Lack of Antibody Response to Invasin in Humans with Yersiniosis

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The Yersinia pseudotuberculosis inv gene encodes invasin, a 103-kDa outer membrane protein allowing bacteria to penetrate mammalian cells. This protein is produced in vitro at below 30°C. In this work, we studied the antibody response against invasin in humans suffering from yersiniosis and in mice orally infected with a virulent strain of Y. pseudotuberculosis. Infection with enteropathogenic Yersinia strains did not induce either a systemic or a gut antibody response to invasin. Our results suggest that the inv gene is not expressed in the gut at 37°C and, therefore, that invasin is not presented to the immune system when microorganisms multiply in the host tissues.

Yersiniosis is a human enteric infection caused by Yersinia pseudotuberculosis or Yersinia enterocolitica. The most common clinical manifestation of Y. pseudotuberculosis infection is acute mesenteric lymphadenitis (pseudoappendicitis), and Y. enterocolitica causes terminal ileitis. The infection is usually self-limited, lasting 5 to 10 days, but postinfection complications such as reactive arthritis or erythema nodosum may arise 1 to 6 weeks after the onset of infection. Septicemia is rare but can occur in elderly or immunocompromised patients (1).

These microorganisms are enteroinvasive pathogens. A gene responsible for bacterial entry into cultured epithelial cells, called inv, is present on the chromosomes of both Y. pseudotuberculosis and Y. enterocolitica (4, 9). The nucleotide sequences of the two inv genes are 75% identical, and the encoded proteins are 77% identical (16). Inv encodes a protein of 103 kDa and 92 kDa in Y. pseudotuberculosis and Y. enterocolitica, respectively, called invasin (7, 16). This molecule is found in the bacterial outer membrane, and its production is thermodiluted: invasin is produced at low temperatures (≤30°C) (6, 7, 11). Invasin-deficient mutants of Y. pseudotuberculosis and Y. enterocolitica exhibit a lower level of virulence in mice than the parental strains, confirming the role of invasin in vivo (12, 13). Invasin is therefore involved in pathogenesis, and it is thought to act only at the first stage of the infectious process (3).

(This work was presented in part at the 93rd General Meeting of the American Society for Microbiology, Atlanta, Ga., 16 to 20 May 1993 [2a].)

The immune response to invasin in patients developing yersiniosis has not previously been studied. In this work, we tested for the presence of antibodies against this protein in human sera containing high titers (≥320) of agglutinins raised against Y. enterocolitica or Y. pseudotuberculosis, as determined by a microagglutination assay. The sera, kindly given by Elisabeth Carniel from the Centre National de Référence des Yersinia (Institut Pasteur, Paris, France), were collected between 1990 and 1992 from 15 patients living in different areas of France. Three of them were indisputably infected by Y. enterocolitica since the bacterium was recovered from stools (Table 1). No enteropathogenic Yersinia strains were isolated from the 12 remaining patients, but the diagnosis of yersiniosis was most likely correct on the basis of the clinical symptoms (pseudoappendicular syndrome or appendicitis, reactive arthritis, and/or erythema nodosum) (Table 1).

Sera were screened for antibodies directed against invasin by immunoblot analysis with a 120-kDa hybrid protein corresponding to an N-terminal-deletion derivative of invasin from Y. pseudotuberculosis (780 amino acids, i.e., 80% of invasin) fused to Escherichia coli maltose-binding protein (MalE). This fusion protein, MalE-invasin, was extracted from the protease-deficient E. coli strain JL369 harboring the recombinant plasmid pJL303, a pCG806 derivative carrying about 80% of the inv gene (8). Briefly, the tac promoter was induced with 1 mM isopropyl-β-D-thiogalactoside, and the bacterial cells were harvested and lyed by heat and osmotic shock. The crude extract was loaded onto a cross-linked amylose column. The fusion protein bound to the column was then eluted with 10 mM maltose. Protein purification was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), electrotransfer of the protein to a nitrocellulose sheet, and then immunoblotting with a monoclonal antibody (Mab), 3A2, raised against invasin, as previously described (13). For detection of serum antibodies directed against invasin, nitrocellulose strips each loaded with 1 μg of purified MalE-invasin were incubated with human sera diluted 1:100, washed, and incubated with a peroxidase-labeled antibody (goat anti-human immunoglobulins G, M, and A; Cappel, Malvern, Pa.) diluted 1:1,000. Antibody binding was revealed by the addition of diaminobenzidine-tetrahydrochloride and hydrogen peroxide. This Mab, obtained by immunizing mice with an extract of E. coli overproducing native invasin (5), recognizes the last 192 amino acids of the invasin C terminus and also the fusion protein MalE-invasin (Fig. 1). Sera collected from 10 newborns and used as negative controls (agglutinin titer < 40) did not react with this antigen. No antibodies binding to invasin were detected in the 15 samples containing anti-Yersinia agglutinins. This result clearly shows that the chromosomally encoded outer membrane protein cannot be used for the serodiagnosis of yersiniosis. The failure to detect antibodies to invasin in patients cannot be explained by a lack of immunogenicity of invasin, since invasin-specific MAb have been successfully prepared (8). It is possible that the immune response is strictly limited to the portal of entry or that invasin is not expressed in vivo during infection. These questions were investigated by infecting mice orally with a virulent strain of Y. pseudotuber-
culosis and following the antibody responses in sera and intestinal secretions during the course of infection. Six- to eight-week-old BALB/c mice were inoculated intragastrically with 10³ bacteria of Y. pseudotuberculosis IP2790 grown at 28°C, as previously described (14). This strain harbored the virulence plasmid pYV (15), and its oral 50% lethal dose was 10²-³ bacteria per mouse. Most animals were clinically ill at the end of the first week of infection, and 4 of the 28 mice died between days 8 and 12 with multiple abscesses in the liver and spleen. On days 3, 8, 16, 21, and 45 following the oral challenge, intestinal secretions and blood were collected (four animals per time point). Briefly, secretions were obtained, as described by Elson et al. (2), by administering a polyethylene glycol saline lavage solution orally and then 1 mg of pilocarpine intraperitoneally. Protease inhibitors were added to intestinal secretions, and samples were frozen at -20°C until assay. Sera diluted 1:10 and undiluted intestinal secretions were tested by immunoblot for the presence of specific antibodies as described above, except that a goat anti-mouse immunoglobulin was used as the second antibody. No antibody response to invasin was detected in any serum or intestinal secretion during the course of experimental infection. Thus, infection of mice with enteropathogenic Yersinia strains does not induce either a systemic or a gut antibody response against invasin. In contrast, we previously found that mice orally immunized with a recombinant Salmonella strain expressing invasin developed a humoral response against invasin (14). This discrepancy is most likely related to differences in inv expression in the two microorganisms, invasin being produced optimally at 28 to 30°C in Yersinia strains and independently of temperature in Salmonella strains (14).

Enteropathogenic Yersinia strains infect humans mostly through foods which are usually stored at 4 to 8°C. It is known, on the basis of in vitro experiments, that Yersinia strains multiply and synthesize invasin at this temperature (10). Thus, humans probably come into contact with bacteria producing invasin. Once ingested, microorganisms move through the stomach, where they encounter a hostile acid environment which kills most of the bacteria. It is probable that a few surviving microorganisms reach the intestine, where they penetrate the epithelial cells because of the activity of invasin and can thus disseminate to the gut-associated lymphoid tissue. Our results suggest that the inv gene is not expressed at 37°C after the bacteria have entered the host and therefore that invasin is not presented to the immune system when bacteria multiply in the host tissues. This view is reinforced by our recent finding that a recombinant Salmonella strain producing invasin at 37°C induces an immune response against invasin (14). In conclusion, virulent Yersinia strains possess a refined system based on thermoregulated genes, which allows them to avoid the development of an immune response to virulence factors that are required only for entry by rapidly stopping the expression of these factors once in the host.

![FIG. 1. Purification of MalE-invasin fusion protein. The fusion protein was detected in total cell proteins of E. coli JL369 (lanes 1) and in eluate from an amylose column (lanes 2) by SDS-PAGE (A) and immunoblotting with an invasin-specific MAb (3A2) (B). This MAb recognizes the recombinant protein and also its degradation products.](http://cvi.asm.org/)

### TABLE 1. Characteristics of the 15 patients studied

<table>
<thead>
<tr>
<th>Patient no., sex, and age (yr)</th>
<th>Clinical symptom(s)</th>
<th>Serum agglutinin titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Female, &gt;60</td>
<td>Enteritis (isolation of Y. enterocolitica from stools)</td>
<td>640⁴</td>
</tr>
<tr>
<td>2. Female, 3</td>
<td>Reactive arthritis and erythema nodosum</td>
<td>1,280⁴</td>
</tr>
<tr>
<td>3. Male, 38</td>
<td>Enteritis (isolation of Y. enterocolitica from stools)</td>
<td>320⁴</td>
</tr>
<tr>
<td>4. Female, 26</td>
<td>Reactive arthritis and erythema nodosum</td>
<td>1,280⁴</td>
</tr>
<tr>
<td>5. Female, 76</td>
<td>Reactive arthritis</td>
<td>640⁴</td>
</tr>
<tr>
<td>6. Female, adult</td>
<td>Enteritis (isolation of Y. enterocolitica from stools) and erythema nodosum</td>
<td>640⁴</td>
</tr>
<tr>
<td>7. Female, 32</td>
<td>Reactive arthritis</td>
<td>1,280⁴</td>
</tr>
<tr>
<td>8. Male, 21</td>
<td>Mesenteric lymphadenitis (pseudoappendicular syndrome)</td>
<td>320⁴</td>
</tr>
<tr>
<td>9. Male, 28</td>
<td>Mesenteric lymphadenitis and peritonitis</td>
<td>320⁴</td>
</tr>
<tr>
<td>10. Male, adult</td>
<td>Enteritis</td>
<td>640⁴</td>
</tr>
<tr>
<td>11. Male, 8</td>
<td>Mesenteric lymphadenitis (pseudoappendicular syndrome)</td>
<td>640⁴</td>
</tr>
<tr>
<td>12. Male, 1</td>
<td>Erythema nodosum</td>
<td>320⁴</td>
</tr>
<tr>
<td>13. Male, 58</td>
<td>Reactive arthritis</td>
<td>320⁴</td>
</tr>
<tr>
<td>14. Male, adult</td>
<td>Mesenteric lymphadenitis (pseudoappendicular syndrome)</td>
<td>320⁴</td>
</tr>
<tr>
<td>15. Male, 34</td>
<td>Appendicitis and liver abscess</td>
<td>320⁴</td>
</tr>
</tbody>
</table>

* Against Y. enterocolitica serotype O9.
* Against Y. enterocolitica serotype O3.
* With HLA-B27 antigen.
* Against Y. pseudotuberculosis serotype I.
* Against Y. pseudotuberculosis serotype IV.
* Against Y. pseudotuberculosis serotype III.
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REFERENCES


