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Influences of strains and environment conditions on lipid A: A case study of Francisella

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Abstract. Tularemia is a zoonosis caused by gram-negative bacteria Francisella tularensis, which has LPS in outer membrane. Lipid A is the most conservative part in LPS and is important for LPS function of bacteria survival and host immune response stimulation. For a certain strain, the lipid A structure is unique, but can also influenced by different environmental conditions. The mechanisms of how lipid A synthesis and modification changes will affect immune mechanism still remain many questions. This article briefly introduces the enzymes participating general lipid A synthesis process. F. novicida, as a specific example, has differences in lipid A-related enzymes from those of ordinary bacteria. Lipid A structure and content can be regulated by some environment conditions like temperature differences between environment and host cells. The structure of lipid A determines its function, so bacterial virulence is various. Some F. novicida knockout mutants have no ability to stimulate immune response in host cells and have lower toxicity than wild-type strains. In therapy, modified lipid A is used as immune adjuvant and some lipid A mutant strains can be vaccines to protect animals against lethal Francisella strain infection. A deeper understanding of Francisella lipid A structure, regulation and immune mechanisms helps to prevent and treat tularemia disease. In future research, more enzymes affecting characteristics of lipid A or the whole LPS can be further studied. More effective vaccines and drug design relies on a better understanding of pathogenic mechanisms.

Keywords: Francisella, lipid A, immunity.

1. Introduction

Infectious diseases are concerned because of their relevance to human health. In existing and new infectious diseases, zoonoses account for a large proportion, such as Ebola virus disease, salmonellosis, and COVID-19. Zoonoses are transmitted from non-human animals to humans, through direct contact with captive or wild animals, contamination in the diet, or presence in the environment, causing public health problems and interfering with industries related to animal products [1]. Tularemia is a zoonosis caused by Francisella tularensis. Tularemia patients present with high fever, malaise, vomiting, local or systemic myalgia, and swollen glands. There are several recognized transmission routes: insect-borne transmission, digestive tract infection, respiratory tract infection, and contact transmission.

F. tularensis is a gram-negative bacterium and has a variety of antigenic components on its surface that can induce immune response in human. Lipopolysaccharide (LPS) is an important component of outer membrane of gram-negative bacteria. Composition of LPS includes three parts, lipid A, a core

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oligosaccharide, and an O antigen. Lipid A, also known as the endotoxin, is important for LPS function, and its structure is unique to a certain strain, although the lipid A structure can change with environmental conditions and other factors during bacterial growth [2]. The change of lipid A can help F. tularensis escape the innate immune recognition of the host, change the inflammation and pathogenesis, and escape immune killing. Mass spectrometry imaging (MSI) and other methods help to determine the structure of the lipid A of Francisella tularensis. Francisella tularensis novicida was found to be a subspecies that rarely exhibits pathogenicity in human, different from F. tularensis, although it can induce an immune response in mouse models. F. novicida is similar to F. tularensis in nucleic acid sequences, so it is used as a model for studying F. tularensis [3]. There are features in lipid A synthesis of F. novicida. Environment condition changes can also influence lipid A structure. Lipid A changes affect immune mechanisms, deeper understanding on Francisella strains and environment conditions can help prevent and treat tularemia disease in humans.

2. Influences of Francisella strains and environment conditions on lipid A

2.1. General lipid A synthesis pathway

Lipid A is on the inside of the LPS molecule and is involved in the formation of outer leaflet of the outer membrane. The structure of lipid A is conservative. It is mainly composed of hydrophilic diglucosamine backbone and hydrophobic long fatty acid chains. The acyl chains can vary in number, from four to eight depending on synthesis pathways under different bacterial and external conditions, and can have differences in the ways they are attached to the backbone glucosamine, either directly or separated by oxygen atom. Most lipid A contains 14-carbon 3-hydroxylated fatty acids, 10~20-carbon fatty acids and side chain fatty acids are also present. Phosphates are linked to diglucosamine by ester or amide bonds [3]. Biosynthesis of lipid A occurs at the cytoplasmic surface of the bacteria cell membrane.

The synthesis starting point is UDP-N-acetylglucosamine (UDP-GlcNAc), and a series of enzymes encoded by Lpx gene cluster are involved in catalyzing the reactions. UDP-GlcNAc can be acylated at the 3-hydroxyl position, by the UDP-GlcNAc O-acyltransferase encoded by the LpxA gene, to produce UDP-3-monouoyl-GlcNAc. In the process, β-hydroxy-14 alkanoic acid is supplied by (β-OH) C14-ACP. The LpxC gene encodes UDP-3-O-N-acetylglucosamine deacetylase, which can catalyze UDP-3-monouoyl-GlcNAc to remove the acetyl group on the C-2 amino group. With the help of acyl-transferase encoded by the LpxD gene, UDP-2, 3-Di-acyl-GlcN can be formed. The LpxH gene encodes pyrophosphatase, which catalyzes the replacement of UDP with a phosphate group to produce lipid X. Lipid X and UDP-2, 3-Di-acyl-GlcN produce four-chain lipid A precursor. Their condensation via pyrophosphate bond is catalyzed by disaccharide synthase encoded by LpxB gene. The precursor consists of one phosphate group, and kinase allows another phosphate group to be added to produce lipid IVA, a lipid A precursor with conserved structures in different strains [3].

2.2. Special features of lipid A of F. novicida

Lipid A can be modified in a variety of ways, including the number and length of acyl chains, phosphate group modifications, backbone substituent modifications, etc [4]. Lipid IVA modification is diverse. It has been confirmed that LpxE and LpxF are involved in phosphate group addition and removal process in Francisella. For example, LpxF helps remove the phosphate group at 4'position. FlmK is found to control the addition of both mannose and galactosamine in F. novicida, and deletion of FlmK will change lipid A carbohydrate modifications [3]. FlmX has multiple regulation effects in LPS remodeling, including addition of a hexose and formation of the wildtype core structure. FlmX has no ATPase domain, but as it is an inner membrane protein that can modify the galactosamine (GalN) of free lipid A1, a kind of lipid that has high content in F. novicida, with the loss-of-function mutant rescued by a known flippase from E. coli, FlmX is confirmed as a flippase. Besides lipid A1, there is lipid A2 in F. novicida with relatively low content. FlmX is also needed for lipid A2 formation [2].

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2.3. Influences of environment conditions on lipid A

Some enzymes involved in the synthesis and modification of lipid A function independently of environmental conditions. For example, transcription of some constitutive lipid A biosynthetic enzymes such as LpxL, encoding acyltransferase to add acyloxyacyl laurate to lipid IVA, is not regulated by environmental temperature [5]. However, environmental conditions can change the synthesis of lipid A. In response to environmental changes, gene expression is adjusted, enzyme content and activity are affected, thus the internal physiological mechanism of Francisella is changed. Lipid A with different molecular structures can cope with different conditions. Francisella moves among environments like water and dust, vectors such as fleas, and its host mammals such as humans over the whole life span. Insects have a temperature of about 25 ℃, environment about 18 ℃, and mammals about 37 ℃. N-acyltransferase enzyme LpxD can add acyl group of 18C- or 16C-length with a hydroxyl group at 3' position. The results of structural analysis show that LpxD1 adds a longer-chain acyl group at higher temperature in mammal hosts, while LpxD2 adds a shorter-chain acyl group at lower environment temperature. F. novicida undergoes temperature transitions between 18 ℃ and 37 ℃, and the tracking results show changes in the content of lipid A with different acyl chain lengths. Remodeling of lipid A in F. novicida by LpxD1/2 indicates that lipid A synthesis and modification can be regulated by environmental condition changes [3].

3. Pathogenicity and immunity associated with lipid A

3.1. Characteristics of immune response processes

Francisella has multiple mechanisms to adapt and successfully spread and reproduce among the environment, vector and human host. It can stimulate and infect related immune cells, resulting in an immune response from the host. Compared with F. novicida, which is less harmful to humans, F. tularensis causes inflammation, necrosis, and eventually ulceration in the site of invasion. Because the invasion and replication of high copy number bacteria will cause a burden on the infected cells, and the strong inflammatory response. If the pathogen infects the respiratory tract, bronchopneumonia forms in the lungs with necrosis of the alveolar walls. F. tularensis follows lymphatic vessels into nearby lymph nodes and causes them to swell. F. tularensis can adhere to macrophages in the lymph nodes, and after being endocytosed into the cytoplasm, it can escape from the phagocytic vesicles and multiply in the cytoplasm, inducing cell cleavage, releasing offspring and infecting neighboring cells. If the bacteria escape along the lymphatic system into the circulation system, it may spread to the whole systemic organs and tissues, causing body metabolism disorder by internal toxic substances [6]. LPS plays multiple roles in the process. It acts as part of the permeability barrier and provides protection against host killing mechanisms, such as complement, antibody, and antimicrobial peptides. LPS can also facilitate adherence and colonization. Special modifications of the membrane has been confirmed to make the bacteria obtain resistance to phagocytosis and elicit mammalian immune response [7]. Lipid A, as the conservative core, plays an important role in these processes.

3.2. Immune mechanism

The classical lipid A is recognized by Toll-like receptor 4 (TLR4) and myeloid differentiation factor (MD-2) protein receptors. The number of phosphate groups and length or number of fatty acyl chains of lipid A play an important role in the activation of TLR4. For example, E. coli lipid A contains two phosphate groups and six fatty acyl chains, with each chain composed of 12 to 14 carbons, and have a strong activation effect on the innate immune system. However, when the host is stimulated by Francisella and produces an inflammatory response, TLR4 pathway is not activated. The lipid A of F. tularensis contains four longer acyl chains with 16 to 18 carbons, and the phosphate group on the backbone is missing or is protected by modification. These differences prevent LPS binding proteins from recognizing LPS of F. tularensis, so host cell TLR-4 signals are not activated after F. tularensis infection [8]. TLR2 is suggested to be involved in Francisella stimulated immune response.

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The characteristics of Francisella lipid A help it evade the recognition of caspase-11. Caspase-11 can be dimerized and activated by binding to lipid A, then catalyze cutting of Gasdermin D. Gasdermin D will aggregate and form pores on the plasma membrane, triggering cell death. Experimental results in mouse macrophages show that LpxF mutant lipid A contains more fatty acyl chains and activates caspase-11. The LpxF mutant also lacks ability to remove phosphate group, contributing to the attenuated virulence of this mutant [6].

3.2.1. Immunity difference between Francisella strains. F. novicida has low or even no toxicity to healthy people or rabbits and does not cause tularemia. However, in mice F. novicida causes a disease similar to tularemia. The low-toxicity in human allows F. novicida to act as a model system for the toxic F. tularensis. F. novicida lipid A stimulates neither human nor mouse cells through TLR2 compared to F. tularensis. F. novicida lipid A also can not act as TLR2 antagonist in human and mouse cells [3]. Some F. novicida knockout mutants of key lipid A synthesis and modification enzymes have lower toxicity to mice. LpxF knockout mutant has lipid A with two phosphate groups and is confirmed to be nontoxic to mice [5].

3.2.2. The environmental influences on immune mechanism. Francisella undergoes migration between environments and different hosts throughout the life cycle. Environment temperature regulates the alteration of acyl chains' length of lipid A, adding a longer acyl group at higher temperature close to mammalian host by LpxD1 and shorter acyl group at lower temperature by LpxD2. LpxD1-null mutant is unable to add longer acyl chains as the wild type strain. In sensitivity tests of antimicrobial peptides, antibiotics, and environmental stresses, LpxD1-null mutant is repressed more effectively but LpxD2-null mutant results are similar to those of F. novicida. LpxD1-null mutant does not proliferate in cells and is less cytotoxic to host cells [9]. Chain length has effects on membrane fluidity, and profoundly affects the degree to which bacteria are recognized by the host immune system, changing bacterial infection ability and the strength of the host immune response. LpxD1/2-null mutants have differences in membrane permeability and pathogenesis compared to wild type F. novicida [3]. Different mutants show enzyme effects. With how the enzyme genes are regulated on genomic, transcriptional and translational levels by environmental conditions, mechanisms of how bacterial virulence be influenced can be interpreted.

Biofilm plays a role in bacterial adhesion, resistance to host immune killing and defense against harsh environments, and is important for bacterial survival and replication. LPS participates in biofilm formation and biofilm initiation is start under specific pH condition [10].

4. Application of lipid A in immunity and therapy

Modified lipid A can be used as immune adjuvant. Lipid A monophosphate can be obtained by removing the phosphate group at position 1, with toxicity reduced but immune activity retained. The lethality of lipid A monophosphate is only 0.08% of that of LPS, indicating the safety and low toxicity. And lipid A monophosphate can stimulate immune cells and produce cytokines, such as TNF, IFN, and interleukin family. These two features allow lipid A monophosphate to act as an adjuvant [11]. When adjuvant lipid A monophosphate is used combined with vaccines, it can significantly enhance the humoral and cellular immune response.

Low-virulent Francisella species can induce protective immune response and serve as vaccines. A vaccine of live attenuated F. novicida applied in rats and monkeys shows to help the animals defense pulmonary F. tularensis infection, implied by proteome array comparison of specific antibodies in animal serum [3]. In mice, a FlmK-null mutant has advantages acting as a vaccine, like easier to do genetic manipulations than F. tularensis and F. novicida, provides protection against lethal strain infection [5]. LpxD1-null mutant has different modifications to lipid A from those of wild type F. novicida, resulting in changes in membrane fluidity and integrity, and further low-virulence. LpxD1-null mutant also provides protection against lethal F. novicida in mice [3].

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5. Conclusion

LPS is an important component of gram-negative bacteria. This article focuses on the toxic lipid A, the core of LPS. Francisella, pathogenic bacteria of zoonotic disease Tularemia, is main object of this article's concern. The synthesis and immunity of lipid A are summarized. Intermediates in the synthesis process and enzymes involved in catalysis are listed in the synthesis section. The roles of some enzymes involved in lipid A synthesis and modification in F. novicida are further discussed. Environment conditions, such as temperature differences between environment and host cells, can have influences on lipid A structure and content. As bacteria can cause disease and stimulate host immune response, pathogenicity and immunity associated with lipid A, including characteristics of immune response processes and some immune mechanisms, are introduced with example Francisella. Among different Francisella strains and same strain under various environmental conditions, enzyme content and function are regulated, causing lipid A structure and further bacterial virulence changes. However, in therapy modified lipid A can also act as immune adjuvant and some lipid A mutant strains can be vaccine to protect animals against lethal Francisella strain infection. This article explains the differences in disease degree among different strains through protein-level regulation of lipid A pathogenicity, focusing on enzyme gene-knockout mutants. Lipid A structure and content level changes influence the characteristics of LPS and bacteria membrane, and influence their ability to help Francisella escape the immune recognition and survive to proliferate. With deeper understanding of Francisella lipid A immune mechanisms, people can better prevent and treat tularemia disease. Vaccine design ideas can refer to application of F. novicida knockout-mutants against wild type F. novicida and F. tularensis. However, in this article many other enzyme genes affecting lipid A synthesis are not mentioned and mechanisms of enzyme gene expression regulation on gene level still remain in questions. In future research, the properties of other knockout mutants can be studied, combining subsequent LPS assemble processes. Similar methods can also be used to study other virulent structures of Francisella, or structures including lipid A of other bacteria. Existing understanding of pathogenic mechanisms helps to develop targeted drugs.

References

[1] Sharma R Patil R D Singh B et al 2023 Tularemia - a re-emerging disease with growing concern The veterinary quarterly 43 1 1-16

[2] Chin C Y Zhao J Llewellyn A C et al 2021 Francisella FlmX broadly affects lipopolysaccharide modification and virulence Cell reports 35 11 109247

[3] Li Y Powell D A Shaffer S A et al 2012 LPS remodeling is an evolved survival strategy for bacteria Proceedings of the National Academy of Sciences of the United States of America 109 22 8716-8721

[4] Hewawaduge C Senevirathne A Sivasankar C & Lee J H 2023 The impact of lipid A modification on biofilm and related pathophysiological phenotypes endotoxicity immunogenicity and protection of Salmonella Typhimurium Veterinary microbiology 282 109759

[5] Powell D A 2012 Immune Responses to a Francisella Lipid A Mutant: Characterization and Therapeutic Potential Order No 3555213 Available from Publicly Available Content Database 1318844900

[6] Degabriel M Valeva S Boisset S & Henry T 2023 Pathogenicity and virulence of Francisella tularensis Virulence 14 1 2274638

[7] Freudenberger Catanzaro K C & Inzana T J 2020 The Francisella tularensis Polysaccharides: What Is the Real Capsule? Microbiology and molecular biology reviews: MMBR 84 1 e00065-19

[8] De Pascalis R Rossi A P Mittereder L et al 2020 Production of IFN-γ by splenic dendritic cells during innate immune responses against Francisella tularensis LVS depends on MyD88 but not TLR2 TLR4 or TLR9 PloS one 15 8 e0237034

[9] Yun J Wang X Zhang L & Li Y 2017 Effects of lipid A acyltransferases on the pathogenesis of F novicida Microbial pathogenesis 109 313-318

DOI: 10.54254/2753-8818/47/2024PJ0110

[10] Mlynek K D Lopez C T Fetterer D P et al 2022 Phase Variation of LPS and Capsule Is Responsible for Stochastic Biofilm Formation in Francisella tularensis Frontiers in cellular and infection microbiology 11 808550
[11] Evans J T Cluff C W Johnson D A et al 2003 Enhancement of antigen-specific immunity via the TLR4 ligands MPL adjuvant and Ribi529 Expert review of vaccines 2 2 219-229