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Review Article

Bacterial endotoxin-lipopolysaccharide; structure, function and its role in immunity in vertebrates and invertebrates

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ABSTRACT

Biotic and abiotic factors shape investment in costly defenses. The immune systems of vertebrates and invertebrates differ in their fitness cost. However, the hygienic behavior of both can result in increased survival rate and fitness cost. The immune response of vertebrates has developed more sophisticated and complicated mechanisms including an immunological memory with the generation of large antigen-recognition receptors and innate immune systems. The invertebrate immune system must rely on non-self-recognition molecules to ensure efficient defense responses against infectious pathogens that continuously threaten their survival. Lipopolysaccharide (LPS) from bacterial endotoxin, has been regarded as having potential molecules involved in immune recognition and immune defense. This review focused on an overview of bacterial endotoxin, LPS, and their structure, function, and elucidation of immune responses in both vertebrates and invertebrates are discussed. In addition, invertebrate defense against LPS is reviewed in detail. The precise mechanisms underlying self and non-self-recognition represent the basis to prevent and control infections from endotoxins as well as to stimulate animal resistance. This is particularly relevant for immune defense against LPS in invertebrates and vertebrates which are frequently constrained by recurrent diseases.

Introduction

Lipopolysaccharide (LPS) is the major constituent of the outer membrane of all Gram-negative bacteria and this is the bacterial-associated substance called endotoxin that elicits septic shock in animals (Beutler and Rietschel, 2003). Immune systems have developed to protect multicellular organisms from self or foreign "nonself" substances. During evolution, two general immune systems have developed to detect foreign substances namely innate (natural) immunity and adaptive (acquired) immunity. The innate immune system is phylogenetically a more ancient defense mechanism and can be found in all multicellular organisms. This system is the first-line of host defense that helps to limit infection in the early stage of infection and relies on germ line-encoded receptors that recognize conserved molecular patterns found in microorganisms (Fearon and Locksley, 1996; Fearon, 1997; Medzhitov and Janeway, 1997; Vishnu Priya, 2015). It is now clear that the innate immune system is very important for self or non-self recognition in vertebrates and plays an important role in adaptive immune systems (Medzhitov and Janeway, 1998a; Vishnu Priya, 2015). Various cells in invertebrates respond to microorganisms by enclosing these infectious agents within aggregates and then destroying them. The innate immune system of invertebrates can respond to the presence of pathogens with cellular and humoral responses (Vishnu Priya, 2015). One of the most abundant sources of LPS encountered by vertebrates is their resident gut microbiota and intestinal alkaline phosphatase detoxify the LPS and prevent intestinal inflammation in response to the resident microbiota (Bates et al., 2007).

Structure of cell wall of Gram-negative bacteria

In Gram-negative bacteria, one of the major important components is endotoxin, which is present in the outer membrane of the cell wall. The cell envelope of Gram-negative bacteria (Fig. 1) consists of the inner membrane (IM), the peptidoglycan (murein) and the outer membrane (OM) (Raetz and Whitfield, 2002). The IM is a phospholipids bilayer, which is similar to the plasma membrane of eukaryotic cells, and is permeable to lipophilic compounds. In 1892, Richard Pfeiffer first defined endotoxin as a heat-stable, toxic substance that was released upon disruption of microbial envelopes (Beutler and Rietschel, 2003). Numerous integral

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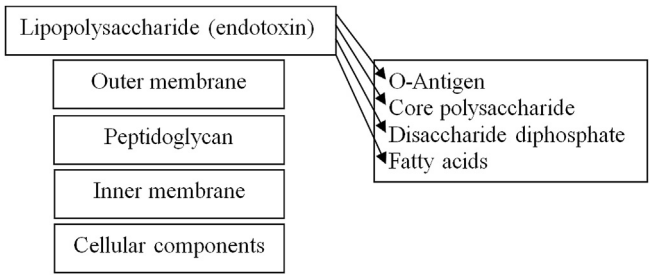

Fig. 1. Basic components of bacterial endotoxin.

transmembrane, helical proteins and peripheral membrane proteins are primarily responsible for transport, cell signaling and metabolic functions (Harald, 2001). The periplasm is the gelatinous material between the outer membrane and the IM. It contains enzymes for nutrient breakdown as well as binding proteins to facilitate the transfer of nutrients across the IM. Peptidoglycan in the periplasmic space is composed of alternating N-acetyl glucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) sugars that are cross-linked by short peptide bridges and maintains the osmotic pressure and cell structure (Holtje, 1998). The outer membrane is unique to Gram-negative bacteria, and its role is to serve as a protective structure. The lipid structures are highly asymmetric. LPSs are arranged in a tightly packed structure in the outer membrane (Kamio and Nikaido, 1976; Vishnu Priya, 2015).

Structure of LPS

The term endotoxin refers to cell-associated toxin and based on their internal compounds it generally refers to lipopolysaccharide (LPS). This LPS is composed of two major important components, hydrophilic lipid A and hydrophilic polysaccharide (O-region). Both are important for endotoxin biological activity. Toxicity is associated with the lipid component (Lipid A) and immunogenicity is associated with the polysaccharide components. The cell wall antigens (O antigens) of Gram-negative bacteria are components of LPS. LPS elicits a variety of inflammatory responses in an animal and it activation is complemented by the alternative (properdin) pathway; hence, it may be a part of the pathology of Gram-negative bacterial infections (Gutsmann et al., 2005; Vishnu Priya, 2015).

LPS has a molecular weight >100,000 D. The lipid A portion of the molecule has been shown to be responsible for numerous *in vivo* and *in vitro* effects of endotoxin. LPS stimulates the immune responses (Yu and Kanost, 2004) and enhances cellular immune reactions (Foukas et al., 1998; Soldatos et al., 2003). However, it has been reported that commercial bacterial endotoxin LPS contains enough peptidoglycan (PGN) to activate antimicrobial peptide (Dziarski and Gupta, 2006). Low activities of endotoxin stimulate the immune response and higher activities can lead to septic shock. *In vivo*, Gram-negative bacteria probably release minute amounts of endotoxin while growing. This may be important in the stimulation of natural immunity. Endotoxins are heat stable (boiling for 30 min does not destabilize endotoxin), but not to certain powerful oxidizing agents such as superoxide, peroxide and hypochlorite, which are used to remove the bacterial endotoxin (Vishnu Priya, 2015).

Detailed view of lipopolysaccharide components

Lipid A is the lipid component of LPS. It contains the hydrophobic, membrane-anchoring region of LPS. Lipid A consists of a phosphorylated N-acetyl glucosamine dimer with 6 or 7 fatty acids attached, usually six fatty acids are found. All fatty acids in Lipid A are saturated. Some fatty acids are attached directly to the N-acetyl glucosamine dimer and others are esterified to the 3-hydroxy fatty acids that are characteristically present. The structure of Lipid A is highly conserved among Gram-negative bacteria (Vishnu Priya, 2015).

Core (R) antigen or R polysaccharide is attached to the six position of one NAG. The R antigen consists of a short chain of sugars. For example: KDO – Hep – Hep – Glu – Gal – Glu – GluNAc. Two unusual sugars, heptose and 2-keto-3-deoxyoctonoic acid (KDO), are usually present in the core polysaccharide. KDO is unique and invariably present in LPS and so it has been used as an indicator in assays for LPS (endotoxin) (Vishnu Priya, 2015).

Vishnu Priya (2015) reported that somatic (O) antigen or O polysaccharide is attached to the core polysaccharide. It consists of repeating oligosaccharide subunits made up of 3–5 sugars. The individual chains vary in length ranging up to 40 repeat units. The O polysaccharide is much longer than the core polysaccharide, and it maintains the hydrophilic domain of the LPS molecule. A major antigenic determinant (antibody-combining site) of the Gram-negative cell wall resides in the O polysaccharide. The cecropins are antimicrobial peptides which have a broad spectrum of activity against Gram-positive and Gram-negative bacteria (Suparna et al., 2011).

Major variation occurs in the composition of the sugars in the O side chain between species and even strains of Gram-negative bacteria. At least 20 different sugars are known to occur and many of these sugars are characteristically unique dideoxyhexoses, which occur in nature only in Gram-negative cell walls. Variations in sugar content of the O polysaccharide contribute to the wide variety of antigenic types of *Salmonella* and *E. coli* and mostly other strains of Gram-negative species. Defined sugars in the structure, especially the terminal ones, elicit immunological specificity of the O antigen. Smooth strains (S-Strain) are produced by the presence of O part whereas the rough strains (R-Strain) are produced by the absence of the O region (Vishnu Priya, 2015).

Virulence/Toxicity

Both Lipid A (the toxic component of LPS) and the polysaccharide side chains (the nontoxic but immunogenic portion of LPS) act as determinants of virulence in Gram-negative bacteria. O-antigens have adhered properties and these are resistance to phagocytes, protection toward to antigens and antigenic variation property. Lipid A act as an immune stimulator, which induces the biological responses of a specific organism (Hancock and Diamond, 2000; Papo and Shai, 2005; Vishnu Priya, 2015).

Biological activity of lipopolysaccharide

An experimental animal's biological immune responses may be analyzed using several parameters, like injection of living or killed Gram-negative cells or purified LPS into experimental animals causes a wide spectrum of nonspecific pathophysiological reactions, such as fever, changes in white blood cell counts, disseminated intravascular coagulation, hypotension, shock and death. The injection of minimum doses of endotoxin results in death in most mammals. The sequence of events follows a regular pattern: (1) latent period; (2) physiological distress (diarrhea, prostration, shock); and (3) death. How soon death occurs varies on the dose of the endotoxin, the route of administration, and the species of animal. Animals vary in their susceptibility to endotoxin. The recent development of transgenic technologies and silkworm genome