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Variability of the Protein Sequences of LcrV Between Epidemic and Atypical Rhamnose-Positive Strains of *Yersinia pestis*

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Abstract. Sequencing of *lcrV* genes and comparison of the deduced amino acid sequences from ten *Y. pestis* strains belonging mostly to the group of atypical rhamnose-positive isolates (non-*pestis* subspecies or pestoides group) showed that the LcrV proteins analyzed could be classified into five sequence types. This classification was based on major amino acid polymorphisms among LcrV proteins in the four "hot points" of the protein sequences. Some additional minor polymorphisms were found throughout these sequence types. The "hot points" corresponded to amino acids 18 (Lys → Asn), 72 (Lys → Arg), 273 (Cys → Ser), and 324-326 (Ser-Gly-Lys → Arg) in the LcrV sequence of the reference *Y. pestis* strain CO92. One possible explanation for polymorphism in amino acid sequences of LcrV among different strains is that strain-specific variation resulted from adaptation of the plague pathogen to different rodent and lagomorph hosts.

3.1 Introduction

LcrV (V antigen) of the medically significant yersiniae is a multifunctional protein involved in modification of innate immune response to these pathogens as well as in regulation and translocation of Yop effectors of the type III secretion system (Brubaker 2003). Moreover, LcrV is a major protective antigen and a principal component of the modern anti-plague subunit vaccines which are currently under development (Titball and Williamson 2004).

Recent studies also revealed some heterogeneity in the sequences of the *lcrV* genes of different origin (Adair et al. 2000; Anisimov, Lindler, and Pier 2004; Hakansson et al. 1993; Motin et al. 1992; Price et al. 1989; Roggenkamp et al. 1997; Sing et al. 2005; Snellings, Popek, and Lindler 2001; Song et al. 2004) especially in *Y. enterocolitica* (Hakansson et al. 1993; Sing et al. 2005; Snellings et al. 2001). Although major types of LcrV antigens of *Y. pestis* and *Y. pseudotuberculosis* are cross-protective (Motin et al. 1994), certain variations in the sequence of LcrV resulted in the reduction of the cross-protectivity against some yersiniae strains (Anisimov et al. 2004; Roggenkamp et al. 1997; Une and Brubaker 1984).

In this study we determined sequences of LcrV of ten strains of *Y. pestis* and compared them with those from the sequenced *lcrV* genes of this pathogen. Most of the strains used for our sequencing experiments belonged to atypical rhamnose-positive

isolates of *Y. pestis* avirulent to guinea pigs and humans (Anisimov et al. 2004; Song et al. 2004). They are generally known as pestoides group, which according to the classification adopted in Former Soviet Union countries are referred as non-main subspecies of *Y. pestis* (Anisimov et al. 2004). In another classification, this category of strains is referred as isolates of the microtus biovar (Song et al. 2004). Accordingly, the isolates of the typical biovars such as antiqua, medievalis and orientalis we designated here as epidemic strains to emphasize their historic role in plague pandemics.

3.2 Materials and Methods

The strains of *Y. pestis* analyzed in this study belonged to five "subspecies" circulating in the Eurasian natural plague foci and differed in their epidemiological significance (Table 1; Anisimov et al. 2004). All *lcrV* genes were sequenced by using primers LcrVF (5'-CAGCCTCAACATCCCTACGA-3'), LcrVFI (5'-GCAAAATGGCATCAAGCGAG-3'), and LcrVR (5'-TGTCTGTCGTCTCTTGTTGC-3') and compared with the data available at GenBank/EMBL/DDBJ (accession numbers, M26405, AF167309, AF167310.1, AE017043.1, CP000311).

3.3 Results and Discussion

3.3.1 Comparative Analysis of the V Antigen Sequence Heterogeneity

To address the genetic variation among LcrV antigens of *Y. pestis* strains of different origin and epidemiological significance, we determined the complete sequence of *lcrV* genes for ten strains. We found that only three sequences were identical to the predominant *lcrV* sequence-type initially reported by Price et al. (1989). Two of the isolates which showed this type of LcrV (I-1996 and I-2638, see Table 1) belonged to the epidemic type of *Y. pestis* strains, biovar antiqua, and one isolate (I-2836, Table 1) was atypical. Furthermore, nucleotide sequence analysis of the amplified *lcrV* alleles indicated that the sizes of the *lcrV* genes ranged from 975 to 981 nucleotides in length (encoding 324 to 326 amino acids). The LcrV proteins analyzed can be classified into sequence types A - biovar microtus strain 91001; subspecies *caucasica* strains: Pestoides F, 1146, C-585 (324 amino acids), B - subspecies *pestis* biovar antiqua strains: Antiqua, I-1996, I-2638, biovar medievalis strain KIM, biovar orientalis strain CO92; subspecies *ulegeica* strain I-2836 (326 amino acids), B/C - subspecies *ulegeica* strain I-2422 (326 amino acids), C - subspecies *hissarica* strain A-1728; subspecies *caucasica* strain C-582 (324 amino acids), and D - biovar antiqua strain Angola (326 amino acids); subspecies *altaica* strains: I-3455 (326 amino acids), I-2359 (325 amino acids). This classification was based on appearance of major amino acid polymorphisms among LcrV antigens in the four "hot points" of the protein sequences. Some additional minor polymorphisms were found through out these sequence types. The "hot points" correspond to amino acids 18 (Lys →

Table 1. *Y. pestis* strains used in these studies

Strain	Geographical origin[a]	Biovar/ subspecies[b]	Main host	*lcrV* accession number	Reference
C-585	Transcaucasian highland (foci #4-6)	antiqua / caucasica	*Microtus arvalis*		This study
C-582	Transcaucasian highland (foci #4-6)	antiqua / caucasica	*M. arvalis*	DQ489557.1	This study
1146	Transcaucasian highland (foci #4-6)	antiqua / caucasica	*M. arvalis*		This study
I-1996	Trans-Baikal focus #38	antiqua / *pestis*	*Citellus dauricus*		This study
I-2638	Mongun-Taigin focus #37	antiqua / *pestis*	*Citellus undulatus*		This study
A-1728	Gissar focus #34, Tadjikistan, Uzbekistan	medievalis / *hissarica*	*Microtus carruthersi*	DQ489552.1	This study
I-2422	Northeast Mongolia, Gobi Desert	medievalis / *ulegeica*	*O. pricei*	DQ489554.1	This study
I-2836	Northeast Mongolia, Gobi Desert	medievalis / *ulegeica*	*Ochotona pricei*	DQ489553.1	This study
I-2359	Mountain-Altai focus #36	medievalis / *altaica*	*O. pricei*	DQ489556.1	This study
I-3455	Mountain-Altai focus #36	medievalis / *altaica*	*O. pricei*	DQ489555.1	This study

For information on [a] geographical location of plague natural foci and [b] biovar-subspecies interrelations see ref. (Anisimov et al. 2004).

Asn), 72 (Lys → Arg), 273 (Cys → Ser), and 324-326 (Ser-Gly-Lys → Arg) in the LcrV sequence of the reference epidemic strain CO92:

type A Lys_{18}, Lys_{72}, Cys_{273}, Arg_{324};
type B Lys_{18}, Lys_{72}, Cys_{273}, Ser-Gly-$Lys_{324\text{-}326}$;
type B/C Asn_{18}, Arg_{72}, Cys_{273}, Ser-Gly-$Lys_{324\text{-}326}$;
type C Asn_{18}, Arg_{72}, Ser_{273}, Arg_{324};
type D Asn_{18}, Arg_{72}, Ser_{273}, Ser-Gly-$Lys_{324\text{-}326}$.

The LcrV of the type B group is a predominant variant among *Y. pestis* strains sequenced so far. It has been found in 23 *Y. pestis* strains belonging to all three biovars of the epidemic *Y. pestis* isolates originated from Asia, Africa and Americas as well as in one representative of *ulegeica* subspecies from Mongolia (Adair et al. 2000; Motin et al. 1992; Price et al.; this study). This type of LcrV is the most homologous to LcrV from *Y. pseudotuberculosis* strains of 1b and 3 serovars available in the GenBank/EMBL/DDBJ (Bergman et al. 1991; Motin et al. 1992). The strains possessing LcrV type B may have "universal virulence" (epidemic strains are pathogenic to many mammals) or be pathogenic only for a few rodent and lagomorph species including their natural host (atypical, subspecies *ulegeica*) (Anisimov et al. 2004). It is unlikely that the "selective virulence" of the atypical rhamnose-positive

Y. pestis strains is a result of variations in the LcrV amino-acid sequence, although other LcrV types were found exclusively in the atypical strains circulating in the populations of *Microtus* species (types A and C) or *O. pricei* (types B/C and D). Data about the natural host of the strain Angola, type D are not available. The strains carrying the same LcrV types are circulating in geographically distant natural plague foci: type A – foci #4-6 (Transcaucasian highland, Armenia, Azerbaijan, Georgia) and focus L (Inner Mongolia, China); type C – foci #4-6 (Transcaucasian highland, Armenia, Azerbaijan, Georgia) and focus #34 (Hissarian Ridge, Tadjikistan, Uzbekistan); type D – focus #36 (Mountain Altai, Russia) and Angola (Africa).
The deletion that was caused by two direct repeats (ATGACACG) at the 3' terminus of *lcrV* gene (Song et al. 2004) is characteristic of the *Y. pestis* types A and C (this study) as well as to the majority of LcrV variants from *Y. enterocolitica* (Gen- Bank/EMBL/DDBJ accession numbers, X96796.1, X96797.1, X96798.1, X96799.1, X96800.1, X96801.1 (Roggenkamp et al. 1997), AF102990.1 (Hakansson et al. 1993), AF336309.1 (Snellings et al. 2001), and AY150843.2). Moreover, other amino-acid replacements such as amino acids 18 (Lys → Asn), 72 (Lys → Arg), 273 (Cys → Ser), are specific only for *Y. pestis*.

Taken together, these observations demonstrate that LcrV can display polymorphism in size and amino acid sequence among atypical rhamnose-positive *Y. pestis* strains. The presence of a modified LcrV protein apparently have not altered lethality of these strains for mice and their natural hosts, since atypical rhamnose-positive *Y. pestis* strains were reported to be highly virulent for these animal species (Anisimov et al. 2004; Song et al. 2004). However, the influence of LcrV sequence polymorphism on the "selective virulence" (host range) of these strains needs to be further investigated.

3.3.2 Conclusion

This study demonstrated that the LcrV antigens analyzed could be classified into five sequence types, according to the appearance of major amino acid polymorphisms among LcrV proteins in the four "hot points" of the proteins with some minor polymorphisms found throughout these sequence types. One possible explanation for variation in amino acid sequences among different strains is that strain-specific variation might result from adaptation for continued transmission of the plague pathogen within different rodent and lagomorph species. Further explorations into functional activity of different *Y. pestis* LcrV sequence types will reveal the biological significance of this phenomenon.

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