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Role of the YadA Protein in Prevention of Opsonization of *Yersinia enterocolitica* by C3b Molecules

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When mixed with normal human serum, wild-type pathogenic *Yersinia enterocolitica*, previously incubated at 37°C, fixed less C3b than its variant cured of the virulence plasmid pYV. Mutants unable to secrete the Yop proteins were still protected against C3b deposition. By contrast, mutants deficient in the production of outer membrane protein YadA fixed more C3b than their YadA$^+$ parent. Gene *yadA*, cloned as a minimal polymerase chain reaction fragment and introduced in *trans*, complemented the mutations. Production of YadA by recombinant *Escherichia coli* LK111 also resulted in a reduction of the amount of C3b deposited on the bacterial surface. The reduction of C3b at the surface of *Y. enterocolitica* YadA$^+$ compared with YadA$^-$ cells correlated with an increase of the amount of factor H fixed at the bacterial surface. The YadA monomer separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane was able to bind factor H. We conclude that factor H bound to YadA reduces the C3b deposition on the bacterial surface, probably by a rapid inactivation of C3b.

Yersinia enterocolitica is an enterobacterium frequently involved in human enterocolitis. The predominant clinical features are diarrhea in young children and mesenteric adenitis characterized by abdominal pain and leukocytosis in older children and adolescents (for a review, see reference 11). Because of its psychrophilic character and its high prevalence in the human population, *Y. enterocolitica* is increasingly involved in massive contamination of blood bags, leading to severe transfusion accidents (41).

Pathogenic strains of *Y. enterocolitica* harbor a 70-kb plasmid called pYV (3, 20, 58). This plasmid encodes several thermodependent properties, including the secretion of 11 proteins called Yops and the production of an outer membrane protein called YadA (6, 49) and of an outer membrane lipoprotein, related to TraT, called YlpA (9). The secretion of Yops occurs only at 37°C in a medium deprived of Ca^{2+} ions and coincides with a severe growth restriction. Both phenomena depend on a 20-kb region of the pYV plasmid containing the loci *virA*, *virB*, *virC*, and *virF* (12–14). The *virC* operon, composed of the 13 genes *yscA* through *yscM*, is involved in Yop secretion (35). The *virF* locus encodes a DNA-binding protein acting as a transcriptional activator of the *yop*, *ysc*, *yadA*, and *ylpA* genes (9, 13, 14, 28).

The roles of some Yops have been elucidated. YopE is a cytotoxin (44), and YopH is a phosphotyrosine phosphatase that inhibits phagocytosis (4, 21, 43). YopM binds the thrombin and so inhibits the aggregation of platelets and the onset of inflammation (29, 30, 42). YopO, now called YpkA, is a serine-threonine protein kinase (19). The roles of the other Yops remain elusive.

Like Yops, YadA is synthesized only at 37°C, but unlike Yops, its production is independent of Ca^{2+} and it does not require the products of the *virA* and *virC* loci (35). YadA is a polymer of about 200 kDa composed of subunits of 45 to 52.5 kDa (48, 49, 57) forming a tiny fibrillar structure at the surface of *Y. enterocolitica* (25, 27, 57). In experimental oral infection of the mouse, YadA contributes to the long-term fecal excretion of the bacteria (25). In vitro, YadA plays various roles in relation to its surface location. It increases the surface hydrophobicity (34) and promotes autoagglutination (48). It confers adherence to epithelial cells (22) and to the extracellular matrix by binding collagen fibers (17, 47) and fibronectin (54). YadA also inhibits the anti-invasive effect of interferon (7). Finally, YadA confers resistance to the bactericidal activity of human serum (2) by inhibiting the formation of the membrane attack complex (40). Although other factors, like the Ail protein (5) and lipopolysaccharide (55), play a role in this phenotype, YadA seems to be the major determinant since YadA-deficient mutants become serum sensitive (2).

The alternative pathway of complement activation can be viewed as a major recognition system for nonspecific defense mechanisms. In this pathway, the initial step is the deposition of C3b, the larger fragment of the serum protein C3. C3b can fix either factor B or factor H. On activating surfaces, C3b binds factor B, forming the C3bB complex, which is processed to C3bBb, the alternative C3 convertase. This convertase then amplifies the activation process and initiates assembly of the membrane attack complex. By contrast, on nonactivating surfaces, C3b binds factor H and becomes accessible to factor I, which cleaves C3b into its inactivated form, C3bi (for a review see reference 37).

One of the major effects of complement activation is opsonization by the deposition of large amounts of C3b molecules on the surface of the activator cell. The target cell then becomes accessible to uptake by professional phagocytes carrying C3 receptors. Some bacterial pathogens have developed mechanisms that prevent this opsonization (for a review, see reference 23). For example, the capsule of group B streptococci contains sialic acid, which can favor the binding of factor H to C3b (16). Protein M of group A

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TABLE 1. Plasmids used in this study

Plasmid	Genotype	Reference(s) or source
pBC7	pYVe227*yadA*::pBC9	This work
pBC9	pGCS82 + internal fragment *Pvu*II of *yadA* (*yadA* mutator)	This work
pGB08	pYL4*yadA*-GB08::Tn*813*-R388	2
pGB63	pYL4-GB63::Tn*813*-R388	2
pGB910	pYL4*yadA*-GB910::Tn*813*-R388	2
pGC217	pGB63*yscC*-GC217::mini-Mud*lac yadA*$^+$	13, 35
pGC403	pGB63*yscD*-GC403::mini-Mud*lac yadA*$^+$	13, 35
pGCS82	pGP704 + *aadB* from Tn*732*	28
pIC20H	*bla*$^+$ *lacZ'*, multiple cloning site	33
pKNG78	P_{tac} *lacI*^{q} *bla*$^+$ Tet^r	24a
pMS150	pIC20H-*yadA*	This work
pMS151	pKNG78-*yadA*	This work
pMS153	pSelect-1 P_{lac}-*yadA*, *oriT* from pTJS82	This work
pSelect-1	*lacZ' ori*F1 Tet^r	Promega
pTJS82	pUC7 + *oriT* from RK2	46
pYV227	Virulence plasmid of *Y. enterocolitica* W22703, *yadA*$^+$	10

streptococci also fixes factor H, which again correlates with a faster inactivation of C3b (24).

Tertti et al. (53) assayed total C3b deposited at the surface of serotype O:3 *Y. enterocolitica* cells incubated with normal human serum (NHS). They detected more C3b on the surface of pYV$^-$ cells than on the surface of pYV$^+$ bacteria grown at 37°C. More recently, Pilz et al. (40) showed that pYV$^+$ strains rapidly inactivate C3b into C3bi and that this process requires YadA.

In this paper, we confirm and extend these observations on the resistance of pYV$^+$ *Y. enterocolitica* to complement, using our serotype O:9 model strain W22703. We confirm that YadA reduces the deposition of C3b on the bacterial cell surface, and we show that this reduction is correlated with a YadA-mediated fixation of factor H, probably leading to a faster degradation of C3b.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Y. enterocolitica* W22703 (nalidixic acid resistant) and W22708 (streptomycin resistant) are two restriction mutants (Res$^-$ Mod$^+$) of the wild-type serotype O:9 strain W227 (10). *Y. enterocolitica* W1024 (serotype O:9) was isolated in 1988 by G. Wauters (Brussels, Belgium) from human stools. *Escherichia coli* strains used are *E. coli* LK111 (C600 Res$^-$ Mod$^+$ *lacY*$^+$ *lacZ*ΔM15 *lacI*) (received from M. Zabeau, Ghent, Belgium), JM101 (*lacZ*ΔM15 *lacI*^{q}) (56), and Sm10 lambda *pir*$^+$. The last strain, constructed by Miller and Mekalanos (36), allows the replication of *pir* mutants of R6K and mobilizes plasmids containing the origin of transfer of RK2. Plasmids used are listed in Table 1.

Bacterial growth for production of YadA and SDS-PAGE of YadA. *Y. enterocolitica* was inoculated at an optical density at 600 nm of 0.1 in a conical flask containing 5 ml of brain heart infusion (Difco, Detroit, Mich.) supplemented with 0.4% glucose, 20 mM $MgCl_2$, and 20 mM sodium oxalate and containing the appropriate antibiotics (nalidixic acid, 35 μg/ml; tetracycline, 10 μg/ml; kanamycin, 50 μg/ml; gentamicin, 20 μg/ml; and merbromine, 20 μg/ml). All antibiotics were purchased from Boehringer (Mannheim, Germany). The cultures were shaken for 2 h at room temperature and then shifted for 3 h at 37°C.

E. coli was inoculated at an optical density at 600 nm of 0.1 in universal bottles containing 5 ml of tryptic soy broth (Gibco, Paisley, Scotland) and the appropriate antibiotics. The cultures were shaken for 5 h at 37°C. If necessary, 1 mM isopropyl-β-D-thiogalactoside (IPTG) was added after 2 h of culture.

Bacteria from 1 ml of culture were harvested by centrifugation in a microcentrifuge tube, and the pellet was dissolved in 200 μl of electrophoresis sample buffer (6.5 mM Tris-HCl [pH 6.8], 3% sodium dodecyl sulfate, 5% β-mercaptoethanol, 10% glycerol, 0.03% bromophenol blue). The whole cell lysate mixture containing the equivalent of 3×10^8 bacteria was subjected to sodium dodecyl sulfate-polyacrylamide (10%, wt/vol) gel electrophoresis (SDS-PAGE).

Disruption of *yadA* by homologous recombination. A 270-bp internal *Pvu*II fragment of the *yadA* gene from *Y. enterocolitica* was extracted from plasmid pACYC184-E4 (2). This fragment, which corresponds to nucleotides 953 to 1222 in the *Y. enterocolitica* serotype O:3 sequence published by Skurnik and Wolf-Watz (49), was cloned into the suicide plasmid pGCS82 (28), giving pBC9. We mutagenized *yadA* by crossing *E. coli* Sm10 lambda *pir*$^+$(pBC9) with *Y. enterocolitica* W22703(pYVe227). The integration of pBC9 into pYVe227 was selected on the gentamicin resistance marker of pBC9. The recombinant plasmid pYVe227*yadA*::pBC9 was called pBC7.

Amplification and cloning of *yadA*. The polymerase chain reaction (38) was used as described previously (15) with the following cycles: cycle 1, 5 min at 93°C, 10 min at 40°C, 5 min at 70°C; cycles 2 to 29, 1 min at 93°C, 2 min at 55°C, 2 min at 70°C; cycle 30, 1 min at 93°C, 2 min at 55°C, 10 min at 70°C. The amplification primers were MIPA 125 (5'-GGGT CGACGTTTTATTCACGGGAA-3') and MIPA 126 (5'-GG TCTAGATAAATGACATTACCACTG-3'). MIPA 125 and MIPA 126 contain a *Sal*I and an *Xba*I site, respectively. The 1.6-kb polymerase chain reaction product was digested by *Sal*I and *Xba*I and cloned in the corresponding sites of plasmid pIC20H. The resulting plasmid was called pMS150. In this construction, the *yadA* gene is under the control of the *lac* promoter. The 1.6-kb *Xba*I-*Sal*I fragment was also cloned in the same restriction sites of plasmids pKNG78 and pSelect-1, generating plasmids pMS151 and pMS152, respectively. In plasmid pMS151, the *yadA* gene is under the control of the *tac* promoter, whereas in plasmid pMS152, the *yadA* gene is downstream from the *lac* promoter. Plasmid pMS152 was made mobilizable by cloning the origin of transfer (*oriT*) of RK2 extracted as a *Bam*HI fragment from pTJS82. The mobile plasmid was called pMS153.

Sera. NHS (Sigma, St. Louis, Mo.) was devoid of anti-Yop antibodies as checked by immunoblot. Fluorescein isothiocyanate (FITC)-conjugated rabbit antibodies to human C3c, FITC-conjugated rabbit antibodies to goat immunoglobulins, horseradish peroxidase (HRP)-conjugated rabbit antibodies to goat immunoglobulins, and HRP-conjugated swine antibodies to rabbit immunoglobulins were from DAKO-Immunoglobulins a/s (Glostrup, Denmark). Goat anti-H serum was received from P. L. Masson (Experimental Medicine Unit, University of Louvain, Brussels, Belgium). The specificity of the serum was checked by immunoelectrophoresis and Ouchterlony immunodifusion, using as a reference a commercial chicken anti-H serum (Sera-Lab, Crawley Town, England). Both sera showed the same precipitation arc in immunoelectrophoresis of NHS and identity response by Ouchterlony analysis.

The goat antibodies specific to factor H were purified from nitrocellulose as described previously (50). Rabbit poly-

clonal serum against YadA was obtained as described by Sory et al. (52).

Fluorometry. Bacteria from 1 ml of culture were harvested, washed twice in phosphate-buffered saline (PBS) (13 mM sodium hydrogenophosphate, 2.8 mM potassium dihydrogenophosphate, 135 mM natrium chloride, pH 7.4) and resuspended in PBS supplemented with 10 mM ethyleneglycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) and 5 mM $MgCl_2$ at an optical density at 600 nm of 1.0. The bacterial suspension (90 μl) was incubated for 30 min at 37°C with 10 μl of NHS. The microcentrifugetube was then centrifuged for 1 min, and the pellet was washed once with PBS. To monitor C3b, we added FITC-labeled anti-C3c antibodies (final dilution, 1/200) and incubated the mixture for 30 min at room temperature in the dark. To monitor factor H, we added goat anti-H serum at a final dilution of 1/100 and incubated the mixture for 30 min at room temperature. The mixture was then centrifuged, and the bacteria were washed twice with PBS. FITC-labeled rabbit antibodies to goat immunoglobulins were added at the final dilution of 1/200, incubated, and washed as described above. In all cases, after the last washing, the bacteria were resuspended in a final volume of 1 ml of PBS and applied to a fluorescence-activated cell sorter (FACS) (Epics Elite; Coulter, Hialeah, Fla.). To circumvent the problem of YadA-mediated autoagglutination, we selected constant size parameters (low scattering angle and side scatter). The number of events counted ranged from 1,000 to 2,000.

Blotting experiment. The bacterial membrane proteins were prepared as described by Achtman et al. (1). Proteins were separated by SDS-PAGE and blotted to a nitrocellulose membrane (51). The membranes were incubated overnight with 10% NHS. After three 5-min washes in PBS containing 0.1% Tween 20, the membranes were incubated for 1 h with 10% anti-factor H serum. After being washed, the membranes were incubated with HRP-labeled rabbit antibodies directed against goat immunoglobulins, washed, and stained as described previously (51).

RESULTS

Deposits of C3b on the surface of *Y. enterocolitica*. Variants, pYV^+ and pYV^-, of *Y. enterocolitica* W22703 were incubated under conditions of expression of the *yop* regulon. After harvest, the bacteria were incubated with 10% NHS, and the amount of C3b fixed was revealed by fluorometry with an FITC-labeled antiserum raised against fragment C3c. This serum recognizes both C3b and C3bi. The amount of total C3b detected on pYV^+ cells (Fig. 1B, panel 1) was lower than the amount detected on pYV^- cells (Fig. 1B, panel 2). This result confirmed the observation made by Tertti et al. (53) with a *Y. enterocolitica* strain of serotype O:3.

Yops and C3b deposition. To determine whether a Yop protein is involved in the inhibition of C3b deposition, we tested our mini-Mud*lac* insertion mutants W22703(pGC217) and W22703(pGC403), which are affected in the *virC* operon (13). These mutants are unable to secrete the Yops, but they still present YadA on their surfaces (35) (Fig. 1A). The two mutants fixed the same amount of C3b as their parental strain (Fig. 1B, panels 3 and 4). We concluded that the secretion of Yops was not involved in the reduction of fixation of C3b by *Y. enterocolitica*.

YadA and C3b deposits. Two *yadA* insertion mutants, W22708(pGB08) and W22708(pGB910), fixed more C3b than the wild type (Fig. 1B, panels 5 and 6), suggesting that YadA was involved in the reduction of C3b deposition. However, mutants carrying pGB08 and pGB910 are also affected in the unlinked *ylpA* gene because of a previous Tn*3* insertion mutation (2, 9). Hence, we constructed a pYV plasmid that was mutated only in *yadA*. The *yadA* gene was disrupted by homologous recombination between the pYV plasmid and a suicide plasmid (36) carrying an internal portion of the *yadA* gene. The recombinant strain, W22703(pBC7), no longer produced YadA (Fig. 1A), and we observed that again the loss of YadA correlated with an increase of the amount of C3b on the bacteria (Fig. 1B, panel 7). To avoid any bias due to the rough character of W22703, we mutagenized *yadA* in W1024, a smooth strain of the same serotype. We obtained the same result (not shown) as with W22703, indicating that the action of YadA on C3b deposition is independent of the length of the lipopolysaccharide.

Since our *yadA* mutations could be polar and affect the expression of uncharacterized downstream genes, we complemented the mutation with a plasmid containing only *yadA* and no other pYV sequence. Plasmid pMS153 contains a polymerase chain reaction-tailored *yadA* gene devoid of its promoter, cloned downstream from the *lac* promoter of vector pSelect-1. The use of the *lac* promoter allowed us to bypass the need for VirF and hence to produce YadA in a pYV^- strain. The three strains W22703(pBC7), W22703(pBC7)(pMS153), and W22703(pMS153) were then tested for their capacities to fix C3b. Complemented strain W22703(pBC7)(pMS153) fixed less C3b than W22703(pBC7). Interestingly, W22703 carrying only pMS153 and no pYV plasmid fixed as little C3b as W22703(pBC7)(pMS153) (Fig. 1B, panels 7, 8, and 9). We concluded from all of these experiments that YadA was involved in the reduction of the amount of C3b observed on the pYV^+ *Y. enterocolitica* cells. Moreover, YadA is presumably the only pYV-encoded factor involved in the protection against C3b.

To confirm the role of YadA in the reduction of C3b fixation, we monitored the response of *E. coli* cells producing YadA. For this analysis, we used plasmid pMS151, which contains the *yadA* gene cloned downstream from the *tac* promoter of vector pKNG78. This plasmid directs the production of YadA at the cell surface upon induction by IPTG (Fig. 2A). For unknown reasons, the amount of YadA varied from one culture to another (Fig. 2A). We monitored the deposition of C3b in two separate cultures of *E. coli* JM101(pMS151) and in one culture of JM101(pKNG78) taken as a negative control. We found that the YadA-producing bacteria fixed less C3b than the parental bacteria (Fig. 2B). Moreover, the reduction of C3b deposition correlated with the amount of YadA present at the cell surface.

Fixation of factor H. In view of the preceding results, we hypothesized that YadA could lead to the fixation of factor H on the bacterial surface and so allow factor I to cleave C3b in C3bi and further in C3dg, C3c, and C3d. We thus monitored the binding of factor H at the cell surface. Bacteria of strains W22703(pYV227), W22703(pBC7), W22703(pBC7)(pSelect-1), and W22703(pBC7)(pMS153) were incubated first with 10% NHS, collected, and incubated with a nonpurified goat anti-H serum. Bacteria were then treated with an FITC-labeled antiserum directed against goat immunoglobulin G (IgG) and analyzed in a FACS. As hypothesized, the bacteria synthesizing YadA fixed more factor H than the mutants (Fig. 3).

We then studied the interaction between YadA and factor H in an affinity blot experiment. Total membrane proteins were extracted from *E. coli* LK111(pMS153) and LK111(pSelect-1), separated by SDS-PAGE, and trans-

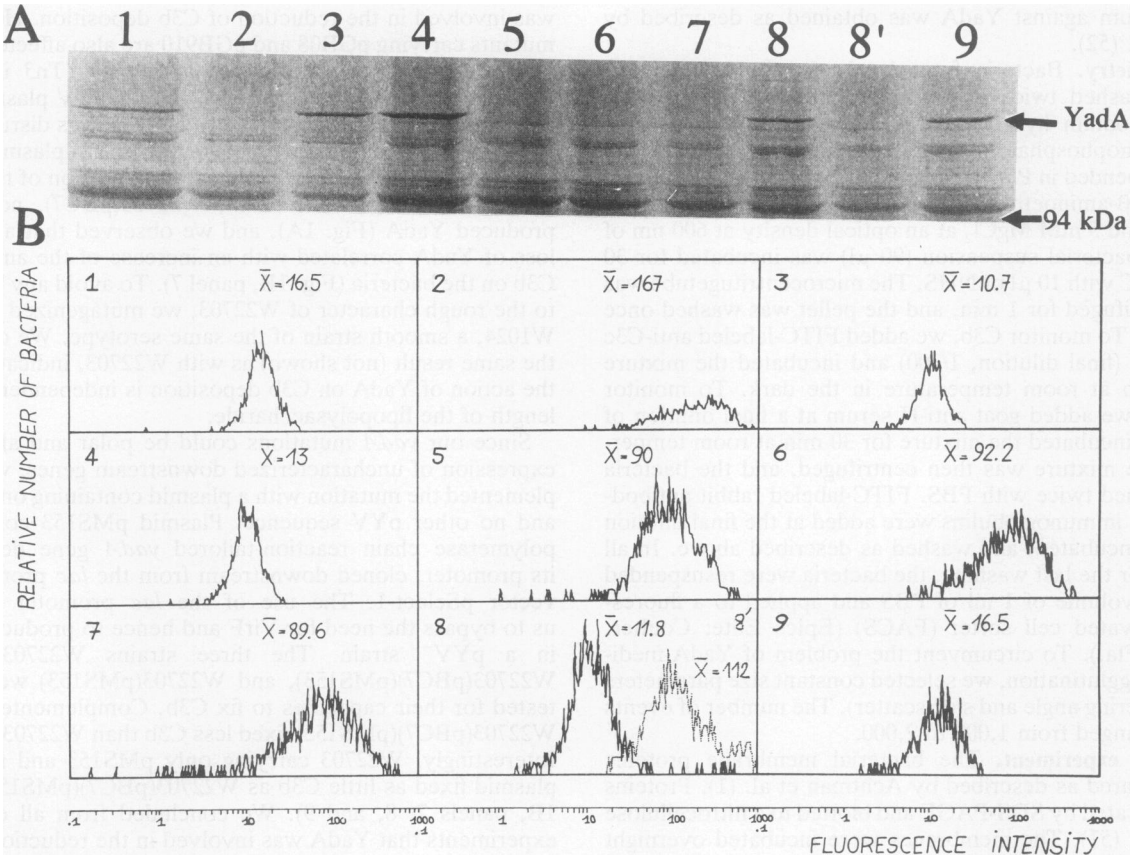

FIG. 1. (A) Coomassie blue-stained SDS-PAGE gel of total *Y. enterocolitica* proteins. Lanes: 1, W22703(pYV227) (YadA$^+$ Yops$^+$); 2, W22703(pYV$^-$) (YadA$^-$ Yops$^-$); 3, W22703(pGC403) (YadA$^+$ Yops$^-$); 4, W22703(pGC217) (YadA$^+$ Yops$^-$); 5, W22708(pGB08) (YadA$^-$ Yops$^+$); 6, W22708(pGB910) (YadA$^-$ Yops$^+$); 7, W22703(pBC7) (YadA$^-$ Yops$^+$); 8, W22703(pBC7)(pMS153) (YadA$^+$ Yops$^+$); 8′, W22703(pBC7)(pSelect-1) (YadA$^-$ Yops$^+$); 9, W22703(pMS153) (YadA$^+$ Yops$^-$). The upper arrow points to the YadA polymer. The lower arrow indicates the position of the molecular weight marker. (B) Fluorometric assay of C3b deposited on the bacterial surface. The same strains as in the respective lanes in panel A were used. The mean fluorescence intensity ($\bar{X}$) is indicated above each peak. The number of events counted for each strain was 1,500. The means were analyzed by a hierarchic F statistic analysis. The difference between values for YadA$^+$ and YadA$^-$ strains was significant ($P < 0.01$).

ferred onto a nitrocellulose membrane. This membrane was incubated first with 10% NHS and then with a specific goat anti-H serum. Factor H was visualized by HRP-labeled anti-goat immunoglobulins. The monomer of YadA extracted from *E. coli* membranes fixed factor H (Fig. 4B). However, the polymer did not react, presumably because of the poor transfer of this large molecule. To confirm this lack of transfer of the polymer, we carried out a Western blot (immunoblot) by using a polyclonal rabbit anti-YadA serum previously shown to recognize both the polymer and the monomer of YadA (52). We observed that this serum reacted only with the monomer in this immunoblot (Fig. 4D). A strong binding of factor H to a 37-kDa band was also observed; this band could correspond to *E. coli* porins. Such a reaction could be explained by the presence of anti-*E. coli* antibodies in the goat anti-H serum. When the anti-H antibodies were purified, binding was restricted to the YadA monomer (Fig. 4C). We concluded from these experiments that YadA fixed factor H by a protein-protein interaction.

DISCUSSION

Nonspecific defense mechanisms such as complement activation and phagocytosis play an important role in the destruction and elimination of invading microorganisms. Pathogenic bacteria have thus developed specific systems allowing them to avoid recognition by the nonspecific defense systems of their host (for a review, see reference 23). For gram-negative bacteria, activation of complement has two consequences: an opsonic effect resulting from the fixation of C3b on the bacterial surface and a lytic effect due to the formation of the membrane attack complex. Opsonization leads to phagocytosis by polymorphonuclear leukocytes via the interaction between the cell surface receptor CR1 or CR3 and C3b or C3bi deposited on the target bacteria. Bacteria can thus avoid phagocytosis by reducing the fixation of C3. In several instances, this reduction results from the fixation of factor H, which leads to the inactivation of C3b by factor I and prevents the formation of the alternative C3 convertase (C3Bb). Pathogenic bacteria adopted different strategies to bind factor H. Some of them cover their surface with sialic acid residues, thus mimicking host cell surfaces: group B strains of *Streptococcus pyogenes* have a sialic acid-containing cell wall (16), while *Neisseria meningitis* and *Neisseria gonorrhoeae* sialylate their lipopolysaccharide, in vivo, by using exogenous CMP–*N*-acetyl neuraminic acid (18). Other bacteria developed surface proteins having affin-

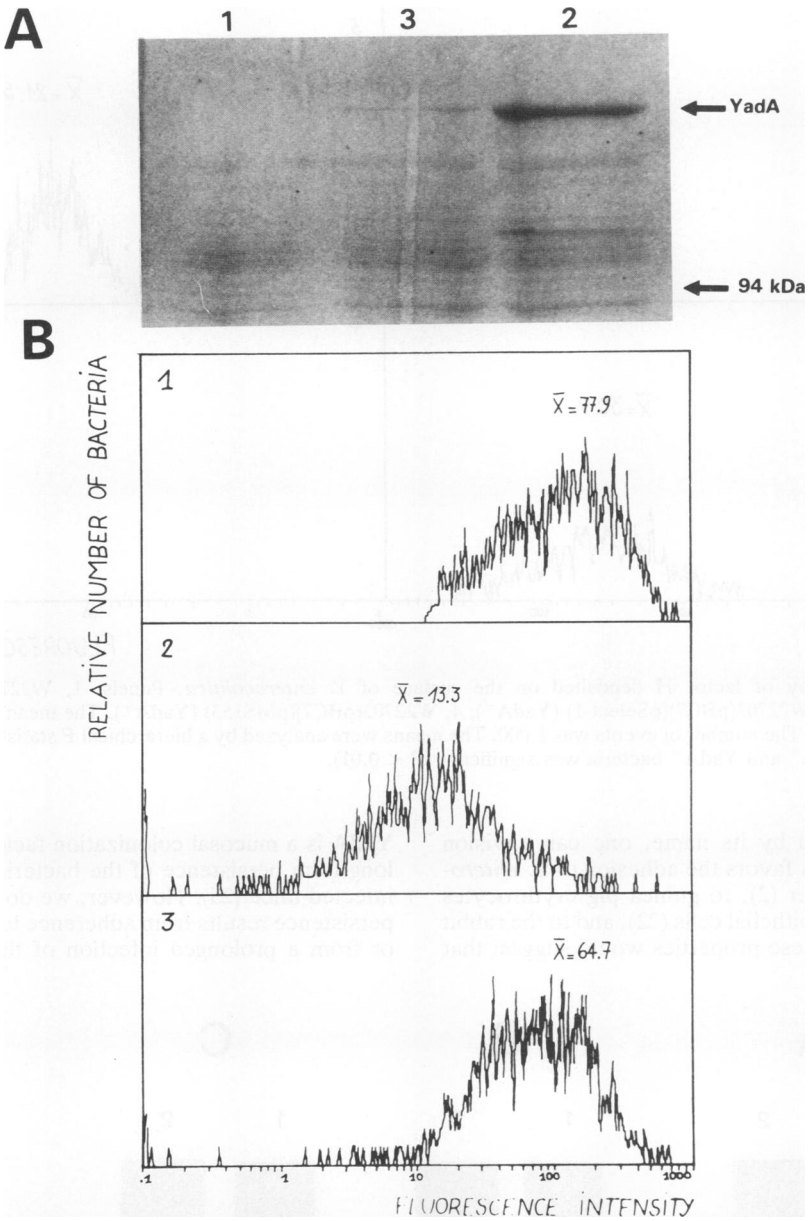

FIG. 2. (A) SDS-PAGE of total proteins of *E. coli* JM101. Lanes: 1, JM101(pKNG78) (YadA$^-$); 2, JM101(pMS151) (YadA$^+$) culture 1; 3, JM101(pMS151) (YadA$^+$) culture 2. The YadA polymer is indicated by the upper arrow; the lower arrow indicates the position of the molecular weight marker. (B) Fluorometric assay of C3b deposited on the bacterial surface. The panels correspond to the lanes in panel A. The mean fluorescence intensity ($\bar{X}$) is indicated above each peak. The number of events was 2,000. A one-way analysis of variance followed by orthogonal contrasts has been performed on the means. The mean for panel 3 was significantly different ($P < 0.01$) from that for panel 2 but not from that for panel 1.

ity for factor H. This is the case for group A *S. pyogenes* strains producing the M protein (24).

It has been known for a long time that virulent *Y. enterocolitica* strains resist the bactericidal activity of human serum (27) and that YadA is involved in this resistance (2). Tertti et al. (53) showed that pYV$^+$ strains do indeed fix less C3b than their pYV$^-$ isogenic variants, confirming that the pYV plasmid encodes a mechanism preventing the activation of complement by the alternative pathway. More recently, Pilz et al. (40) showed that YadA prevents the fixation of the membrane attack complex and increases the rate of C3b inactivation. Our data confirmed that the pYV plasmid indeed encodes a function reducing C3b deposition, and they demonstrated that YadA is responsible for this phenotype. They also showed that the reduction of opsonization is associated with the fixation of factor H. There is thus a functional parallel between YadA of *Y. enterocolitica* and protein M of *S. pyogenes* in spite of the fact that we have found no sequence homology between these two proteins. By reference to the known antiphagocytic activity of protein M, we can hypothesize that the resistance of *Y. enterocolitica* to phagocytosis by polymorphonuclear leukocytes (31, 32) is mediated by YadA.

The multiplicity of the functions attributed so far to YadA

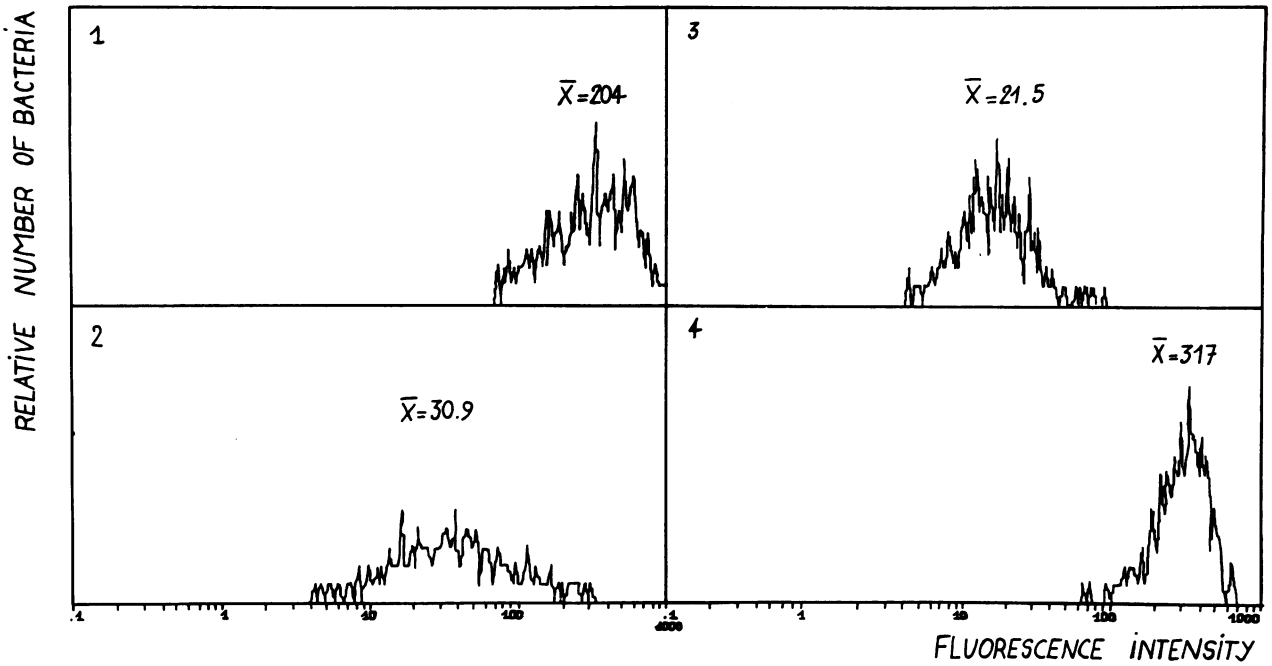

FIG. 3. Fluorometric assay of factor H deposited on the surface of *Y. enterocolitica*. Panels: 1, W22703(pYV227) (YadA$^+$); 2, W22703(pBC7) (YadA$^-$); 3, W22703(pBC7)(pSelect-1) (YadA$^-$); 4, W22703(pBC7)(pMS153) (YadA$^+$). The mean fluorescence intensity ($\bar{X}$) is indicated above each peak. The number of events was 1,000. The means were analyzed by a hierarchical F statistic analysis. The difference between the values for YadA$^+$ and YadA$^-$ bacteria was significant ($P < 0.01$).

is puzzling. As suggested by its name, one can envision YadA as an adhesin:YadA favors the adhesion of *Y. enterocolitica* cells to each other (2), to guinea pig erythrocytes (26), to in vitro-cultured epithelial cells (22), and to the rabbit intestinal mucosa (39). These properties would suggest that YadA is a mucosal colonization factor, as illustrated by the long-term persistence of the bacteria in the ileum of orally infected mice (25). However, we do not know whether this persistence results from adherence to the mouse enterocytes or from a prolonged infection of the Peyer's patches. No

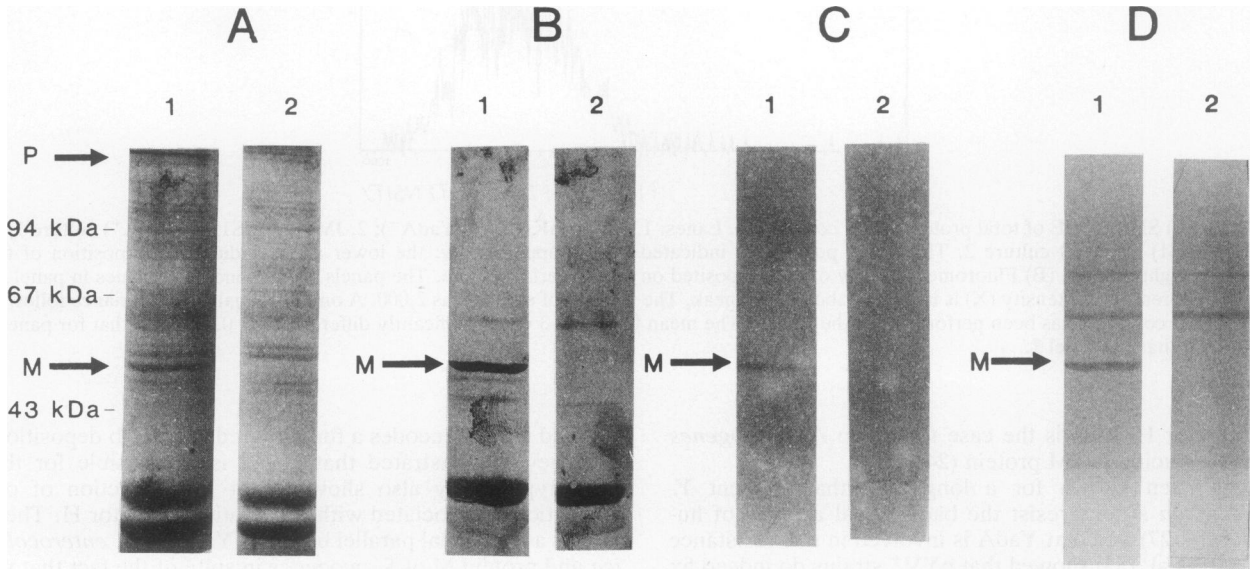

FIG. 4. (A) SDS-PAGE of external membrane proteins of *E. coli* LK111. Lanes: 1, LK111(pMS153) (YadA$^+$); 2, LK111(pSelect-1) (YadA$^-$). Arrows indicate the polymer (P) and the monomer (M) of YadA. (B) Nitrocellulose membrane after gel transfer, incubated with 10% NHS and revealed by anti-H antibodies. The monomer of YadA is indicated by the arrow. Lanes are as in panel A. (C) Same as panel B but with purified goat anti-H antibodies. (D) After SDS-PAGE, the proteins have been transfered onto a nitrocellulose membrane, incubated with rabbit anti-YadA serum, and revealed by HRP-labeled antirabbit antibodies. The arrow indicates the monomer of YadA. Lanes are as in panel A.

specific enterocyte receptor has been described so far. It must also be remembered that the structure of YadA is different from that of the majority of the adhesins involved in mucosal colonization. The following other properties of YadA seem to be related to a later phase in the invasion process. (i) YadA adheres to components of the extracellular matrix, such as collagen (17, 47) and fibronectin (54). How these adhesion properties contribute to the tissue invasion is not very clear yet, because *Yersinia pestis* gains in invasiveness while loosing YadA (44). (ii) YadA interferes with the alternative complement pathway activation, i.e., killing by complement (2) and opsonization (53) leading to phagocytosis by polymorphonuclear leukocytes (31). This property, which is achieved by binding factor H, as shown in this paper, is clearly relevant to the pathogenesis of *Y. enterocolitica*. One could wonder why a *Yersinia* function favoring the bacterial invasion is missing in *Y. pestis*. It should be remembered that *Y. pestis* produces a capsule, called fraction I antigen, which has an antiphagocytic activity (8). YadA is thus dispensable, in this regard, for *Y. pestis*, which might explain its loss by a point mutation (45).

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