*Proc. Natl. Acad. Sci. USA*
Vol. 89, pp. 3561–3565, April 1992
Microbiology

# Bacterial resistance to complement killing mediated by the Ail protein of *Yersinia enterocolitica*

**(adherence/serum/epithelial cell)**

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*Contributed by Stanley Falkow, January 6, 1992*

**ABSTRACT** **Ail is a 17-kDa outer membrane *Yersinia* protein that mediates bacterial attachment to, and invasion of, cultured epithelial cells. We report here an alternative role for Ail in the pathogenesis of *Yersinia* infection. We found that *Escherichia coli* HB101 harboring the 4-kilobase recombinant *ail* clone pVM102 were highly resistant to killing in up to 50% normal human serum. A 674-base-pair fragment of DNA from pVM102, which encodes the *ail* gene, was inserted into pUC18 and shown to promote full resistance to complement killing in *E. coli* HB101. Cellular attachment and resistance to complement killing in a plasmid-cured *inv*$^-$ strain of *Yersinia enterocolitica* (0:8) was correlated with the thermoinduced expression of Ail at 37°C. Insertional inactivation of *ail* in *Y. enterocolitica* resulted in loss of both thermoinduced bacterial properties. Cellular attachment and serum resistance were restored by complementation of the defect by plasmid-encoded *ail*. Complementation of cell attachment activity required bacterial growth at 37°C, indicating that an additional thermoinduced factor is required for this Ail function. In addition, these studies reveal that functional homology exists between Ail and the structurally related protein Rck, which promotes resistance to complement killing in *Salmonella typhimurium*.**

Bacterial pathogens have evolved several strategies to neutralize normal host defense mechanisms. Resistance to the bactericidal activity of complement and the ability to enter eukaryotic cells are two distinct strategies commonly used for this purpose (1). *Yersinia* that cause disease in mammals, including the enteropathogen *Yersinia enterocolitica*, utilize both of these pathogenic strategies (2).

*Yersinia* attachment to, and invasion of, eukaryotic cells is thought to play a crucial role in allowing the bacteria to colonize and penetrate host cell barriers (3). Two chromosomally encoded genes have been isolated as recombinant clones of *Yersinia* DNA that function in *Escherichia coli* to promote bacterial entry into eukaryotic cells (3). The *inv* gene encodes invasin, a surface protein that promotes efficient bacterial uptake into cultured animal cells expressing a $\beta$1 integrin receptor (3). A second gene called *ail* (attachment-invasion locus) allows laboratory strains of *E. coli* to attach to several epithelial cell types and efficiently enter select cell lines such as Chinese hamster ovary (CHO) cells (4). The *ail* gene has been epidemiologically linked exclusively to pathogenic species of *Y. enterocolitica* (5) and encodes the 17-kDa outer membrane Ail protein (6). Neither the identity of the host cell receptor(s) for Ail nor the nature of this cellular specificity is known.

In addition to the chromosomally encoded *inv* and *ail* determinants, factors encoded on a highly conserved 70-kilobase (kb) virulence plasmid called pYV (7) have been shown to mediate *Yersinia*–host cell interactions. The thermoinduced outer membrane protein YadA (previously called Yop1 or P1) promotes bacterial attachment to eukaryotic cells (8) and collagen (9) and may act in concert with other plasmid-encoded factors to promote an alternative pathway of *Yersinia* invasion (10).

After entry into the host, resistance to killing by serum is important for extracellular growth and survival of the bacteria in deeper tissues (11, 12). Resistance to complement killing by *Y. enterocolitica* has been shown to depend on bacterial growth temperature (13, 14). When grown at 37°C, the temperature encountered during infection of the mammalian host, the bacteria become resistant to killing by normal human serum (NHS), while bacteria grown at 28°C are sensitive. YadA contributes to this phenotype (13, 15), a function seemingly unrelated to its role in bacterial adherence. However, strains of *Y. enterocolitica* deficient in YadA or entirely lacking the virulence plasmid do retain detectable levels of resistance to complement when grown at 37°C (13, 14). In addition, thermoinducible resistance to the bactericidal action of complement in *Yersinia pseudotuberculosis* is not plasmid dependent (16). These data indicate that chromosomally encoded factors, as well as YadA, can contribute to the complement-resistance phenotype in *Yersinia*.

In this report, we show that, in addition to mediating bacterial adherence and invasion, Ail promotes high levels of resistance to complement killing. Ail is structurally related to several other bacterial outer membrane proteins that do not appear to share common cellular functions. Proteins homologous to Ail but of unknown function include the bacteriophage $\lambda$ Lom protein (6, 17) and OmpX from *Enterobacter cloacae* (18). Two Ail-related proteins are found in the pathogenic bacterium *Salmonella typhimurium*: PagC is required for full virulence and resistance to macrophage killing (19), and Rck promotes resistance to complement killing (20, 21). Our results demonstrate that at least two members of this protein family, Ail and Rck, share functional as well as structural homology.

## MATERIALS AND METHODS

**Bacterial Strains and Growth Conditions.** The bacterial strains and plasmids used are listed in Table 1. Bacteria were maintained on LB agar (GIBCO) medium at 28°C or were stored at −70°C in 50% LB broth (GIBCO)/50% (vol/vol) glycerol. Overnight cultures of bacteria were prepared by growth in LB for 18 hr at 28°C or 37°C with aeration. The cell density of a bacterial culture was adjusted to the desired colony-forming units (cfu)/ml by OD measurements at 600 nm.

**Construction of Plasmids.** The techniques used for molecular cloning were as described (22). The plasmid pLOM1 was constructed by subcloning a fragment of $\lambda$ DNA into the multiple cloning region of pUC18 essentially as described

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Abbreviations: cfu, colony-forming unit(s); NHS, normal human serum; IF, internal fragment; LPS, lipopolysaccharide.

Table 1. Bacterial strains and plasmids

| Strain or plasmid | Relevant properties | Ref. or source |
|---|---|---|
| *E. coli* | | |
| HB101 | Laboratory strain | 22 |
| SM10(λ*pir*) | *recA*::RP4, *pir*R6K | 23 |
| *Y. enterocolitica* | | |
| DP5102 | *inv*::*cam*, pYV cured | D. Pierson |
| YE5103, -4 | DP5102, *ail*::pJM703-IF | This study |
| pUC18 | *ori*ColE1, *lac*I'OPZ' | 22 |
| pIL14 | pBR322::*afa* | 24 |
| pPH18 | pUC18::*rck* | 21 |
| pLOM1 | pBR322::*lom* | This study |
| pVM102 | pBR322::*ail* | 4 |
| pAIL2 | pUC18::*ail* | This study |
| pAIL-IF | pUC18::*ail* IF | This study |
| pDP112 | pSC101::*ail* | D. Pierson |
| pJM703.1 | *ori*R6K, *mob*RP4 | 23 |
| pJM703-IF | pJM703.1::*ail* IF | This study |

(17). To construct pAIL2, a DNA fragment encoding the *ail* gene was subcloned from pVM102 into the multiple cloning region of pUC18 as follows. pVM102 was cleaved with *Dde* I, which releases a 674-base-pair (bp) fragment containing the *ail* open reading frame and promoter [coordinates 358–1032 (6)]. The fragment was purified, treated with the Klenow fragment of DNA polymerase to generate blunt DNA ends, and ligated to pUC18 cleaved with *Hin*cII. In pAIL2, *ail* is in the same transcriptional orientation with respect to *lac*. The plasmid pAIL-IF, which contains a 265-bp internal fragment (IF) of *ail* [coordinates 549–814 (6)] bounded by *Eco*RI sites, was constructed by unidirectional deletion of pAIL2 (25). The plasmid pJM703-IF was derived by ligating the IF *Eco*RI fragment of pAIL-IF with the large *Eco*RI fragment of the mobilizable suicide vector pJM703.1 (23).

**Insertional Inactivation of *ail* in *Y. enterocolitica*.** SM10(λ*pir*) encodes the conjugation functions of plasmid RP4 and the replication protein π of R6K to allow stable maintenance of the ampicillin-selectable plasmid JM703-IF (23). SM10(λ*pir*) harboring pJM703-IF was mated with *Y. enterocolitica* DP5102 *inv*::*cam* on LB agar plates at 28°C for 4 hr, and transconjugants were selected at 28°C on LB agar plates containing ampicillin (100 μg/ml) and chloramphenicol (30 μg/ml). Chromosomal DNA was isolated from transconjugants, digested with *Eco*RV, and analyzed by filter hybridization using the IF as a probe (4). The transconjugant YE5103 was transformed with the tetracycline-selectable plasmid pDP112 by electroporation (26).

**Complement Resistance Assay.** NHS from healthy volunteers was collected, aliquoted, and stored at −70°C. Overnight bacterial cultures (1.0 ml) were centrifuged and washed twice in phosphate-buffered saline containing 5 mM $MgCl_2$ (PBSM). Bacteria were diluted in PBSM to ≈1 × $10^8$ cfu/ml. An aliquot of diluted bacteria (50 μl) was mixed with an equal volume of various dilutions of NHS in PBSM and incubated at 37°C for 60 min. As a control, bacteria were mixed with 50 μl of diluted heat-inactivated (56°C, 30 min) NHS and incubated as described above. Serial dilutions of the mixtures were prepared and cfu were determined by plating on LB agar plates. Resistance to complement killing was quantitated by the method of Joiner (27). The extent of killing was expressed as $\log_{10}$ kill, calculated as $\log_{10}$ cfu/ml surviving in heat-treated NHS minus $\log_{10}$ cfu/ml surviving in unheated NHS.

**Hep-2 Cell Attachment Assay.** The human laryngeal epithelium (Hep-2) cell line was maintained and prepared for attachment assays as described (4). Glass coverslips were placed in a 24-multiwell tissue culture dish and seeded with 1 × $10^5$ Hep-2 cells. The following day, 5 μl (2 × $10^7$ cfu) of an overnight bacterial culture was inoculated into the well and incubated in a 5% $CO_2$/95% air incubator at 37°C for 3 hr. The culture medium was then removed and the monolayer was washed three times with PBS to remove nonadherent bacteria. The monolayer was then fixed in methanol and stained with Giemsa for microscopic examination and photography (10).

**SDS/PAGE and Immunoblot Analysis.** Overnight bacterial cultures were washed in PBS and adjusted to 1 × $10^9$ cfu/ml. Equal volumes (1.0 ml) were centrifuged and the cell pellet was resuspended in 100 μl of 2× sample buffer (28) containing 8 M urea. Whole-cell extracts were prepared (28) and 10 μl of each sample was resolved on an SDS/8–15% polyacrylamide gel. Gels were stained with Coomassie blue or immunoblots were prepared by electrophoretic transfer of protein from the gels to nitrocellulose filters (28). The filters were incubated sequentially with a mouse monoclonal antibody, 2B2, that is specific for Ail (unpublished data) and anti-mouse IgG antibody conjugated to alkaline phosphatase as described (28).

## RESULTS

**Resistance to Complement Killing in *E. coli* Mediated by *ail*.** *E. coli* HB101 transformed with the recombinant *ail* plasmid pVM102 bind with high efficiency to Hep-2 cells (4). To investigate whether *ail* could also mediate resistance to complement killing, HB101 harboring pVM102 was incubated in various dilutions of NHS for 1 hr at 37°C, and after this treatment viable bacteria were quantitated. Bacterial survival in NHS treated with heat to inactivate complement action was calculated and used to normalize the data (Fig. 1). HB101(pVM102) was highly resistant to complement killing over the range tested, with only a 1.5 log reduction in viability in 50% NHS. In contrast, HB101(pBR322) was killed to completion in only 10% NHS.

To demonstrate that this complement-resistance activity was mediated by the product of *ail*, a fragment of DNA containing the gene and promoter region of *ail* was isolated from pVM102 and cloned into the plasmid pUC18. Expression of Ail protein in HB101 from this plasmid, called pAIL2, was confirmed by immunoblot analysis (Fig. 2*A*, lane 1). For antigen detection, we used a mouse monoclonal antibody called 2B2, which was raised against purified Ail (ref. 6; unpublished data). When tested for complement resistance, HB101(pAIL2) showed similar high levels of resistance to complement killing as compared to HB101(pVM102), with a

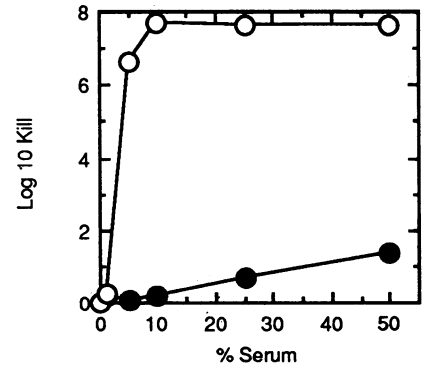


FIG. 1. Measurement of complement resistance in *E. coli* HB101. HB101 harboring pBR322 (○) or pVM102 (●) was grown at 28°C, diluted to 1 × $10^8$ cfu/ml in PBSM, and mixed with an equal volume of various dilutions of NHS. After incubation at 37°C for 1 hr, serial dilutions of each mixture were prepared and viable bacteria (cfu/ml) were quantitated by plating. Results are expressed as $\log_{10}$ kill, which is calculated as $\log_{10}$ cfu/ml surviving in heat-inactivated serum minus $\log_{10}$ cfu/ml surviving in fresh serum.

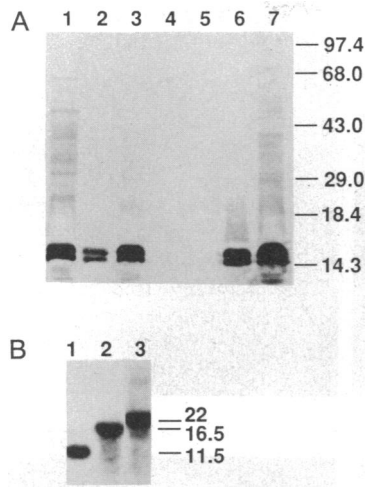


FIG. 2. Analysis of the *ail* gene and Ail protein expression in *E. coli* and *Y. enterocolitica*. (*A*) Immunoblot analysis of Ail expression. From *E. coli* HB101(pAIL2) (lane 1) and *Y. enterocolitica* (lanes 2–7), total cell extracts were prepared from equal cfu of bacteria and were analyzed by immunoblotting. Bacteria were grown at 28°C (lanes 1, 2, 4, and 6) or 37°C (lanes 3, 5, and 7). For *Y. enterocolitica*, the strains tested were DP5102 (lanes 2 and 3), YE5103 (lanes 4 and 5), and YE5103(pDP112) (lanes 6 and 7). The sizes in kDa of the protein molecular mass standards are indicated on the right. (*B*) Filter blot hybridization analysis of the *ail* locus. Chromosomal DNA was isolated from DP5102 (lane 1), YE5104 (lane 2), and YE5103 (lane 3); digested with *Eco*RV, which does not cleave pJM703-IF (6 kb); and analyzed by filter blot hybridization. The probe used was the *ail* IF of pAIL-IF. The 11.5-kb *Eco*RV fragment detected in lane 1 corresponds to the wild-type *ail* locus. Insertion of either monomeric (YE5104; lane 2) or dimeric (YE5103; lane 3) pJM703-IF into *ail* results in *Eco*RV fragments of ≈16.5 and ≈22 kb, respectively.

reduction in viability of ≈1 log in 50% NHS (Table 2). HB101(pAIL2) also exhibited cell binding activity for Hep-2 cells (Table 2).

**Expression of Complement Resistance and Cell Binding Activity in *Y. enterocolitica*.** We next sought to determine whether the cell binding and complement resistance activities of Ail thus far characterized in *E. coli* were relevant to its normal function in *Y. enterocolitica*. To avoid competing activities of proteins that might mask the activity of Ail, we analyzed the strain DP5102 (Table 1), a derivative of *Y. enterocolitica* 8081 (7), which is cured of pYV and lacks a functional *inv* gene. Previous reports indicated that plasmid-cured strains of *Y. enterocolitica* were more resistant to complement action when grown at 37°C than at 28°C (13, 14). Therefore, DP5102 was grown at 28°C or 37°C and tested for the capacity to resist complement killing in various dilutions of NHS. Our results confirmed the previous observations, as DP5102 grown at 37°C was resistant to killing over the range tested, while bacteria grown at 28°C were significantly more sensitive and were all killed in 50% NHS (Fig. 3*A*). In the Hep-2 cell binding assay, DP5102 exhibited binding activity only when grown at 37°C (Fig. 4). This activity in *Y. enterocolitica* is likely analogous to the thermoinduced cell binding activity observed in a plasmid cured *inv* strain of *Yersinia pseudotuberculosis* (10). Concurrent with the observed increase in complement resistance and cell binding in DP5102 grown at 37°C was an increase in the expression of Ail as detected by immunoblotting (Fig. 2*A*, lanes 2 and 3).

Table 2. Measurement of complement resistance and attachment to Hep-2 cells by HB101 expressing proteins related to Ail

| Strain | Protein | $Log_{10}$ kill* | Attachment† |
|---|---|---|---|
| HB101(pUC18) | | 7.69 ± 0.25 | − |
| HB101(pAIL2) | Ail | 0.99 ± 0.15 | + |
| HB101(pIL14) | Afa-1 | 7.46 ± 0.14 | + |
| HB101(pLOM1) | Lom | 7.46 ± 0.31 | − |
| HB101(pPH18) | Rck | 3.73 ± 0.08 | (+) |

*Values represent means ± SD of three experiments in 50% NHS.
†Results of Hep-2 cell attachment assays as described in Fig. 4.

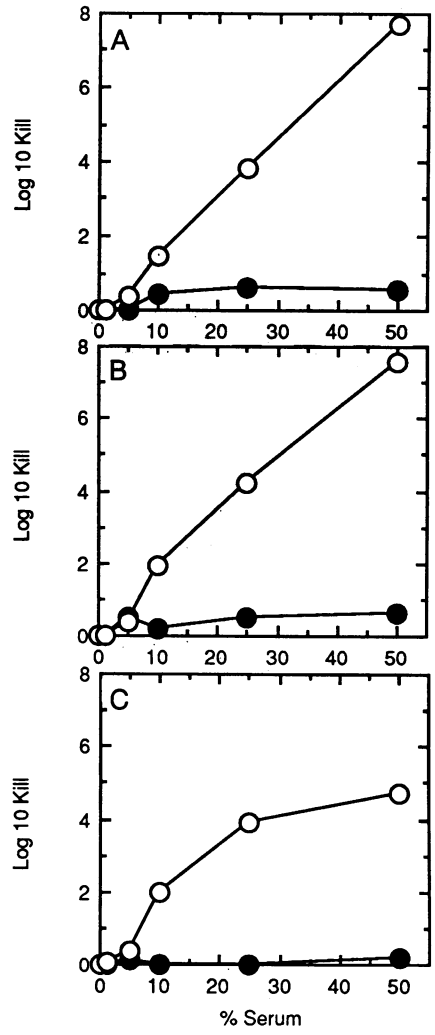


FIG. 3. Measurement of resistance to complement killing in *Y. enterocolitica*. (*A*) Effect of growth temperature of complement resistance in DP5102. DP5102 was grown at 28°C (○) or at 37°C (●) and resistance to killing in various dilutions of NHS was determined as described in Fig. 1. (*B*) Effect of *ail* on complement resistance in YE5103 (○) or YE5103(pDP112) (●) grown at 28°C. (*C*) Effect of *ail* on complement resistance in YE5103 (○) or YE5103(pDP112) (●) grown at 37°C.

**Disruption of *ail* Leads to Loss of Thermoinducible Resistance to Complement Killing and Cell Binding in *Y. enterocolitica*.** To prove that these thermoinduced activities were attributed to Ail, we constructed a strain of *Y. enterocolitica* lacking functional *ail*. By the technique of Miller and Mekalanos (ref. 23; see *Materials and Methods*), directed single crossover insertions into *ail* were obtained. Chromosomal insertions of the suicide vector pJM703-IF into *ail* were characterized by filter blot hybridization (Fig. 2*B*). As shown, two classes of insertion mutants were obtained. The sizes of the chromosomal DNA restriction fragments indicate that the two recombinant classes, represented by YE5104 and YE5103, arose from the integration into *ail* of monomeric or dimeric pJM703-IF, respectively. YE5103 was chosen for further study, as its growth rate under selection was equal to that of the unselected parent. Immunoblot analysis of

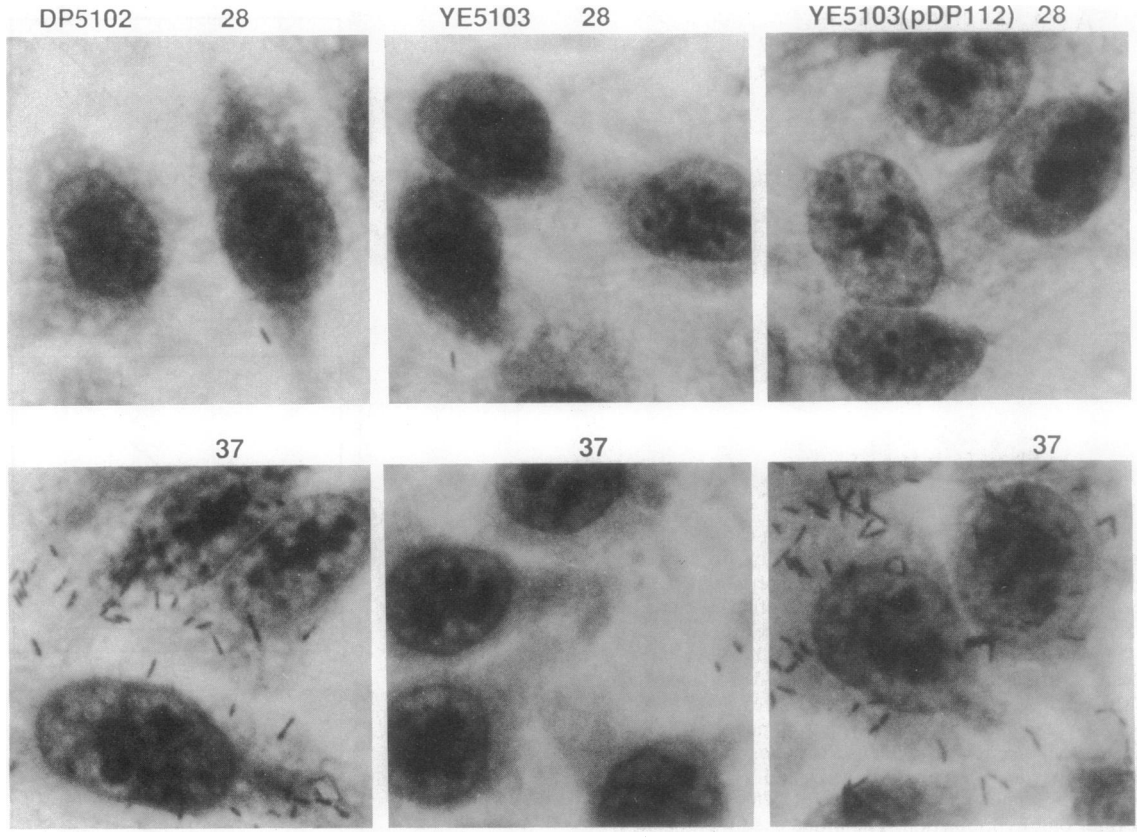


FIG. 4. Attachment of *Y. enterocolitica* to Hep-2 cells. The indicated strains, grown at 28°C (*Upper*) or 37°C (*Lower*), were tested for attachment activity to Hep-2 cells. After the bacteria were allowed to associate with Hep-2 monolayers for 3 hr, the monolayers were washed to remove unbound bacteria, stained with Giemsa to visualize attached bacteria, and photographed. (×55.)

YE5103 for production of intact Ail confirmed that the *ail* gene was disrupted (Fig. 2*A*, lanes 4 and 5).

YE5103 cultivated at 28°C or 37°C was tested in standard fashion for cell binding and complement resistance. As expected, YE5103 grown at 28°C was sensitive to complement killing (Fig. 3*B*), and lacked Hep-2 cell binding activity (Fig. 4). Similar results were obtained with YE5103 grown at 37°C: in NHS concentrations ranging from 0% to 25%, these cells had levels of complement resistance comparable to YE5103 grown at 28°C (Fig. 3*C*). Ail therefore played a major role in thermoinduced resistance to complement. Other surface components may also have contributed, since at the highest NHS concentration (50%), YE5103 retained residual complement resistance. YE5103 grown at 37°C did not bind to Hep-2 cells (Fig. 4), demonstrating that Ail was also necessary for this thermoinduced activity in *Y. enterocolitica*.

The *ail* mutation in YE5103 was complemented in trans with the low copy *ail* plasmid pDP112 (Table 1). Expression of Ail from the plasmid was confirmed by immunoblotting (Fig. 2*A*, lanes 6 and 7). Because the *ail* gene was present in multiple copies, Ail was expressed at greater than normal levels in YE5103(pDP112) at both growth temperatures. Overexpression of Ail was sufficient to promote complement resistance regardless of growth temperature (Fig. 3 *B* and *C*). However, overexpression of Ail in YE5103(pDP112) grown at 28°C did not restore cell binding; growth of the bacteria at 37°C was required for this phenotype (Fig. 4). These results indicated that Ail was necessary and sufficient for complement resistance in *Y. enterocolitica* but that an additional thermoinduced factor(s) was required for Ail-mediated cell binding.

**Ail Shares Structural and Functional Homology to the Rck Protein of *S. typhimurium*.** To explore the possibility that the homology among the proteins related to Ail might result in functional similarities with regard to cell binding and complement resistance, we compared the properties of HB101 expressing Ail, Lom, and Rck. HB101 expressing Afa-1, an X-type afimbrial bacterial adhesion that is unrelated to Ail, was examined as a control. Plasmids encoding these proteins (Table 1) were introduced into HB101 and high-level expression of each protein was confirmed by SDS/PAGE analysis (data not shown). Each strain was then tested for resistance to complement killing and binding activity toward Hep-2 cells (Table 2). The control strain HB101(pUC18) as well as HB101(pIL14) and HB101(pLOM1) were killed to completion in the serum assay. HB101(pPH18) was resistant to killing, confirming that Rck can mediate resistance to complement in *E. coli* (21). Results from the cell binding assay indicated that Rck could also mediate a low level of bacterial attachment to Hep-2 cells. Quantitative assays of Hep-2 binding (4) revealed that the level of HB101(pPH18) attachment was significantly above that of HB101(pUC18), but approximately 10- to 100-fold lower than that of HB101(pAIL2) (unpublished data). As shown for HB101(pIL14), high-level expression of a bacterial adhesin does not always promote complement resistance. These results imply that Ail and Rck share some level of functional homology in the context of the *E. coli* background. Since Lom does not promote cell attachment or complement resistance, this type of functional similarity may be restricted to the related proteins found normally in pathogenic bacteria.

## DISCUSSION

In response to appropriate signals from the host, a bacterial pathogen may promote infection by synthesizing specialized surface proteins that interact with host-encoded factors. By

the application of both molecular and genetic techniques to this multifactorial process in *Yersinia*, we are beginning to define the nature of several of these specialized proteins and to identify the host proteins with which they interact. The major conclusion of this work is that at the growth temperature likely encountered during infection of the mammalian host, Ail is highly expressed in *Y. enterocolitica* and it functions in a dual capacity to promote cellular interactions and resistance to complement.

The *ail* gene was initially isolated and its product was characterized in *E. coli* (4). In this innocuous genetic background, the function of Ail could be analyzed without concern for competing activities from proteins with redundant activities, such as invasin and YadA. In addition to promoting bacterial attachment activity in *E. coli*, we found that high-level expression of Ail provided significant resistance to complement-mediated killing. Although Ail could theoretically play a role in these pathogenic phenotypes, it was unclear whether differences in the surface architectures of *E. coli* and *Yersinia* contributed to the observed activity of Ail. Therefore, to extend these results to *Yersinia*, a reverse genetics approach was taken to examine the activities of Ail in its normal environment.

In a *Y. enterocolitica* strain lacking invasion and YadA, it was determined that Ail activity was detectable and that it promoted both bacterial attachment and resistance to complement killing. However, these activities were dependent on growth of the bacteria at 37°C, which was required to trigger high-level expression of Ail. Mutational complementation indicated that Ail was necessary and sufficient for complement resistance, but that an additional thermoinduced factor(s) was required for Ail-mediated attachment and, most likely, invasion. One possibility is that the structure of lipopolysaccharide (LPS) is important in the function of Ail as an adhesion/invasin. For example, the presence of hydrophobic sugars in the O-group side chains might mask one domain or region of Ail that is required for receptor binding. It is known that growth of enteropathogenic *Yersinia* at 37°C leads to the loss of O-group sugars that are present during cultivation at 28°C (2, 29). Ail may function well as an adhesin/invasin in laboratory strains of *E. coli* because the LPS of such strains generally lacks O-group side chains. Differences in the structure of LPS among the *Yersinia* might also explain why introduction of *ail* into nonpathogenic strains of *Y. enterocolitica* promotes complement resistance, but not cell attachment (unpublished data).

As demonstrated *in vitro*, invasin and YadA act to promote cellular interactions, resistance to complement killing, or both. However, the role these factors play in the *Yersinia* infection process is less obvious. In fact, it has been shown that for lethal oral infections of mice with *Y. pseudotuberculosis*, either determinant is dispensable, and that strains lacking both YadA and invasin are enhanced in their virulence (30). As we have shown that Ail has the capacity to promote cellular interactions as well as bacterial resistance to complement killing, it is possible that Ail can compensate for the absence of both YadA and invasin in an animal infection model of acute disease.

It is of interest to determine how Ail facilitates bacterial resistance to complement killing. A number of other enterobacterial surface structures have been implicated in this activity (reviewed in ref. 27). These include the acidic polysaccharide KI capsule, proteins such as Iss and TraT, and LPS. Only for LPS has the mechanism of complement resistance been rigorously defined (27). The LPS of complement-resistant *Salmonella* contains particularly long O-group side chains, which leads to activation of terminal complexes at a safe distance from the bacterial surface (27). Since Ail is a small protein and does not affect the gross structure of LPS (unpublished data), it is likely that it acts in a different capacity. High numbers of molecules on the surface could simply sterically hinder terminal attack complexes from insertion into the hydrophobic regions of the bacterial membrane. However, it is tempting to speculate that the complement resistance activity mediated by Ail is more specific and related to the capacity of this protein to bind host factors. In theory, by binding a serum factor or complement component, Ail could subvert the stable formation of active attack complexes. Attachment to a host factor that is bound to the surface of a eukaryotic cell might similarly permit uptake of bacteria. Identification of the protein(s) bound by Ail under different bacterial growth conditions should reveal the molecular function of this protein as well as provide insights into the function of the related members of the family.

We thank E. Heffernan, D. Guiney, and D. Pierson for supplying plasmids and for communicating their results prior to publication. We also thank A. Hromockyj and M. Joffe for critical reading of the manuscript. This work was supported by Public Health Service Grant AI26195-02 from the National Institutes of Health. J.B.B. was supported by National Institutes of Health Fellowship AI07851-01.

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