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Yersinia High-Pathogenicity Island Contributes to Virulence in *Escherichia coli* Causing Extraintestinal Infections

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The *Yersinia* high-pathogenicity island (HPI) encodes an iron uptake system mediated by the siderophore yersiniabactin (Ybt) and confers the virulence of highly pathogenic *Yersinia* species. This HPI is also widely distributed in human pathogenic members of the family of *Enterobacteriaceae*, above all in extraintestinal pathogenic *Escherichia coli* (ExPEC). In the present study we demonstrate a highly significant correlation of a functional HPI and extraintestinal virulence in *E. coli*. Moreover, using a mouse infection model, we show for the first time that the HPI contributes to the virulence of ExPEC.

Highly pathogenic strains of *Yersinia pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* biotype IB possess a genomic island designated the high-pathogenicity island (HPI). This 35- to 45-kb island carries a siderophore-mediated iron uptake system named the yersiniabactin (Ybt) locus, which is required for full virulence expression in *Yersinia* (4). A unique characteristic of the HPI is its wide distribution in different members of the family of *Enterobacteriaceae*, especially in extraintestinal pathogenic *Escherichia coli* (ExPEC) causing urinary tract infection, septicemia, and meningitis in newborns (5, 8, 16). The Ybt siderophore system has been shown to be functional in the vast majority of HPI-positive ExPEC strains, indicating a selective pressure for maintenance of this genetic module (16). However, experimental evidence for a direct impact of the HPI on virulence of ExPEC has not yet been obtained. In this study we determined the distribution and the functionality of the HPI and its relation to virulence in a previously described collection of commensal and extraintestinal *E. coli* strains that had been tested for extraintestinal virulence in a mouse model (14). Our data indicate a high rate of association of a functional HPI with mouse virulence. The same mouse model was used to evaluate the impact of a functional HPI on virulence by examining two wild-type strains from the collection together with their isogenic Ybt synthesis mutants. In this study, we provide first-time evidence of an impact of the HPI on the virulence of ExPEC.

All 82 *E. coli* strains of the previously described collection (14), representing isolates with different phylogenetic and virulence traits, were examined (i) for the presence of the *Yersinia* HPI using PCR and Southern hybridization (16, 17) and (ii) for the functionality of the HPI by analysis of Ybt synthesis using a reporter gene-mediated bioassay (16). The strain collection had previously been surveyed for virulence factors, such as attachment factors (*pap*, *sfa*/*foc*, and *afa*), an *E. coli* transmembrane protein involved in neonatal meningitis (*ibe10*), the alpha-hemolysin (*hly*), and the aerobactin siderophore (*aer*) (14). Associations between the presence of HPI, the expression of Ybt, the occurrence of other virulence factors, and mouse lethality were determined by χ^2 tests and calculations of odds ratios using SigmaStat software (version 2.03; SPSS Inc., Richmond, Calif.). The threshold for statistical significance was a P of <0.05. Comparison of the prevalence of HPI within the different phylogenetic groups A, B1, B2, and D (7) was performed by determining Spearman's rank order correlation using SigmaStat software (9).

First we determined the distribution of HPI within the *E. coli* strain collection with regard to phylogenetic groups. The HPI was found significantly more frequently in *E. coli* strains of the phylogenetic group B2 (92%) than in strains of groups A (35%) and B1 (48%) (Table 1). This finding corresponds with that of previous studies on the phylogenetic distribution of the HPI (5) and supports the idea that virulence determinants are accumulated in the phylogenetic group B2, to which most of the EXPEC isolates belong (2, 3, 10). More strikingly, the presence of HPI was significantly associated with lethality in mice (Table 2). As the HPI codes for the Ybt siderophore system, we looked at a functional HPI with regard to Ybt production. The HPI was found to be functional in HPI-positive B2 strains (94%) more often than in corresponding strains of group A (71%) and B1 (45%). Ybt production was significantly associated with *E. coli* strains that were lethal to mice, which killed 2 to 10 out of 10 mice ($\chi^2 = 37.28$; $P < 0.001$). Interestingly, of all the virulence factors examined (*pap*, *sfa*/*foc*, *aer*, *hly*, K1 antigen, *afa*, and *ibe10*), the functional HPI showed the highest association with lethality in mice (Table 2) (14).

Two HPI-positive *E. coli* strains of the collection (IAI51 and IAI52) (14) with a high-virulence phenotype were selected to determine the impact of HPI on mouse virulence. These strains lack some of the potential virulence factors, such as aerobactin and hemolysin (14). The HPI-encoded Ybt system of the strains was inactivated by insertion of a kanamycin resistance gene cassette into the *irp1* gene coding for Ybt synthesis protein HMWP1 as described previously (12). The

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TABLE 1. Correlation between presence of HPI, production of Ybt, and affiliation to each of the phylogenetic groups of *E. coli*

Phylogenetic group (no. of strains)	Presence of HPI			Ybt production		
	No. of strains	CC[a]	*P*	No. of strains	CC[a]	*P*
A (20)	7	−0.37	<0.001	5	−0.31	0.009
B1 (23)	11	−0.24	0.032	5	−0.40	0.008
B2 (37)	34	0.50	<0.001	32	0.58	<0.001
D (2)	2	0.31	NS[b]	2	0.15	NS

[a] Correlation coefficient (CC) measured by Spearman rank order correlation represents the strength of correlation, with the maximum value for positive association at 1 and that for negative association at −1.
[b] NS, not statistically significant.

presence of the kanamycin cassette within *irp1* was confirmed by PCR and Southern hybridization. In order to recomplement the mutation of the *irp1* gene, plasmid pCP1 was introduced into the mutant strains as described recently for *Y. enterocolitica* (11). The pCP1 plasmid carries the entire 35-kb core region of the HPI coding for the Ybt siderophore system. Original strains, mutants, and recomplemented strains were examined for the structure of HPI by PCR and Southern hybridization (12) and for Ybt synthesis using a reporter gene-mediated bioassay (16). Pathogen-free female mice of the outbred white Swiss mouse lineage (Swiss OF1-Caw) (age, 4 weeks; body weight, 14 g) were purchased from IFA Credo (Orléans, France). *E. coli* strains were prepared and isolated as described previously (14), except for the mutant strains, which were grown with kanamycin (40 μg/ml). Groups of 30 mice were subcutaneously inoculated with either wild-type, or mutant, or recomplemented mutant strains (10^8 CFU). After inoculation, the mice were observed daily for up to 1 week to score for killed mice.

The results of the mouse virulence tests of wild-type strains and mutants correspond entirely with the statistical correlation of HPI with a virulent phenotype. Sixteen out of 30 mice infected by *E. coli* wild-type strain IAI52 were killed, whereas none of the mice infected with the *irp1* mutant died ($\chi^2 = 21.82$; $P < 0.001$). Even more pronounced results were obtained using the *E. coli* IAI51 strain, which killed 28 of 30 mice in comparison to the *irp1* mutant (1 of 30; $\chi^2 = 48.65$; $P < 0.001$). Introduction of the pCP1 plasmid partially restored the mouse-lethal phenotype of the *irp1* mutants with 2 of 10 mice killed by the recomplemented strains. This partial restoration is likely due to the fact that the pCP1 plasmid is rather instable in vivo, as has been reported for *Y. enterocolitica* previously (11). Thus, the results demonstrate the rather clear-cut impact of the HPI on virulence.

TABLE 2. Relationship between different virulence traits and mouse lethality in *E. coli* strains of the IA collection ($n = 82$)

Associated trait	No. (%) of strains	Lethality			
		χ^2 value	Odds ratio	95% CI[b]	*P*
Ybt production	44 (53.7)	37.28	35.00	7.96–178.65	<0.001
pap	32 (39.0)	34.90	24.60	6.57–99.92	<0.001
HPI	54 (65.9)	33.28	ID	ID	<0.001
sfa/foc	15 (18.3)	23.46	ID	ID	<0.001
hly	15 (18.3)	13.63	12.43	2.34–87.68	<0.001
K1 antigen	14 (17.1)	5.19	4.04	1.01–17.29	0.023
aer	24 (29.3)	2.94	4.77	0.99–8.87	0.029
ibe10	5 (6.1)	2.82	5.63	0.55–138.67	NS
afa	3 (3.6)	0.14	0.62	0.02–9.41	NS

[a] Values represent χ^2 test results and odds ratios without corrections. The χ^2 value for each trait represents the strength of association with a mouse-lethal phenotype. Associations with a P of <0.05 are considered statistically significant. ID, indeterminable; NS, not statistically significant.
[b] 95% CI, 95% confidence interval limits for odds ratio.

The HPI codes for a siderophore Ybt-mediated iron uptake system. The iron limitation during the colonization and infection of a host organism provides a good reason why an endogenous iron uptake system would be advantageous. This is true for yersiniae, for which the HPI-encoded Ybt is the only endogenous siderophore system. However, *E. coli* possesses at least one other endogenous siderophore system (enterobactin), and in many pathogenic isolates the aerobactin system is detectable, too. One reason for the predominance of Ybt in ExPEC could be the high iron-binding affinity with an Fe^{3+}-Ybt formation constant (pK) of 36.6 compared to those of Fe^{3+}-enterobactin (35.5) and Fe^{3+}-aerobactin (23.3), which are the other endogenous siderophores in *E. coli* (13, 15). Interestingly, the *irp1* mutant strains that were defective for Ybt siderophore synthesis revealed no growth impairment under iron depletion compared to the parental strains (data not shown). Previously demonstrated has been a dual role of siderophores with (i) delivery of iron to the microbial pathogen and (ii) immunosuppression of the host (1). The iron-binding constants of siderophores alone might therefore not be sufficient to explain their contribution to virulence. An intriguing hypothesis for the association of functional HPI with mouse virulence as well as with the presence of other virulence factors is the gene-regulatory effect of Ybt. It has been shown for yersiniae that, in complex with the HPI-encoded protein YbtA, Ybt acts as a positive regulator of gene expression (6). It is conceivable that genes outside the HPI, e.g., other virulence factors, could be influenced by the Ybt-mediated regulation. Thus, cross talk between different virulence determinants could lead to a regulatory network of virulence expression in *E. coli* sensed by an iron-limited environment. The present study may encourage further investigations of ascending urinary tract infection or meningitis in animal models to determine the impact of a functional HPI on virulence in these routes of ExPEC infections.

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