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Hidden attributes of zymosan in the pathogenesis of inflammatory diseases: A tale of the fungal agent

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ABSTRACT

Inflammation triggers immune system-mediated actions that contribute to the development of multiple diseases. Zymosan, a polysaccharide derived from the Saccharomyces cerevisiae cell wall, is mainly made up of glucan and mannan residues and is used as an inflammatory agent. Zymosan is a fungal product that activates the immune system through the activation of inflammatory signaling pathways, and releases a variety of harmful chemicals including pattern recognition receptors, reactive oxygen species (ROS), and the excitatory amino acid glutamate, cytokines, adhesion molecules, etc. Furthermore, we will dive into the molecular mechanistic insights through which this fungal agent induces and influences various inflammatory diseases such as cardiovascular, neuroinflammation, diabetes, arthritis, and sepsis. Based on the evidence, zymosan appears to be a promising inflammatory-inducing agent. Nonetheless, more animal data is the need of the hour to catch a glimpse and unravel the capacity of zymosan.

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Introduction

Inflammation is the physiologic response of the immune system that can be induced by infections, fungi, microbial agents, and toxic chemicals. The development of several inflammatory disorders, including cardiovascular, neuroinflammation, diabetes, arthritis, sepsis, and cancer, have been linked to inflammation and the immune system (1). Zymosan is a fungal substance generated from the *Saccharomyces cerevisiae* yeast cell wall that has been used as an inflammatory compound for more than 50 years. Zymosan is a glucan containing 1, 3-glycosidic linkages connecting repeated glucose units. It is made up of polysaccharide chains with varying molecular mass, with around 73% polysaccharides, 15% proteins, 7% lipids, and inorganic substances. It is in powder form with minute lumps having an off-white to light brown color, insoluble in water and its storage temperature is 2-8 ˚C (2). Researchers discovered that zymosan enhances the inflammatory response by activating immune-specific receptors in immune cells such as macrophages, dendritic cells, and neutrophils, receptors including toll-like receptors (TLR2 and TLR6), dectin-1 receptor, and complement receptor 3 (CR3). Both innate and acquired immunity are activated by them (3). Researchers recently discovered that zymosan causes inflammation by stimulating multiple macrophage receptors such as TLR2, TLR-6, NLRP3, and dectin-1, as well as neutrophil infiltration by synergistically activating inflammatory pathways, resulting in increased TNF-α, IL-6, and IL-1β production and an oxidative burst which leads to neuroinflammation, and cardiovascular and metabolic disorders (4, 5). Zymosan also exerts its effect via other mechanisms that play an important role in the induction of inflammation-related diseases such as atherosclerosis, hypertension, sepsis, and arthritis through increased plasma levels of nitric oxide, increased cyclooxygenase activity, up-regulating the production of free radicals reactivity of oxygen species (ROS) by the formation of active oxygen polymorphonuclear (PMN) cells (6). Arya and colleagues reported that administration of zymosan to C57BL/6 mice induces inflammation through activation of the TLR2/Nf-ƙB signaling pathway, which leads to the production of various cytokines and degradation of low-density lipoprotein receptors, leading to impaired cholesterol levels and arterial stiffness (7). Harrigan and coworkers reported that zymosan administration induces neuroinflammation via activation of microglial cells, which leads to the release of ROS and increased glutamate release and results in neuronal death (8).

Researchers also demonstrated the involvement of zymosan in peritoneal inflammation, which leads to impaired adipokine levels by increasing the levels of IL-17A which leads to the development of obesity (9). An early-phase 1 clinical trial study (NCT04674306) is currently underway, in which participants are being treated with α-lactalbumin and zymosan in combination to produce an immunologic response through vaccination against non-metastatic triple-negative breast cancer. This study is proposed to evaluate the safe and effective dose of the above-mentioned vaccine. However, the outcome is still awaited, as the study will end in September 2023 (10). The present review aims to describe zymosan as a good experimental preclinical model tool for inducing inflammatory diseases. This review provides the

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mechanistic pathogenesis of inflammatory diseases induced by zymosan, which will be helpful for researchers and pharmacologists to better understand the pathogenesis of inflammatory diseases.

Search method

In the current review, the online search engines and databases we used to obtain scientific literature are Science Direct, Scopus, PubMed, Google Scholar, and Web of science. Keywords used to search are "Zymosan", "Neuroinflammation" Alzheimer's disease", "Cardiovascular disease", "Atherosclerosis", "hypertension" "Obesity", "diabetes", "Arthritis", and "Sepsis".

Zymosan role in neuroinflammation

Neurological diseases are the primary contributors to morbidity and disability throughout the world. The progression of the disease is characterized by a series of events involving the immune system's molecular and cellular components, as well as their interactions with cells and structures in the central nervous system (11). Because of this, there has been a great interest in developing new models to explore the pathogenesis, which will help the researchers to develop a neuroprotective therapeutic approach for a specific target of neuroinflammation pathways.

Neuroinflammation is an inflammatory response that occurs in the brain or spine. The release of cytokines, chemokines, free radical species, and secondary messengers triggers inflammation. These mediators are produced in the CNS by resident glial cells such as microglia and astrocytes, as well as endothelial cells and immune cells (12). These neuroinflammatory reactions have physiological, biochemical, immunological, and psychological implications. Furthermore, the severity of neuroinflammation is regulated by the stimulus or insult's context, duration, and course. Inflammation leads to immune cell accumulation, which results in edema, tissue damage, and lately, cell death (13).

Prior research revealed that microglia, together with astrocytes, are the key players in neuroinflammatory reactions because they are essential for maintaining the function of brain tissue (14). These cells are found in both the white as well as the grey matter of the brain and spinal cord. The central nervous system contributes 10% to the population of microglia cells (11). When these cells become activated due to infection, inflammation, or trauma, harmful chemicals such as ROS and then glutamate (an excitatory amino acid AA) are released which leads to neuronal death (Figure 1). In line with this, a researcher has reported the involvement of zymosan in the development of neuroinflammation via stimulation of microglial cells, and leads to the release of endogenous oxidative free radicals and increases the release of glutamate via activation of volume-regulated anion channels (VRACs) that result in neuronal death (8).

The researcher examined the TLR2/3/4 agonists (LPS, zymosan, and poly (I:C)) and found a decrease in TLR2 and TLR4 mRNA levels, while treatment with LPS and zymosan (but not poly(I:C)) decreased in TLR3 transcript levels at 24 hr only. Understanding the relative roles of different TLRs in neurological illnesses associated with neuroinflammation could be aided by studying TLR2 gene expression (15).

In another study, the role of the CNS, TLR2 activation in eliciting neuro-inflammatory responses was evaluated in intact and experimental autoimmune encephalomyelitis (EAE)-infected rats. On naive versus EAE rats, zymosan, a TLR2 agonist, was injected intra-cerebro-ventricularly (ICV). The results showed that zymosan administration in naive mice resulted in a significant neuro-inflammatory response without any clinical signs. In EAE mice, ICV Zymosan produced a potent acute toxic reaction with a mortality rate of 80% (16).

Using stereotaxic injection, a single bolus of zymosan (75 nl) was injected into the lateral white matter of the rat spinal cord at the thoracic level. Inflammatory lesions were brought on by zymosan in the lateral white matter

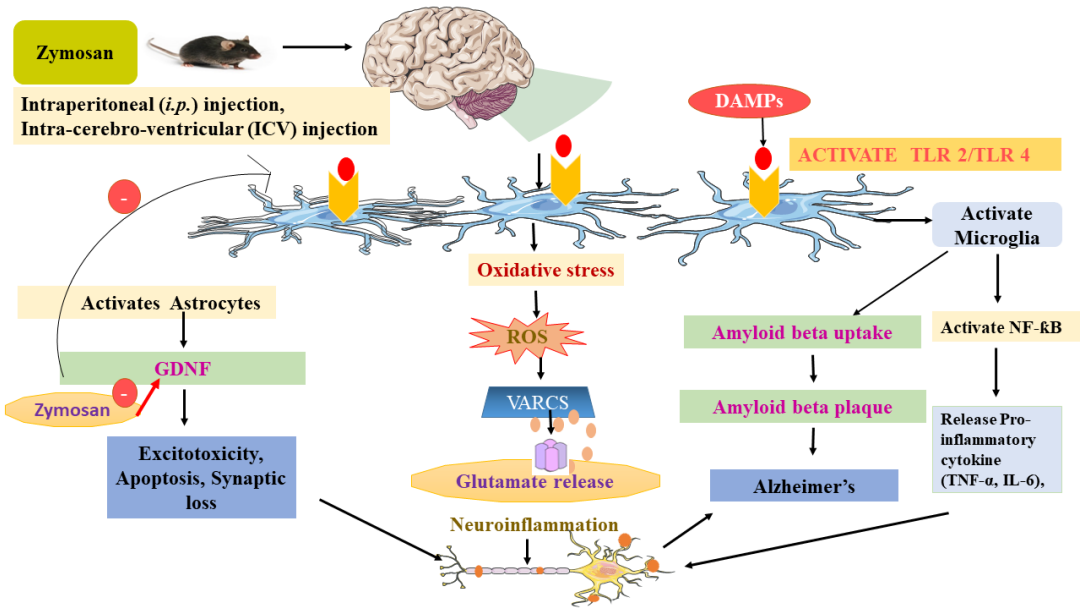

Figure 1. Insights into the multi-target action of zymosan in neuroinflammation and Alzheimer's disease
DAMPs: Damage-associated molecular patterns, ROS: Reactive oxygen species, GDNF: Glial cell-derived neurotrophic factor, TLR: Toll-like receptors, TNF-α: Tumor necrosis factor, IL: Interleukins, NF-Kb: Nuclear factor kappa B, VRACs: Volume-regulated anion channels

of the spinal cord. According to this work, phagocytic microglia/macrophages gradually arise in response to zymosan-induced inflammation in the white matter of the rat spinal cord and the delayed FJB staining of the inflammatory cells. A large number of proteins and/or axonal myelin debris must gradually arise in the cytoplasm of phagocytic microglia/macrophages as one explanation for the progressive appearance of FJB-stained cells in the inflammatory area (17).

It caused full oligodendrocyte loss without increasing NG2 cell proliferation, oligodendrocyte replacement, or ciliary neurotrophic factor expression through the activation of macrophages. Zymosan also promoted delayed lesion extension and primary demyelination of myelinated axons in the periphery of the lesions (18). The most common neurodegenerative diseases are Alzheimer's disease (AD) and Parkinson's disease (PD). Interestingly, researchers reported that microglia highly express all TLRs and their adapter proteins as demonstrated in mice, rats, and human studies; such as TLR1, TLR6, TLR2, TLR3, TLR5, TLR7, TLR8, TLR4, and TLR9 [19, 20, 21]. In microglia, the coupling of TLR2 and TLR4 stimulates the induction of an immune response. Moreover, it was reported that activation of TLR2 in microglia cells enhances pathological amyloid beta (Aβ) uptake, which leads to the accumulation of amyloid beta and results in neurodegeneration (Figure 1) (22). Interestingly, Lax and colleagues demonstrated that systemic (IP) administration of TLR2 agonist zymosan caused cortical neuron death in 5xFAD transgenic mice with Alzheimer's disease (23).

AD pathology suggests the involvement of microglial activation and inappropriate clearance of Aβ. Neurodegeneration is not merely Aβ-induced microglial activation but rather a repeated systemic microbial insult that facilitates its progression in AD (22). Another study by Renata C. Gonçalves reported that (IP) administration of zymosan (30 g/100 g body weight) showed an early alteration in pro-inflammatory cytokines in the hippocampus. However, at the cortex, a late effect was noted, the anti-inflammatory cytokine IL-10 decreased from 4 to 15 days. Oxygen consumption was decreased in the hippocampus and prefrontal cortex, but not the cortex, only at 7 days. Furthermore, a late (15-day) increase in oxidative damage parameters and mild cognitive impairment were observed in Wistar rats (23).

Astrocytes influence microglial activation in the nigrostriatal system, through specific neurotropic factors (24, 25). Moreover, astrocytes can modulate microglia via their phagocytic and reactive oxygen species production activities; zymosan administration stimulated midbrain microglia cultures, and both parameters were greatly reduced in cells incubated with astrocytes conditioned media (ACM). When the GDNF contained in the ACM was removed with a particular antibody, the effect of the ACM on Zymosan A-induced microglial activation was eliminated. Furthermore, media conditioned by GDNF-deficient astrocytes failed to inhibit microglial activation, but GDNF supplementation of non-conditioned media reduced zymosan A-induced microglial activation (26). Based on these mechanisms, zymosan has the potential to induce neuroinflammation.

Zymosan's role in cardiovascular pathology

A growing body of research suggests that there is a fundamental relationship between inflammation and cardiovascular events. Peptidoglycan and lipoteichoic acids from Gram-positive bacteria, lipopolysaccharides and lipoproteins from Gram-negative bacteria, lipoproteins from mycoplasma, and zymosan from yeast have all been implicated in the etiology and pathogenesis of certain cardiovascular events like atherosclerosis and hypertension. Atherosclerosis is a major contributor to cardiovascular events (27).

Despite lifestyle changes and new treatment approaches, the prevalence of atherosclerosis continues to rise, and it remains the world's top cause of mortality. Atherosclerosis is an inflammatory condition of the arteries stimulated by a chemical agent or the adherence of lipids and fibers to the innermost layer of the vessel walls. This, in turn, increases the recruitment of adhesion molecules, cytokines, chemokines, monocytes, and T cells, which migrate within the intima and boost the uptake of modified lipoprotein particles, resulting in the formation of foam cells and leading to arterial stiffness (Figure 2) (6). There are various inflammatory models to develop atherosclerosis, such as a high-fat diet or high-

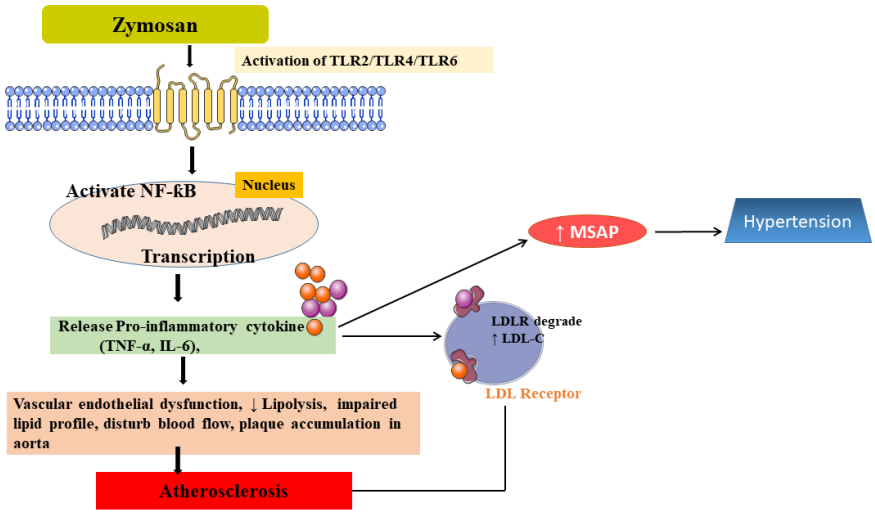

Figure 2. Insights into the multi-target action of zymosan in atherosclerosis
TLR: Toll-like receptors, NF-Kb: Nuclear factor kappa B, MSAP: Mean systolic arterial pressure, TNF-α: Tumor necrosis factor alpha, IL: Interleukins, LDLR: Low-Density Lipoprotein Receptor, LDL: Low-Density Lipoprotein, LDL-C: Low-Density Lipoprotein Cholesterol

cholesterol diet but they are time-consuming. Researchers are working on new models to induce atherosclerosis. The advantage of the zymosan model is time saving since single intraperitoneal injection induces atherosclerosis. Arya and colleagues discovered that administering zymosan 80 mg/kg as a single intraperitoneal injection induces vasculitis in C57BL/6 mice by activating the TLR2 and Nf-B pathways, resulting in the release of various inflammatory mediators such as IL-6 and TNF-α. These inflammatory mediators lead to the degradation of hepatic low-density lipoprotein receptors which leads to impaired cholesterol levels and causes arterial stiffness (Figure 2) (7, 28). A study reported that zymosan induces inflammation and impairs the liver's reverse cholesterol transport mechanism when administered intraperitoneally in C57BL/6 mice (29).

Researchers recently reported that a high-fat diet for 30 days combined with a single IP injection of zymosan (80 mg/kg) induces the vascular event via activation of the pathogen recognition receptor, i.e., TLR2, which further activates the transcription factor, leading to the secretion of inflammatory mediators such as IL-6 and TNF-α. This, in turn, led to impaired cholesterol levels by the degradation of cholesterol receptors, i.e., hepatic low-density lipoprotein receptors, which resulted in plaque or fat accumulation in the aorta, which disrupts blood flow and causes arterial hardening (28). Zhang *et al.*, in 2008 reported that zymosan-induced inflammation in Wistar rats when injected IP 20 mg/kg, once every 3 days for 3 consecutive weeks. They found that zymosan boosted the expression of the genes of various inflammatory factors, including integrins proteins, interleukin IL-18, IL-1**β,** and matrix metalloproteinases 2 and 9. Besides these, they up-regulated expression of NF-ƙB/p65 and alterwd cholesterol levels, after treatment with zymosan in rats. Histopathological changes were observed in aorta tissues by using Sudan IV staining and transmission electron microscopy. The formation of foam cells was observed in aortic tissues. Thus researchers concluded that zymosan leads to atherosclerotic development (30).

Interestingly, the effects of zymosan have also been reported in hypertension. A 2008 study evaluated the effect of zymosan in the hearts of gestational rats. They discovered that giving zymosan (2.37 mg/kg, IP) to gestational rats increased their mean systolic arterial pressure (MSAP) and NF-kB (p65) levels, as well as their inflammatory cytokine (TNF- α) levels, which leads to hypertension in offspring of rats (TNF-α) (Figure 2) (31). Thus, these findings suggest that zymosan has the potential to induce cardiovascular diseases through different mechanistic approaches, such as impairing reverse cholesterol transport, pattern pathogen recognition receptor pathway, transcription factor pathway. Zymosan is suggested as a promising candidate to induce cardiovascular disease.

Zymosan's role in obesity

Obesity is defined as the accumulation of abnormal or excessive fat that makes maintaining a healthy weight difficult. Overload of macronutrients in the adipose tissues initiates the secretions of inflammatory mediators such as TNF-α and IL-6, as well as a decrease in adiponectin synthesis, resulting in a pro-inflammatory and oxidative stress state (Figure 3)(32). Adipokines (adipose-derived secretory factors) are a class of bioactive compounds with both pro- and anti-inflammatory actions that are secreted by adipose tissue, an essential endocrine organ (33). Alterations in the secretions of adipokines due to adipose tissue malfunctioning resulted in the pathogenesis of obesity-linked complications. A 2008 study discovered that zymosan administration causes an inflammatory response in mice, as evidenced by increased levels of serum adipokines including leptin, resistin, adiponectin IL-6, TNF-α, IL-10, and chemokine (C-X-C motif) ligand 2 (Figure 3) (34). Another study demonstrated that zymosan administration triggers the release of proinflammatory cytokines (IL17A) which leads to further production of neutrophil-attracting CXC and CCL2 chemokines and results in peritoneal inflammation in obese mice (9). In the context of this research, it is possible that zymosan can cause obesity via an inflammatory pathway. One promising possibility for causing obesity is zymosan.

Zymosan's role in rheumatoid arthritis

Rheumatoid arthritis is categorized by joint inflammation in later stages and cartilage destruction (35). Several inflammatory mediators are responsible for rheumatoid

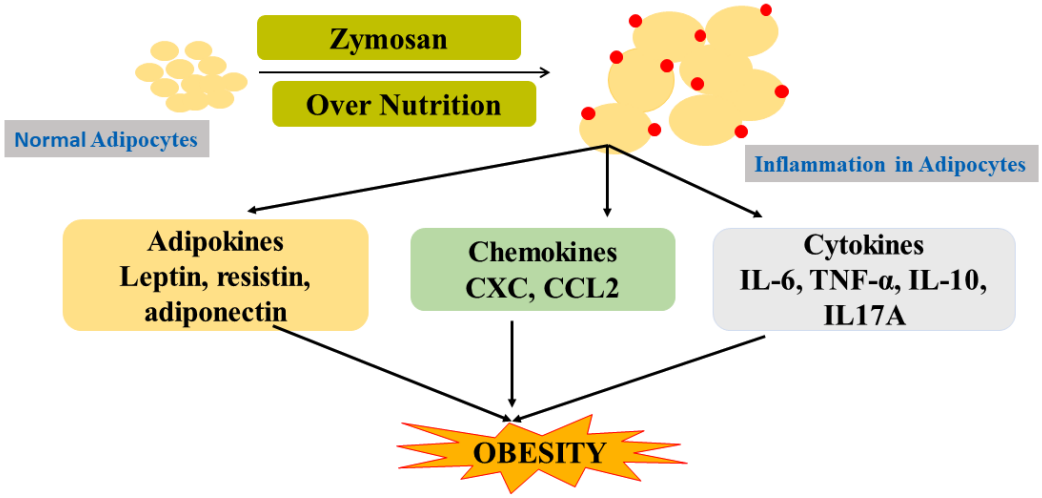

Figure 3. Insights into the multi-target action of zymosan in obesity
IL: Interleukins, TNF-α: Tumor necrosis factor alpha, CCL2: Chemokine (C-C motif) ligand 2

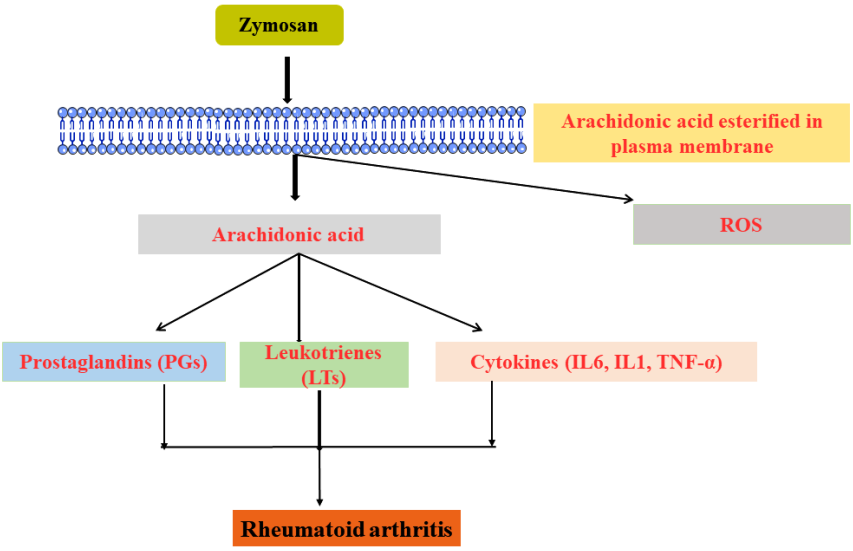

Figure 4. Insights into the multi-target action of zymosan in rheumatoid arthritis
TNF-α: Tumor necrosis factor alpha, IL: Interleukins, ROS: Reactive oxygen species, PGs: Prostaglandins

arthritis and include prostaglandins and leukotrienes, tumors, necrosis factors, and oxygen radicals (Figure 4) (36). Many agents, such as zymosan, carrageenan, and dextran, accelerate joint diseases by inducing inflammation in the articular joint, and in particular, they stimulate match by a different or conventional pathway and using a C3b (complement component) cleavage product. Hence, they encourage the secretion of macrophage lysosomal enzymosanes (37). Researchers reported that zymosan induced inflammation in mice, when an intraarticularly sterile suspension of zymosan was injected (10 µl of 15 mg/ml). The histopathological changes in the joint, such as synovitis, vasculitis, and, sometimes, pannus development were detected. Histological examination of the Zymosan-treated group revealed severe cartilage injury, loss of the common joint architecture, and pannus formation. Researchers concluded that bone erosion was accompanied by severe inflammation of articular tissues. They also suggest that zymosan is a potent releaser of arachidonic metabolites that contribute actively to the primary phase of the inflammatory response (38). In another study, when zymosan was injected intra-articularly (15 mg/ml) into the knee joints, it caused arthritis in mice, resulting in a significant increase in -glucuronidase levels when compared with the control group. Histopathological and ultrastructural studies revealed the inflammatory response, where cartilage destruction, as well as abnormal rough endoplasmic reticulum and mitochondria, were observed (39). Based on these mechanisms, zymosan has the potential to induce rheumatoid arthritis.

Zymosan role in sepsis

Sepsis is characterized by a systemic proinflammatory response that includes sepsis, non-septic shock, septic shock, and ultimately multiple organ dysfunction syndrome and death (40). In 2006, Di and co-workers reported that sepsis contains proinflammatory cytokines that may trigger systemic inflammatory cascades mediated by vasoactive amines, chemokines, free radicals, nitrogen species, and complement and the coagulation systems (Figure 5) (41). As an inflammatory syndrome, septic shock and sepsis are associated with a systemic inflammatory cascade stimulated by innate and adaptive immunity, respiratory, gastrointestinal, renal, and circulatory dysfunction, leading to multiple organ failure syndromes and ultimately death. Importantly, the number of cases of non-septic shock, severe sepsis, and fungal infections has increased significantly. Non-septic shock is caused by non-bacterial and non-endotoxin agents such as zymosan, which is characterized by a systemic inflammatory response that also occurs in septic shock (42, 43).

Zymosan has been revealed to encourage a signal through the TLR2/6 heterodimer and Dectin-1 receptors, leading to the beginning and translocation of NF-κB to the nucleus and modifying cytokine production. Proinflammatory cytokines, such as pro-inflammatory lipid mediators, TNF-, platelet-activating factor, and prostaglandin metabolites, play an important role in the pathophysiology of zymosan-induced shock (Figure 5) (44). These results and mechanisms reveal the possible potential of zymosan to induce sepsis.

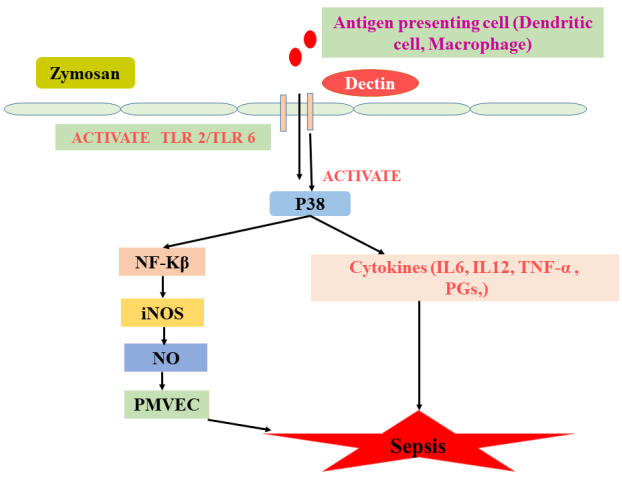

Figure 5. Insights into the multi-target action of zymosan in sepsis
TLR: Toll-like receptors, NF-KB: Nuclear factor kappa B, TNF-α: Tumor necrosis factor alpha, IL: Interleukins, PGs: Prostaglandins, iNOS: Inducible nitric oxide synthase, NO: Nitric oxide, PMVEC: Pulmonary microvascular endothelial cells

Zymosan's role in diabetes

Diabetes mellitus is categorized by type 1 diabetes mellitus (absolute) or type 2 diabetes mellitus (relative). Insulin deficiency is a major pathway disruption. Insulin resistance is a loss of insulin sensitivity in insulin-targeted tissues and is the most important indicator of type 2 diabetes mellitus. Type 2 diabetes mellitus-like or the stimulation of insulin resistance are widely preferred methods in animal models. Diet-induced insulin resistance has mostly been used to justify the consumption of a high-fat or fructose-rich diet. (45). But, the generation of this model required a long time. Formerly, the minor dose of streptozotocin (STZ) in addition to a high-fat diet has been recognized as an altered method (46).

However, the late stage of type 2 diabetes mellitus is close to this model. Hence, other methods for the induction of type 2 diabetes mellitus, like animal models are necessary. Wang and coworkers suggest that the disease models include insulin resistance induced in mice introduced with zymosan via the IP method by giving 100 mg/kg dose once a week for four consecutive weeks. This results in efficient and structural alterations in remote organs including the intestine, liver, kidneys, and lungs due to an extreme inflammatory response (47). Another mechanism of inflammation is caused by zymosan through the initiation of macrophages excited by TLR-2 and TLR-6, which increases the intracellular ROS production due to the initiation of PMN cells. In this type of insulin resistance, zymosan injection has also been held accountable for the reduced expression of numerous protein markers involved in insulin signaling like phosphorylated GSK-3, PI3-Kinase, Akt, and IRS-1 (Figure 6) (48). The general inflammatory response induced by zymosan revealed numerous metabolic and hormonal changes, including increased levels of several stress hormones, increased whole-body glucose turnover, and insulin resistance. Glycogen content was reduced by an elevated rate of hepatic glucose production, and increased glucose uptake was described too (Figure 6). An increase in insulin resistance by zymosan has been recognized (49). But, the use of this cytokine to raise type 2 diabetes mellitus in animal models is still not recognized. Hence this animal model is better than the diet-induced method, and this model also convinces as well as quickly creating insulin resistance.

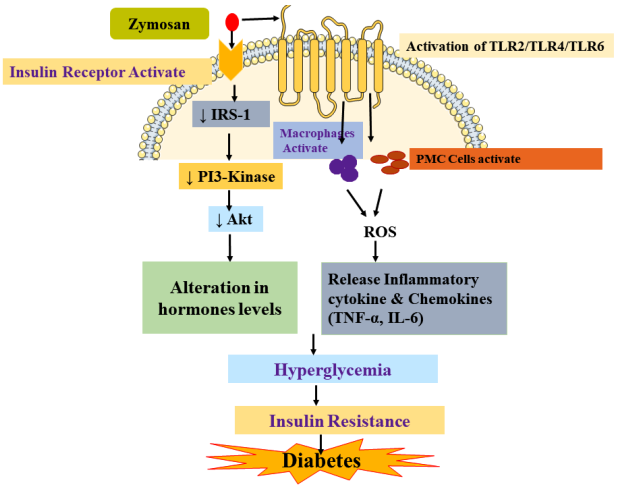

Figure 6. Insights into the multi-target action of zymosan in diabetes TLR: Toll-like receptors, ROS: Reactive oxygen species, TNF- α: Tumor necrosis factor, IL: Interleukins, IRS-1: Insulin receptor substrate 1, PI3: Phosphoinositide 3, PMC: Pollen mother cell, Akt: Protein kinase B

Potential toxicity and side effects of zymosan

Zymosan has documented toxic effects. Many studies have found that zymosan administration causes inflammatory responses, including shock and development of severe multiple organ dysfunction syndromes (MODS), kidney dysfunction, vasodilatation, lethargy, and anorexia (50-56). Moreover, even in experimental studies, motility in 20-40% of animals was observed with the intraperitoneal injection of zymosan due to its lethal immunological response (50). The most common side effects are an inflammatory reaction and granuloma formation at the site of administration (intramuscularly) (50).

Conclusion

This review describes and abridges various studies on zymosan, a fungal agent that instigates a wide range of inflammatory diseases, including cardiovascular, neuroinflammation, diabetes, arthritis, and sepsis. The results cogently highlighted the pleiotropic effects of zymosan on multiple inflammatory signaling pathways, which might explain its concealed, potent inflammatory potential. The goal of the present review is to develop a new, less time-consuming, and therapeutically relevant animal model for assessing and understanding the pathophysiology of numerous inflammatory and metabolic problems. The overwhelming majority of the studies mentioned in this review suggest that zymosan could trigger several inflammatory signaling pathways that result in the development of inflammatory disorders.

Acknowledgment

None.

Authors' Contributions

PA and UB conceived the study or design and performed literature search. NK critically reviewed the final manuscript. ST outlined the study and writing. VS provided the study concept, literature search, and drafting. PA, UB, NK, ST, and VS approved the final manuscript.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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