, subsequently, three mice died on days 9 and 15 postinfection. These differences in *Y. pseudotuberculosis* growth in the intestine that occurred within 24 to 48 h postinfection suggest that the pYV$^-$ strain did not inhibit phagocytosis in the gut epithelium.

The capacity of *Y. pseudotuberculosis* strains to invade the intestinal mucosa was assessed by viable counts in the mesenteric lymph nodes, spleen, liver, lung, and cardiac blood (Tables 1 and 2). In mice inoculated for 1 h, strain 149C (pYV$^-$) was recovered from the whole body as early as 1 h postinfection, but this organism was rapidly eliminated from the mesenteric lymph nodes, spleen, liver, lung, and cardiac blood within 3 h postinfection. Three strains were recovered from mesenteric lymph nodes at 3 h (pYV$^+$ strain) and 6 h (pYV$^-$ strains) postinfection, and then their numbers increased gradually for up to 48 h. A pYV$^+$ strain was isolated from the spleen, lung, and the cardiac blood at 48 h. In mice inoculated for 24 h, two pYV$^-$ strains in the mesenteric lymph nodes gradually decreased in number in contrast with the prominent increase in a pYV$^+$ strain in the mesenteric lymph nodes, spleen, liver, lung, and the cardiac blood. Although the strains recovered from mice infected with strain 149 were autoagglutination positive, the strains recovered from mice infected with strains Pa12983 and 149C were autoagglutination negative, thereby indicating that plasmid pYV was absent in our patient and in mice inoculated with the pYV$^-$ strains. Cornelis et al. (4) stated that the three virulent yersiniae release large amounts of a set of proteins called Yops, encoded by a plasmid pYV, and that the loss of this property always correlates with the loss of pathogenicity. Rosqvist et al. (21) showed that Yop2b from *Y. pseudotuberculosis* inhibits phagocytosis by mouse peri-

TABLE 2. Recovery of *Y. pseudotuberculosis* Pa12983, 149, and 149C from the internal regions of mice after 24 h of oral ingestion of drinking water containing 10^9 viable bacteria

Internal region	No. of bacteria ($\log_{10}$ CFU/g) recovered at time (days) after administration														
	Pa12983 (pYV$^-$) (n = 2 each)					149C (pYV$^-$) (n = 2 each)					149[a] (pYV$^+$) (n = 2 each)				
	2	4	7	14	21	2	4	7	14	21	2	4	7	14 (n = 1)	15[b] (n = 1)
Cardiac blood												1.0	2.6		
Kidney													5.2		
Liver								2.3				1.3	5.2		5.2
Lung		2.6					2.6	2.6			2.5	2.1	3.3	4.2	5.2
Spleen			3.4	3.7							3.1	3.4	6.2		6.2
Mesenteric lymph nodes	4.6	4.6	4.2	3.7	1.3	5.1	4.2	3.4		1.3	5.2	3.2	4.1		6.2
Peyer's patches	3.9	6.2	4.7	4.7	4.2	3.3	4.4	4.1	3.3	4.2	8.2	7.2	5.2	6.8	7.0
Ileal tissue	6.1	3.2	2.7	3.1	3.1	4.1	4.2	2.2	2.3		8.2	7.2	8.3	6.2	6.6
Ileal contents	3.3	4.3	2.7	1.1		5.1	4.2	2.2	2.2		7.3	7.2	8.2	7.2	8.0
Cecal contents	3.7	3.1	2.7	1.7	3.0	5.3	4.5	3.2	2.1	2.4	7.2	8.1	8.1	7.1	8.2
Rectal contents	3.7	3.4	2.6			6.4	4.1	3.2	2.2	2.8	8.1	7.6	8.3	6.4	8.2

[a] All mice had diarrhea on day 4 postinfection, and three mice died on days 9 (two mice) and 15 (one mouse) postinfection.
[b] Data for dead mouse.

toneal macrophages. These findings suggest that the pYV$^-$ *Y. pseudotuberculosis* serotype 4a strain did not inhibit phagocytosis in the gut epithelium and did not produce gastroenteritis in our patient because of the absence of plasmid pYV in vivo.

The virulence of *Y. pseudotuberculosis* also involves a chromosomal gene (18). Simonet et al. (25) reported that pYV$^-$ bacteria are still capable of multiplying in host tissues during the early phase of infection and produce a strong inflammatory response, in contrast to pYV$^+$ bacteria, which severely inhibit granuloma formation in the case of intravenously induced infection of mice. Similar results have been noted with plasmidless derivatives and plasmid mutants of *Y. pestis*, *Y. pseudotuberculosis*, and *Y. interocolitica*, which induced the granulomatous response (26, 27). The present report seems to be the first of a case of oral infection of mice. pYV$^-$ *Y. pseudotuberculosis* sero- type 4a strains (Pa12983 and 149C), which maintained chromosome-mediated properties, invaded the intestinal mucosa and Peyer's patches and were recovered from the mesenteric lymph nodes, spleen, and liver (Tables 1 and 2). Moreover, the level of invasion by strains Pa12983 and 149C into the intestinal mucosa and lymph follicle was lower than that seen with strain 149. These findings suggest that pYV$^-$ organisms readily invade the intestinal mucosa and Peyer's patches and are then transported to the lymph follicle of the mesenteric lymph nodes, spleen, and liver via lymph flow.

Vesikari et al. (28) reported that, in *Yersinia* species, there may be dual resistance mechanisms against phagocytosis: plasmid-mediated adherence and possibly non-plasmid-mediated survival within the phagocytes. Invasion by strains Pa12983 and 149C into the intestinal mucosa and lymph follicle was more severe than that of pYV$^-$ *Y. enterocolitica* serotype O3 strains reported by Bakour et al. (1), Robins-Browne et al. (20), and Lian et al. (16). Maki et al. (17) stated that *Y. pseudotuberculosis* may have additional non-plasmid-associated virulence factors that are missing in *Y. enterocolitica*, since pYV$^-$ *Y. pseudotuberculosis* led to a keratoconjunctivitis very similar to that caused by pYV$^+$ *Y. enterocolitica* serotype O8 in the Serény test and which was also positive in pYV$^-$ *Y. pseudotuberculosis* serotype 4a strains in this study. Thus, acute mesenteric lymphadenitis without gastroenteritis in humans may be caused by additional chromosome-associated virulence factors that are present in *Y. pseudotuberculosis* but not in *Y. enterocolitica*.

Strains of pYV$^-$ *Y. pseudotuberculosis* occurred in animals such as wild mice (6, 8) and moles at a higher frequency than pYV$^+$ strains in the eastern Shimane Prefecture, Japan (7). Three strains of pYV$^-$ *Y. pseudotuberculosis* serotype 4a were isolated from wild mice trapped in the mountains near the residence of a patient. These findings suggest a close relationship between human infections with pYV$^-$ *Y. pseudotuberculosis* and the harboring of this organism in wild animals.

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