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Role of structural variations of polysaccharide antigens in the pathogenicity of Gram-negative bacteria

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ABSTRACT

Pathogenic bacteria employ many strategies to overcome the host immune system for extended survival and propagation in their hosts. Components of the bacterial outer-membrane play an important role in this process. When invading the host, Gram-negative bacteria often use a strategy, known as phase variation, that involves a reversible change in antigenic determinants, frequently polysaccharides. This means that the genes encoding the outer-membrane antigens undergo reversible changes within repeated simple DNA sequence motifs. The antigenic structure of the bacterial outer-membrane is influenced by the character of the host immune system, as well as by the targets for bacterial invasion. When the selection pressure of the immune system is absent or weak, bacteria can fail to synthesise the outer-membrane antigens, which are not needed at that time. Smooth-to-rough (S-R) mutation, an economical and often irreversible process in some Gram-negative bacteria, involves the gradual shortening of the lipopolysaccharide (LPS) O-chain. Under certain conditions, e.g., propagation in embryonated eggs or cell lines, some bacteria will cease synthesis of the complete LPS O-chain because it is an energy-demanding process. A type of gradual shortening of the LPS O-chain by *Coxiella burnetii*, traditionally called phase variation, is used in serological tests for the diagnosis of Q fever. This review discusses the role and function of polysaccharides, especially LPS produced by some Gram-negative bacteria, in bacterial survival.

Keywords Antigens, lipopolysaccharide, mutation, outer-membrane, phase variation, review

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INTRODUCTION

Pathogenic bacteria invading a host are under strong selective pressure from the host immune system and use a range of different strategies to escape its destructive effects. Bacteria can even change the structure of their outer-membrane components depending on the target for invasion, e.g., skin, blood or inner organs. This is a consequence of high-frequency reversible on–off switching of phenotypic expression, which also influences the potential for bacterial pathogenicity. Examples are Gram-negative bacteria, in which lipopolysaccharide (LPS), with lipid A as its endotoxic component and a structurally versatile O antigen (O-chain), is known to be an important virulence factor [1]. The O-chain, which is the exposed part of LPS, is a highly variable surface antigen [2]. If the bacterium lives in the absence of any immune system intervention or stress, it can adapt to this environment, and certain bacteria may even stop the synthesis of some protective compounds that are necessary for survival under more 'difficult' circumstances. This is the case for the smooth-to-rough (S-R) mutations of enteric Gram-negative bacteria, which gradually stop the synthesis of the LPS O-chain, as this is an energy-demanding process. However, the genetic deletions found in R mutants can also be irreversible. Although they save the expenditure of unnecessary energy, these mutants are not able to start the reverse process under changed environmental circumstances. Both processes, i.e., high-frequency reversible on–off switching of phenotypic expression and

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step-by-step S-R mutations, are bacterial responses to changing conditions brought about by the host immune system.

ON–OFF SWITCHING OF PHENOTYPIC EXPRESSION

High-frequency reversible on–off switching of phenotypic expression of cell surface components (phase variation) is a common feature of many virulence determinants. Bacteria have a range of phase-variable mechanisms, but not all phase-variable genes are associated with outer-membrane antigens [3]. Genetic diversity and mutations in bacteria may also have unfavourable consequences [4]. Phase variation is associated with reversible changes within repeated simple DNA sequence motifs that exhibit high mutation rates by loss or gain of repeat units during DNA replication. The switch can be influenced by the extracellular conditions. Such reversible changes are heritable and reversible between generations, and the switching frequency is characteristic for the gene, the bacterial species and the regulatory mechanism [5]. The mechanisms involved in on–off switching of target genes include genomic rearrangement, slipped-strand mispairing (SSM), or variation mediated by differential methylation that changes the expression of a bacterial genome component sequence [6]. Saunders *et al.* [7] described an analysis approach used to identify DNA sequence motifs within a complete bacterial genome sequence. This approach can identify and locate sequence repeats with motifs of 1–10 bases repeated at least five times, and allows detection of candidate genes for phase variation.

The most-studied pathogenic organisms that express this type of phase variation are *Neisseria* spp., *Haemophilus influenzae*, *Campylobacter jejuni* and *Helicobacter pylori*. Of these, *Neisseria meningitidis* and *Neisseria gonorrhoeae* are phase-variable human pathogens that have been studied extensively. Outer-membrane components, e.g., pili [8], LPS [9], outer-membrane proteins, capsules and haemoglobin receptors [10], of these bacteria show high-frequency variations in their expression. LPS of *Neisseria*, as well as that of *Haemophilus*, does not possess an O-chain.

The range of phase variation possibilities for *N. meningitidis* is illustrated in the study by Martin *et al.* [11], which identified 33 potentially phase-variable genes in *N. meningitidis* strain MC58. Bacterial structures that come into contact with parts of the host immune system can change their antigenic determinants in accordance with their immediate situation, thereby increasing antigenic variability and virulence, and concomitant penetration into the host organs. LPS contains terminal units that can quickly change from one structure to another. Investigation of clinical isolates of meningococci has revealed a wide LPS repertoire.

The phase-variable nature of the biosynthesis of LPS molecules produces a mixed population of organisms with respect to their genotype and phenotype [9]. Invasive strains of *N. meningitidis* are able to exchange DNA by transformation and recombination [12], and possess sophisticated mechanisms for adaptation to a rapidly changing microenvironment in the host, depending on locality, e.g., nasopharynx, blood or the central nervous system (with or without pili and capsules). Several mechanisms of *N. meningitidis* capsular phase variation have been described. The synthesis of capsular polysaccharides is increased in clinical isolates from patients with invasive disease, and is down-regulated in *N. meningitidis* isolates recovered from the nasopharynx [10].

A single strain of *N. gonorrhoeae* can produce a limited repertoire of LPS molecules that are required for passing through the human mucosal barrier [13]. However, LPS phase variation during natural infection has also been associated with another phase-variable surface component in order to create an invasive gonoccocal phenotype. *N. gonorrhoeae* uses sialylation of LPS to mediate its entry into epithelial cells and for serum resistance [14]. *N. gonorrhoeae* LPS containing low amounts of sialic acid allows the entry of the bacterium into human mucosal epithelial cells, whereas variants with highly sialylated LPS resist killing by complement and antibodies, but fail to enter human mucosal epithelial cells. There are differences in the expression of sialyltransferase between *N. gonorrhoeae* and *N. meningitidis*, with *N. gonorrhoeae* expressing significantly more sialyltransferase than *N. meningitidis* [15].

Inter- and intra-strain heterogeneity is demonstrated by the LPS produced by *H. influenzae*. Different LPS surface structures produce phase-variants of LPS, which is a principal component of the *H. influenzae* outer-membrane,

following interactions with the host immune system. O-acetylation of LPS is also subject to phase variation. Such acetylation would contribute to the hydrophobicity of LPS, which the bacterium can utilise during infection of the human respiratory tract [16]. Phase variation in LPS also correlates with phase-variable resistance–sensitivity to phage infection [17]. A possible molecular mechanism for the control of this high-frequency spontaneous gain or loss of epitopes is similar to that used by *Neisseria* [18,19]. Variation in the number of CAAT hyper-mutable repeated sequences in genomic loci *lic-1* and *lic-2*, which encode enzymes involved in the synthesis of LPS, can generate a translational switch [20,21]. The phase-variants produced during the course of infection also enhance the virulence of the bacterium. An individual strain of *H. influenzae* can express one or more glycosyl transferases, and *H. influenzae* phase variation mediated by tandem octanucleotide repeats within the *losA* gene has been described [22].

The wide variety of ways in which phenotypic expression can be altered in the bacterial world has been reviewed previously by Torres-Cruz and van der Woude [23]. These authors described the possibility of length variation of short repetitive DNA sequences in *Escherichia coli* as a result of SSM, mediated by the SSM-containing regions of *H. influenzae*. The SSM-containing regions were amplified from *H. influenzae* genomic DNA and integrated into the *E. coli* chromosome in the form of a recombinant phage.

High-frequency phase variation observed in the expression of capsule by a *C. jejuni* strain provides a demonstration of the glycan structure variation in the capsule produced by this pathogen. The loss of expression of high molecular mass glycan is the result of an insertion into the *kpsM* gene. This insertion increased the serum sensitivity and reduced the virulence of this particular strain of *C. jejuni* [24].

H. pylori has to survive the acidic environment of the human stomach in which it resides. Phase variation of *H. pylori* genes may contribute to evasion, colonisation and persistent infection of the stomach. Most LPS biosynthetic genes in *H. pylori* are associated with phase variation, and the genetic location of these genes can vary among strains. Of eight sequenced LPS biosynthetic genes, seven were associated with phase variation [25]. The O-chain of *H. pylori* LPS contains fucosylated oligosaccharides and the Lewis antigens, which mimic human blood group antigens. The expression of these antigens may be variable within the cell population of a single strain [26]. This type of antigenic variation is probably important for *H. pylori* during long-term colonisation. It is possible that the Lewis antigens continually change their phase, and that the expression of certain antigens is beneficial for colonisation and infection. It has been shown that a rough LPS mutant of *H. pylori* without an O-chain has reduced colonising ability [27].

Phase variation in *H. pylori* also affects membrane lipid composition. The switch of *H. pylori* membrane lipid composition genes is spontaneous and reversible, and is associated with a change in the membrane phospholipids, from a low content of lysophospholipids in one variant (normal L variant) to a high content in another variant (lyso-S variant). Prolonged growth at pH 3.5 results in an almost complete conversion to the S variant. L variants survive and grow at pH 5.0, whereas only a few colonies are formed at pH 3.5, all of them as S variants. The change in membrane lipid composition is caused by phase variation in the *pldA* gene, which encodes the outer-membrane phospholipase. Variants with active phospholipase are better able to survive acidic conditions. The change from L to S colonies in *H. pylori* seems to be independent of the LPS variation, although shortening of LPS from smooth to rough LPS always followed the switch from L to S morphology. However, the rough LPS was retained after the reverse switch from the S to L variant [28].

Phase variation in *Franciscella novicida* LPS also affects the antigenic structure of the O-chain. Regulation of O-chain length in *Franciscella* spp. seems to be essential for serum resistance as well as for intracellular growth. The reversible LPS phase variation occurred following diminished expression of one type of LPS that was unable to stimulate nitric oxide production in rat macrophages, with expression of a different type of LPS that induces nitric oxid production in rat macrophages [29,30].

Some bacteria are able to form plasmids that carry certain genetic elements as a reserve source of genetic material for changing their virulence. A study of the molecular mechanism of LPS phase variation in *Legionella pneumophila* has revealed an unstable 30-kb genetic element of phage origin. Whereas the 30-kb element was located on the

chromosome in the virulent wild-type strain, its excision from the chromosome and replication as a high-copy-number plasmid resulted in a mutant phenotype, characterised by alteration of an LPS epitope and loss of virulence. However, the mutant phenotype could revert to the wild-type by re-insertion of the 30-kb element into the chromosome [31].

S-R MUTATION

The types of phase variation described above, all of which are associated with high-frequency spontaneous gain and loss of antigenic LPS epitopes, are all reversible. Other changes in the structure of the outer-membrane involve irreversible changes in the polysaccharide molecules of some Gram-negative bacteria. These include mutations resulting in successive antigenic deletions in LPS, but some proteins in the bacterial outer-membrane can also be affected. The degenerative process in this case consists of a step-by-step mechanism that results in the outer O-chain of LPS decreasing in length.

Examples of such changes can be seen in the LPS structure of enteric bacteria. The genes for the synthesis of the core and O-chain LPS in Enterobacteriacae are organised in contiguous clusters. In *E. coli* and *Salmonella enterica* Typhimurium, these clusters are present in two regions of the chromosome [32]. Following intervention of the host immune system, the S-form of bacterial colonies, possessing complex LPS structures with a complete O-chain and full antigenic structure, are present. The R-form appears in the absence of an effective host immune system, or following propagation *in vitro*, and contains truncated LPS with a varying core structure, the synthesis of which requires less expenditure of energy by the bacterial cell. In addition to alterations in the structure of the LPS, the degenerative process includes deep changes in the outer-membrane, with a dramatic reduction in the number of porin proteins, particularly OmpA [33]. LPS is associated closely with the trimeric OmpF and LamB proteins of the outer-membrane, which enable diffusion of substrates into and out of the cell. Therefore, the S-R variation also influences the content of surface proteins. In deep R mutants of *E. coli*, the rate of porin assembly was retarded in comparison with the rate observed in the wild-type strain [34].

The interaction between S-LPS and Omp proteins in *E. coli* is stronger than that observed for R-LPS–protein complexes of *Acinetobacter calcoaceticus* [35]. The mobility and banding pattern of OmpF porin trimers of *E. coli* in SDS-PAGE are influenced by the chemotype of the associated LPS. OmpF molecules associated with S-LPS migrate with a slower mobility in electrophoresis gels than that of R-LPS [36]. The genetic nature of the deep R mutants in enteric bacteria is now well-understood, in that mutations resulting in defects in the backbone heptose region of the inner core affect modification of this region [37]. However, the mutations in other Gram-negative bacteria have not been so thoroughly studied. All clinical isolates of *H. pylori* have S-LPS with O-chains of relatively homogeneous length, but *H. pylori* strains from culture collections show SDS-PAGE profiles with R-LPS [38].

A good example of step-by-step mutation is the LPS of the obligate intracellular pathogen *Coxiella burnetii*, a close relative of *L. pneumophila*, which is the causative agent of Q fever in humans [39]. Wild-type *C. burnetii* strains possess a typical Gram-negative outer-membrane, with a complex structure of LPS, proteins and phospholipids. The degenerative mutations in *C. burnetii* LPS are traditionally referred to in the scientific literature as 'phase variation', and phase I and phase II LPS molecules have been distinguished. Phase I LPS (S-like form) is a complete LPS molecule with O-chain saccharidic units that is typical of wild-type *C. burnetii* strains. Phase variation is employed in the diagnosis of Q fever. During acute Q fever, *C. burnetii* induces antibodies against phase II (protein antigens), while chronic Q fever, often manifested as endocarditis, is associated with the production of high titres of antibodies directed against phase I (LPS antigen) [40]. Following repeated passage in chick embryo yolk sacs or in cell lines, i.e., in the absence of immune system intervention, *C. burnetii* wild-type strains convert to phase II and produce the R-like form of LPS. Results of SDS-PAGE and immunoblot analysis have shown that the LPS of *C. burnetii* shows a gradual shortening of its O-chain following repeated passage [41]. *C. burnetii* strains with higher numbers of passages in chick embryo yolk sacs had decreased antigenic properties and virulence against guinea-pigs and mice [42], but there was no difference in phase-variation steps between *C. burnetii* strains

of high and low virulence [43]. It can be speculated that phase variation in *C. burnetii* resembles the S-R mutation process observed in Enterobacteriaceae. However, studies of phase I and II *C. burnetii* proteins using SDS-PAGE have failed to reveal any significant decrease in the protein content during phase variation [44,45]. These results indicate differences between *C. burnetii* phase variation and the S-R mutations found in enteric bacteria, where the decrease in the porin protein content in the R-form is clearly apparent (see above).

Hotta *et al.* [46] studied phase variation in *C. burnetii* with three types of monoclonal antibodies. Four *C. burnetii* groups were distinguished during serial passages of *C. burnetii* in cell lines, and other antigenic groups could be distinguished upon further passaging. Vodkin and Williams [47] found large chromosomal deletions associated with phase variation in *C. burnetii* LPS, and it seems that DNA deletions are a characteristic feature of the generation of phase II variants of *C. burnetii*. When mapping the deletion in the O-antigen cluster in two antigenic variants of the Nine Mile strain of *C. burnetii*, the deleted sequences in both variants were revealed to be in genes involved in the biosynthesis of specific sugars forming part of the O-chain [48]. Phase II deletions have now been characterised in 14 isolates of *C. burnetii* by microimmunofluorescence tests and PCR amplification of selected open reading frames important for O-chain biosynthesis [49]. Phase I isolates did not display deletions in this region but, as expected, some phase II isolates contained large deletions in LPS biosynthesis genes. However, other phase II isolates did not contain such deletions, and it has been suggested that LPS truncation in such cases can occur via small genetic changes [50]. Data from comparative genome hybridisation studies have revealed that truncated LPS is fully responsible for the avirulence of the Nine Mile phase II strain, since deletions of open reading frames in other regions were not detected. Further genetic studies of phase II isolates without large deletions in the LPS biosynthesis genes will help to explain their outer-membrane antigenic structure. It is known that phase I LPS of *C. burnetii* possesses low antigenic and immunogenic properties [51] and thus the phase I LPS–protein complex of *C. burnetii* is used as a vaccine against Q fever [52].

CONCLUSIONS

Pathogenic bacteria have developed many strategies to avoid attack by the host immune system. One of these is phase variation, which enables a change in virulence factors according to the environmental situation and the needs of the bacterium, but without increasing the overall mutability of the remainder of the genome. Thus, phase variation is a way to maximise the chance of survival during host colonisation and propagation. This reversible process is characteristic of mostly surface-associated antigens, e.g., LPS, capsule and glycosylated pili, but also for outer-membrane proteins, encoded by contingency genes that have key roles in invasion and colonisation.

In contrast, S-R mutation is a step-by-step process that results in an irreversible change. The resulting R mutants are unable to revert to their original state, and fail to survive in a host with an intact immune system. The so-called phase variation observed in *C. burnetii* is misnamed, as it actually involves a process resembling S-R mutation, except for the preservation of outer-membrane proteins that show a significant decrease following S-R mutation of enteric bacteria.

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REFERENCES

1. Gronow S, Brade H. Lipopolysaccharide biosynthesis: which steps do bacteria need to survive? *J Endotoxin Res* 2001; **7**: 3–23.
2. Lerouge I, Vanderleyden J. O-antigen structural variation: mechanism and possible roles in animal/plant-microbe interactions. *FEMS Microbiol Rev* 2001; **26**: 17–47.
3. Hallet B. Playing Dr Jekyll and Mr Hyde: combined mechanisms of phase variation in bacteria. *Curr Opin Microbiol* 2001; **4**: 570–581.
4. Radman M. Enzymes of evolutionary change. *Nature* 1999; **401**: 866–869.
5. Van der Woude M, Bäumler AJ. Phase and antigenic variation in bacteria. *Clin Microbiol Rev* 2004; **17**: 581–611.
6. Henderson IR, Owen P, Nataro JP. Molecular switches—the ON and OFF of bacterial phase variation. *Mol Microbiol* 1999; **33**: 919–932.
7. Saunders NJ, Peden JF, Hood DW, Moxon ER. Simple sequence repeats in the *Helicobacter pylori* genome. *Mol Microbiol* 1998; **27**: 1091–1098.

8. Power PM, Roddam LF, Rutter K, Fitzpatrick SZ, Srikhanta YN, Jennings MP. Genetic characterization of pilin glycosylation and phase variation in *Neisseria meningitidis*. *Mol Microbiol* 2003; **49**: 833–847.
9. Berrington AW, Tan YC, Srikhanta Y *et al*. Phase variation in meningococcal lipooligosaccharide biosynthesis genes. *FEMS Immunol Med Microbiol* 2002; **34**: 267–275.
10. Tzeng Y-L, Stephens DS. Epidemiology and pathogenesis of *Neisseria meningitidis*. *Microbes Infect* 2000; **2**: 687–700.
11. Martin P, van de Ven T, Mouchel N, Jeffries AC, Hood DW, Moxon ER. Experimentally revised repertoire of putative contingency loci in *Neisseria meningitidis* strain MC58: evidence for a novel mechanism of phase variation. *Mol Microbiol* 2003; **50**: 245–257.
12. Taha M-K, Degmane A-E, Antignac A, Zarantonelli ML, Larribe M, Alonso J-M. The duality of virulence and transmissibility in *Neisseria meningitidis*. *Trends Microbiol* 2002; **10**: 376–382.
13. Schneider H, Hammack CA, Apicella MA, Griffiths JM. Instability of expression of lipooligosaccharides and their epitopes in *Neisseria gonorrhoeae*. *Infect Immun* 1988; **56**: 942–946.
14. Van Putten JPM. Phase variation of lipopolysaccharide directs interconversion of invasive and immuno-resistant phenotypes of *Neisseria gonorrhoeae*. *EMBO J* 1993; **12**: 4043–4051.
15. Packiam M, Shell DM, Liu SV *et al*. Differential expression and transcriptional analysis of the α-2,3-sialyltransferase gene in pathogenic *Neisseria* spp. *Infect Immun* 2006; **74**: 2637–2650.
16. Fox KL, Yldirim HH, Deadman ME, Schweda EKH, Moxon ER, Wood DW. Novel lipopolysaccharide biosynthetic genes containing tetranucleotide repeats in *Haemophilus influenzae*: identification of a gene for adding O-acetyl groups. *Mol Microbiol* 2005; **58**: 207–216.
17. Zaleski P, Wojciechowski M, Piekarovicz A. The role of Dam methylation in phase variation of *Haemophilus influenzae* genes involved in defence against phage infection. *Microbiology* 2005; **151**: 3361–3369.
18. Peak IRA, Jennings MP, Hood DW, Bisercic M, Moxon ER. Tetrameric repeat units associated with virulence factor phase variation in *Haemophilus* also occur in *Neisseria* spp. and *Moraxella catarrhalis*. *FEMS Microbiol Lett* 1996; **137**: 109–114.
19. Peak IRA, Jennings MP, Hood DW, Moxon ER. Tetranucleotide repeats identify novel virulence determinant homologues in *Neisseria meningitidis*. *Microb Pathog* 1999; **26**: 13–23.
20. Weiser JN, Lindberg AA, Manning EJ, Hansen EJ, Moxon ER. Identification of a chromosomal locus for expression of lipopolysaccharide epitopes in *Haemophilus influenzae*. *Infect Immun* 1989; **57**: 3045–3052.
21. Weiser JN, Williams A, Moxon ER. Phase variable lipopolysaccharide structure enhances the invasive capacity of *Haemophilus influenzae*. *Infect Immun* 1990; **58**: 3455–3457.
22. Erwin AL, Bonthuis PJ, Geelhood JL *et al*. Heterogeneity in tandem octanucleotides within *Haemophilus influenzae* lipopolysaccharide biosynthetic gene *losA* affect serum resistance. *Infect Immun* 2006; **74**: 3408–3414.
23. Torres-Cruz J, van der Woude M. Slipped-strand mispairing can function as a phase variation mechanism in *Escherichia coli*. *J Bacteriol* 2003; **185**: 6990–6994.
24. Bacon DJ, Szymanski CM, Burr DH, Silver RP, Alm RA, Guerry P. A phase-variable capsule is involved in virulence of *Campylobacter jejuni* 81–176. *Mol Microbiol* 2001; **40**: 769–777.
25. Salaün L, Saunders NJ. Population-associated differences between the phase variable LPS biosynthetic genes of *Helicobacter pylori*. *BMC Microbiol* 2006; **6**: 79.
26. Appelmelk BJ, Shiberu B, Trinks C *et al*. Phase variation in *Helicobacter pylori* lipopolysaccharide. *Infect Immun* 1998; **66**: 70–76.
27. Logan SM, Conian JW, Monteiro MA, Wakarchuk WW, Altman E. Functional genomics of *Helicobacter pylori*: identification of a β-1,4-galactosyltransferase and generation of mutants with altered lipopolysaccharide. *Mol Microbiol* 2000; **35**: 1156–1167.
28. Tannaes T, Dekker N, Bukholm G, Bilsma JJ, Appelmelk BJ. Phase variation in the *Helicobacter pylori* phospholipase. A gene and its role in acid adaptation. *Infect Immun* 2001; **69**: 7334–7340.
29. Cowly SC, Gray CJ, Nano FE. Isolation and characterization of *Francisella novicida* mutants defective in lipopolysaccharide biosynthesis. *FEMS Microbiol Lett* 2000; **182**: 63–67.
30. Kieffer TL, Cowley S, Nano FE, Elkins KL. *Franciscella novicida* LPS has greater immunological activity in mice than *F. tularensis* LPS, and contributes to *F. novicida* murine pathogenesis. *Microb Infect* 2003; **5**: 397–403.
31. Lüneberg E, Mayer B, Daryab N *et al*. Chromosomal insertion and excision of a 30 kb unstable genetic element is responsible for phase variation of lipopolysaccharide and other virulence determinants in *Legionella pneumophila*. *Mol Microbiol* 2001; **39**: 1259–1271.
32. Schnaitman CA, Klena JD. Genetics of lipopolysaccharide biosynthesis in enteric bacteria. *Microbiol Rev* 1993; **57**: 655–682.
33. Parker CT, Kloser AW, Schneitman CA, Stein MA, Gottesman S, Gibson BW. Role of the *rfaG* and *rfaP* genes in determining the lipopolysaccharide core structure and cell surface properties of *Escherichia coli* K-12. *J Bacteriol* 1992; **174**: 2525–2538.
34. Laird MW, Kloser AW, Misra R. Assembly of LamB and OmpF in deep rough lipopolysaccharide mutants of *Escherichia coli* K-12. *J Bacteriol* 1994; **178**: 2259–2264.
35. Borneleit P, Blechschmidt B, Kleber H-P. Lipopolysaccharide–protein interactions: determination of dissociation constants by affinity electrophoresis. *Electrophoresis* 1989; **10**: 848–852.
36. Diedrich DL, Stein MA, Schnaitman CA. Associations of *Escherichia coli* K-12 OmpF trimers with rough and smooth lipopolysaccharides. *J Bacteriol* 1990; **172**: 5307–5311.
37. Helander IM, Vaara M, Sukupolvi S *et al*. *rfa* mutants of *Salmonella typhimurium*. *Eur J Biochem* 1989; **185**: 541–546.
38. Moran AP, Helander IM, Kosunen TU. Compositional analysis of *Helicobacter pylori* rough-form lipopolysaccharides. *J Bacteriol* 1992; **174**: 1370–1377.
39. Kazár J. *Coxiella burnetii* infection. *Ann NY Acad Sci* 2005; **1063**: 105–114.
40. Peacock MG, Philip RN, Williams JC, Faulkner RS. Serological evaluation of Q fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. *Infect Immun* 1983; **41**: 1089–1098.

41. Lukáčová M, Kazár J, Gajdošová E, Vavreková M. Phase variation of lipopolysaccharide of *Coxiella burnetii*, strain Priscilla during chick embryo yolk sac passaging. *FEMS Microbiol Lett* 1993; **113**: 285–290.
42. Kazár J, Brezina R, Schramek S, Úrvölgyi J, Pospíšil V, Kováčová E. Virulence, antigenic properties and physicochemical characteristic of *Coxiella burnetii* strains with different chick embryo yolk sac passage history. *Acta Virol* 1974; **18**: 434–442.
43. Quevedo Diaz MA, Lukáčová M. Immunological consequences of *Coxiella burnetii* phase variation. *Acta Virol* 1998; **42**: 181–185.
44. Hackstadt T. Steric hindrance of antibody binding to surface proteins of *Coxiella burnetii* by phase I lipopolysaccharide. *Infect Immun* 1988; **56**: 802–807.
45. Lukáčová M, Kazár J, Gajdošová E. *Coxiella burnetii* phase I and II proteins studied by SDS-PAGE. *Acta Virol* 1994; **38**: 263–267.
46. Hotta A, Kawamura M, To H *et al*. Phase variation analysis of *Coxiella burnetii* during serial passage in cell culture by use of monoclonal antibodies. *Infect Immun* 2002; **70**: 4747–4749.
47. Vodkin MH, Williams JC. Overlapping deletions in two spontaneous phase variants of *Coxiella burnetii*. *J Gen Microbiol* 1986; **132**: 2587–2594.
48. Hoover TA, Culp DW, Vodkin MH, Williams JC, Thompson HA. Chromosomal DNA deletions explain phenotypic characteristic of two antigenic variants, phase II and RSA 514 (Crazy), of the *Coxiella burnetii* Nine Mile strain. *Infect Immun* 2002; **70**: 6726–6733.
49. Denison AM, Massung RF, Thompson HA. Analysis of the O-antigen biosynthesis regions of phase II isolates of *Coxiella burnetii*. *FEMS Microbiol Lett* 2007; **267**: 102–107.
50. Beare AP, Samuel JE, Howe D, Virtaneva K, Porcella SF, Heinzen RA. Genetic diversity of the Q fever agent, *Coxiella burnetii*, assessed by microarray-based whole-genome comparisons. *J Bacteriol* 2006; **188**: 2309–2324.
51. Hitchcock PJ, Leive L, Makela PE, Rietchel ET, Strittmatter W, Morrison DC. Lipopolysaccharide nomenclature—past, present, and future. *J Bacteriol* 1986; **166**: 699–705.
52. Lesná J, Lesný M, Lukáčová M, Kazár J. Reactogenicity and immunogenicity of three Q fever chemovaccine preparations. In: Kazár J, Raoult R, eds, *Proceedings of the IV international symposium on rickettsiae and rickettsial diseases*. Bratislava: Slovak Academy of Sciences, 1990; 674–677.