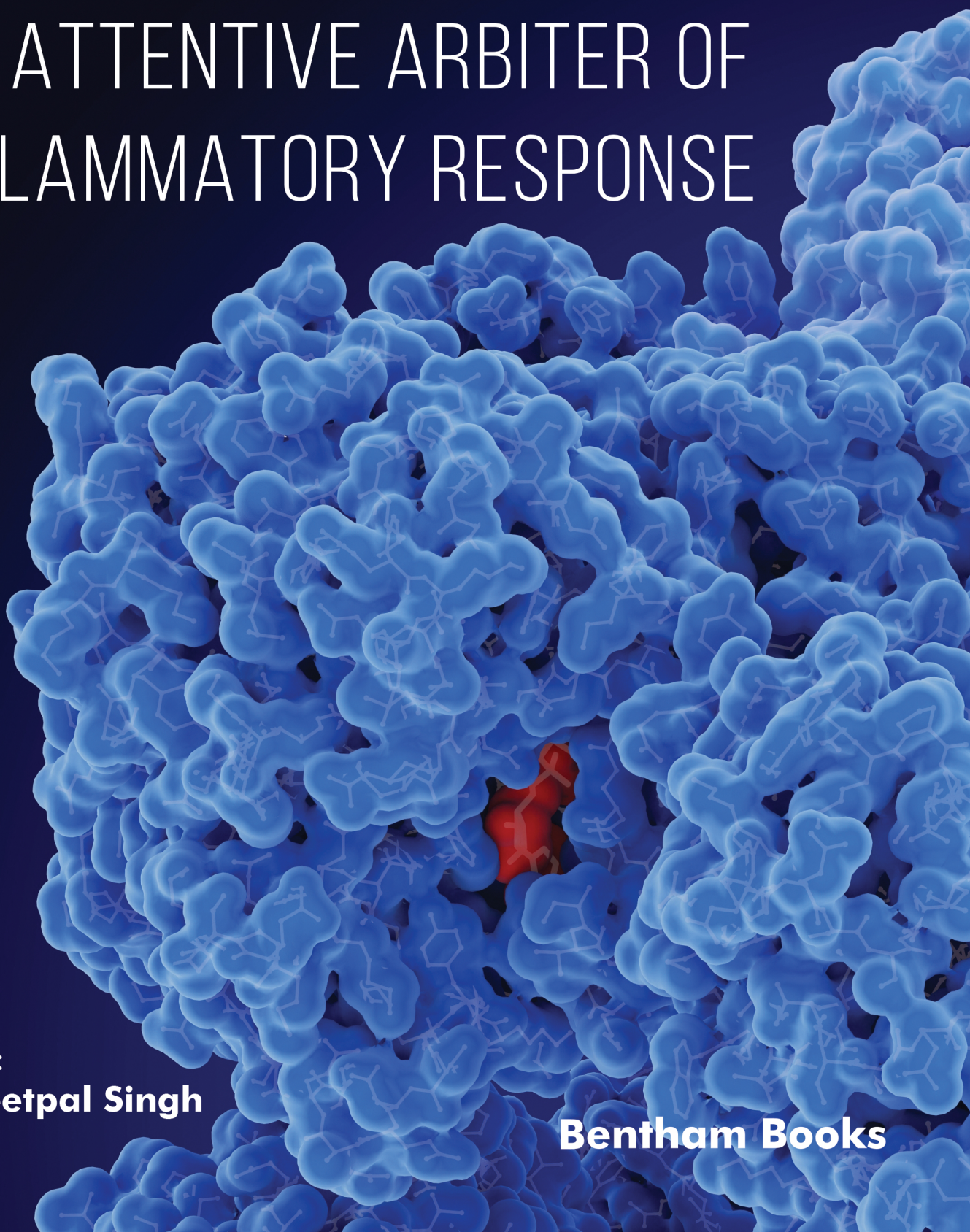
THE NLRP3 INFLAMMASOME: AN ATTENTIVE ARBITER OF INFLAMMATORY RESPONSE



Editor:
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The NLRP3 Inflammasome: An Attentive Arbiter of Inflammatory Response

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ISBN (Online): 978-981-5223-94-1

ISBN (Print): 978-981-5223-95-8

ISBN (Paperback): 978-981-5223-96-5

First published in 2024.

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PREFACE

The human body is an intricate and wondrous system, constantly engaged in a delicate balance of maintaining health and combating various threats. Inflammation, a fundamental process of the immune system, plays a critical role in the body's defense against infection and injury. While inflammation is an essential mechanism for maintaining tissue homeostasis, dysregulated or chronic inflammation can lead to the development and progression of numerous diseases. Within the realm of inflammation, the NLRP3 inflammasome has emerged as a captivating protagonist in recent years. This book, "The NLRP3 Inflammasome: An Attentive Arbiter of Inflammatory Response," delves deep into the intricate workings of this molecular complex and explores its pivotal role in shaping the inflammatory landscape.

Our understanding of the NLRP3 inflammasome has undergone significant advances since its initial discovery. This enigmatic protein complex, comprised of NLRP3 (NOD-like receptor family, pyrin domain-containing 3), ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), and pro-caspase-1, acts as a sensor of danger signals within the cell, orchestrating a cascade of events that culminate in the production and release of pro-inflammatory cytokines, particularly interleukin-1β (IL-1β) and IL-18.

"The NLRP3 Inflammasome: An Attentive Arbiter of Inflammatory Response" is a comprehensive exploration of the intricacies surrounding the NLRP3 inflammasome. Through the collaboration of esteemed scientists, researchers, and clinicians, this book seeks to shed light on the various aspects of NLRP3 biology, regulation, and its involvement in a wide array of diseases, ranging from metabolic disorders to neurodegenerative conditions.

In the first chapter, writer Dr. Rashmi Singh, along with her co-authors, has made a significant contribution to the understanding of the role of the NLRP3 inflammasome in airway inflammation and fibrosis. In their research, they have focused on elucidating the mechanisms of NLRP3 inflammasome activation and its impact on respiratory diseases such as asthma. Their work has highlighted the crucial role of NLRP3 in mediating caspase-1 activation and the secretion of proinflammatory cytokines, which contribute to the progression of asthma by promoting excessive inflammation, extracellular matrix accumulation, and airway remodeling. Moreover, they have identified endotoxin (lipopolysaccharide, LPS) as one of the activators of NLRP3, linking environmental factors to the incidence of asthma and allergic diseases. This chapter provides a comprehensive summary of their research findings, shedding light on the mechanisms underlying NLRP3 inflammasome activation and its regulation in asthmatic exacerbations.

In the second chapter, Dr. Sushweta Mahalanobish, along with co-authors Noyel Ghosh and Parames C. Sil, has made a significant contribution to the understanding of the role of NLRP3 inflammasome in pulmonary hypertension (PH). Their research focuses on the progressive pulmonary vasculopathy characterized by increased mean pulmonary arterial pressure, adverse vascular remodeling, and right ventricular failure. They explore the involvement of inflammation as a crucial factor in the onset and development of PH, specifically highlighting the NLRP3 inflammasome as a key mediator in the signaling cascade that regulates PH-associated conditions through inflammatory mechanisms. The activation of NLRP3 and the subsequent release of proinflammatory cytokines IL-1β and IL-18 contribute to adverse consequences on pulmonary vasculature and the onset of PH. The chapter delves into current PH therapies and their limitations and introduces the potential therapeutic targeting of NLRP3 inflammasomes to modulate inflammation in PH pathobiology. The authors provide a comp-

rehensive insight into the role of NLRP3 inflammasome in PH and its implications for future therapeutic interventions.

Dr. Abhinav Kanwal and his team have provided a comprehensive exploration of the modulatory mechanism of the NLRP3 inflammasome in heart diseases. Despite significant advancements in therapy, heart failure remains a leading cause of mortality worldwide. The authors highlight the crucial role of the inflammasome in the progression of various cardiovascular diseases, including heart failure, abdominal aortic aneurysm, atherosclerosis, diabetic cardiomyopathy, hypertension, dilated cardiomyopathy, cardiac remodeling, and calcific aortic valve disease. Specifically, they focus on the NLRP3 inflammasome, a multi-protein signaling platform that tightly regulates inflammatory responses and antimicrobial host defense, leading to the generation of pro-inflammatory cytokines through the activation of caspase-1 and subsequent pyroptosis. By investigating the NLRP3 inflammasome in different cardiovascular diseases, the authors aim to uncover critical disease triggers and endogenous modulators with the aimof developing new therapeutic interventions in the future. The chapter provides a summary of recent literature, emphasizing the activation mechanism of the NLRP3 inflammasome and its implications in the pathophysiology of heart failure, shedding light on this complex and intriguing aspect of heart diseases.

In the fourth chapter, Monika Joon and Manisha Yadav explore the intricate relationship between Mycobacterium tuberculosis (Mtb) and the inflammasome. Mtb is known as a highly successful human pathogen, capable of evading the host immune response through the development of robust effectors. It can survive and multiply within the host's immune system, even in the presence of immune tools meant to eliminate it. Granuloma formation, a compensatory mechanism, offers partial benefits to both the host and the pathogen. While extensive research has been conducted on various mycobacterial virulence factors, the relatively newer concept of inflammasomes requires further investigation. Insights into the inflammasome-Mtb interaction may open up new avenues for the development of host-directed therapy (HDT) strategies to combat TB. By comprehending the intricate dynamics between the inflammasome and Mtb, novel approaches for managing this disease can be explored.

The fifth chapter by Syed Ehtaishamul Haque, Aamir Khan, and Ashif Iqubal deals with an overview of the NLRP3 inflammasome's activation mechanism, its association with cardiovascular complications, and the potential of NLRP3 inhibitors as cardioprotective agents. They highlight the positive correlation between NLRP3 inflammasome activation and various cardiovascular disorders, including hypertension, angina, arrhythmia, cardiac fibrosis, myocardial infarction, and heart failure. By discussing the structural components of the NLRP3 inflammasome and its molecular activation pathway, the authors underline its crucial role in the pathogenesis of cardiovascular diseases. Furthermore, they shed light on promising outcomes from studies exploring NLRP3 inflammasome inhibitors in cardiovascular disorders. Overall, the manuscript underscores the importance of targeting the NLRP3 inflammasome as a potential therapeutic approach for managing and treating cardiovascular diseases.

In the sixth chapter, Sonal Yadav examines the role of NLRP3 in protozoan parasitic infections. They discuss the activation of the NLRP3 inflammasome by various protozoan parasites, including *Giardia duodenalis*, *Entamoeba histolytica*, *Trichomonas vaginalis*, Plasmodium species, *Trypanosoma cruzi*, *Schistosomes*, *Toxoplasma gondii*, and Leishmania species. The authors highlight the protective effects of NLRP3 against certain infections, such as Giardia, *Trypanosoma cruzi*, and *Entamoeba histolytica*, while also noting its contribution to pathology in Schistosomes and Malaria parasite infections. They emphasize the need for

further research to better understand the precise mechanisms and roles of NLRP3 in host defense and inflammatory pathology in parasitic protozoan infections, which could pave the way for the development of innovative treatment strategies.

The seventh chapter by Dr. Adekunle Babajide Rowaiye and his colleagues focuses on the NLRP3 inflammasome as a target for anti-inflammatory drugs. They highlight the crucial role of the NLRP3 inflammasome in the innate immune response and its association with various inflammation-related diseases. The authors discuss the activation of the NLRP3 inflammasome and the production of proinflammatory cytokines, emphasizing the importance of inhibitory mechanisms to decrease inflammation and inflammasome-mediated cell death. They further explore the potential of targeting signaling molecules along the NLRP3 inflammasome pathway as drug targets for effective inhibition and downregulation of proinflammatory cytokines. The chapter provides insights into the classes of NLRP3 inflammasome inhibitors, their anti-inflammatory effects, and underlying mechanisms of action.

Dr. Agnès Hamzaoui and co-authors, in the eighth chapter, investigate the potential value of sputum levels of Interleukin-38 (IL-38) and NLRP3 inflammasome in severe childhood asthma. Asthma is known to be an inflammatory airway disorder with varying expression of cytokines based on disease severity. The transition from exacerbation to remission involves a complex interplay between inflammatory and anti-inflammatory mediators. The authors focus on the expression of IL-38 and NLRP3 inflammasome in severe asthmatic children. They find that NLRP3 inflammasome is upregulated in severe asthma, while levels of IL-38 are low. The inflammatory profile of severe asthma in children is characterized by the expression of IL-17, IL-32, IL-1β, and NLRP3 inflammasome. This study sheds light on the potential role of IL-38 and NLRP3 inflammasome as biomarkers in severe childhood asthma. It contributes to a better understanding of the inflammatory mechanisms involved in the disease.

The ninth chapter by Lokesh Sharan, Anubrato Pal, Priya Saha, and Ashutosh Kumar explores the role of inflammasomes, specifically NLRP1, NLRP3, NLRC4, and AIM2, in inflammation and neuropathic pain. These inflammasomes play a crucial role in the development of autoimmune and metabolic disorders, cancer, and various inflammatory conditions. The activation of inflammasomes is triggered by molecular changes, such as mitochondrial dysfunction, neuroinflammation, lysosomal damage, oxidative stress, sensitization, and disinhibition, leading to the activation of proinflammatory pathways and subsequent development of inflammasome-related neuropathic pain. Among these inflammasomes, NLRP3 has been extensively studied and identified as a key player in neuropathy. This chapter provides an overview of the involvement of inflammasomes, particularly NLRP3, in neuropathic pain. Based on available evidence, targeting inflammasome activity is proposed as a potential cutting-edge approach for the successful treatment of neuropathic pain. The understanding of inflammasome-mediated mechanisms in neuropathic pain may pave the way for the development of novel therapeutic strategies in the future.

The chapters in this book provide in-depth analyses of the mechanisms underlying NLRP3 inflammasome activation, the signaling pathways involved, and the interplay between NLRP3 and other cellular processes. Additionally, the authors delve into the consequences of dysregulated NLRP3 activation, highlighting the implications for disease pathogenesis and potential therapeutic interventions. From the role of NLRP3 in sterile inflammation to its contribution to the pathogenesis of autoimmune disorders, each chapter offers valuable insights into this captivating field of research.

I hope that "The NLRP3 Inflammasome: An Attentive Arbiter of Inflammatory Response" serves as a valuable resource for scientists, clinicians, and students alike, fostering a deeper understanding of the NLRP3 inflammasome and its impact on human health. It is our sincere belief that by unraveling the mysteries surrounding this vigilant arbiter of inflammation, we can unlock novel therapeutic strategies that harness its potential for the betterment of patients worldwide.

I would like to express my sincere gratitude to Mr. Nitin Kumar for his invaluable assistance in the editing and refining of the book. I am also immensely thankful to Ms. Humaira Hashmi, In-charge of the eBook Department, and Ms. Asma Ahmed, Manager of the eBooks Publication Department for her support in publishing this book and Mr. Mahmood Alam, Director of Publications at Bentham Science Publishers, for their unwavering support, encouragement, and assistance. Their contributions have been instrumental in bringing this book to fruition.

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CHAPTER 1

Role of NLRP3 Inflammasome in Airway Inflammation and Fibrosis

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Abstract: The NLRP3 inflammasome is a critical component of the innate immune system that mediates caspase-1 activation and the secretion of proinflammatory cytokines IL-1β/IL-18 in response to microbial infection and cellular damage. Nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain 3 (NLRP3), one of the members of the NLR family, consists of NLRP3, the adaptor molecule, apoptosis-associated speck-like protein containing a caspase and recruitment domain (ASC) and an inflammatory caspase-1 that causes excessive inflammasome activation in respiratory diseases like asthma and could exacerbate the progression of asthma by considerably contributing to ECM accumulation and airway remodeling. NLRP3 is closely associated with airway inflammation and asthma exacerbations as endotoxin (lipopolysaccharide, LPS) is one of its activators present in the environment. Asthma is a complex immunological and inflammatory disease characterized by the presence of airway inflammation, airway wall remodeling and bronchial hyperresponsiveness (BHR). Symptomatic attacks of asthma can be caused by a myriad of situations, including allergens, infections, and pollutants, which cause the rapid aggravation of respiratory problems. The presence of LPS in the environment is positively correlated with the incidence of asthma and allergic diseases. In this chapter, we summarize our current understanding of the mechanisms of NLRP3 inflammasome activation by multiple signaling events in asthmatic exacerbations and their regulation.

Keywords: Caspases and NLRP3 regulators, Fibrosis, Inflammation, Inflammasomes, Pyroptosis, ROS.

INTRODUCTION

The fundamental elements of the innate immune system are physical as well as chemical obstacles to infection and a number of cellular components that identify invasive pathogens and trigger antimicrobial immune responses. The mucosal surfaces with antimicrobial secretions, vascular endothelium, ciliated respiratory

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epithelium, and epidermis all serve as examples of physical and chemical defensive systems. The air, along with a mixture of gases, carries other substances, such as dust particles, smoke and biological contaminants, such as dust mites, fungi, bacteria, spores, pollen grains and viruses. Lungs remain in continuous contact with the environmental air and several stimuli; hence, the quality of breathing air has a great impact on the health of an individual (Fig. **1**). The overall risk assessment of respiratory diseases is further complicated by socioeconomic status, lifestyle, age, nutritional status, environmental exposure to pollutants and genetic factors of the individual. These factors altogether may predispose or alter the prognosis of the disease (Fig. **2**). The extent of lung damage is also determined by the toxicity, intensity, duration and route of exposure as well as the physical state, such as the size or characteristics of the inhaled substances.

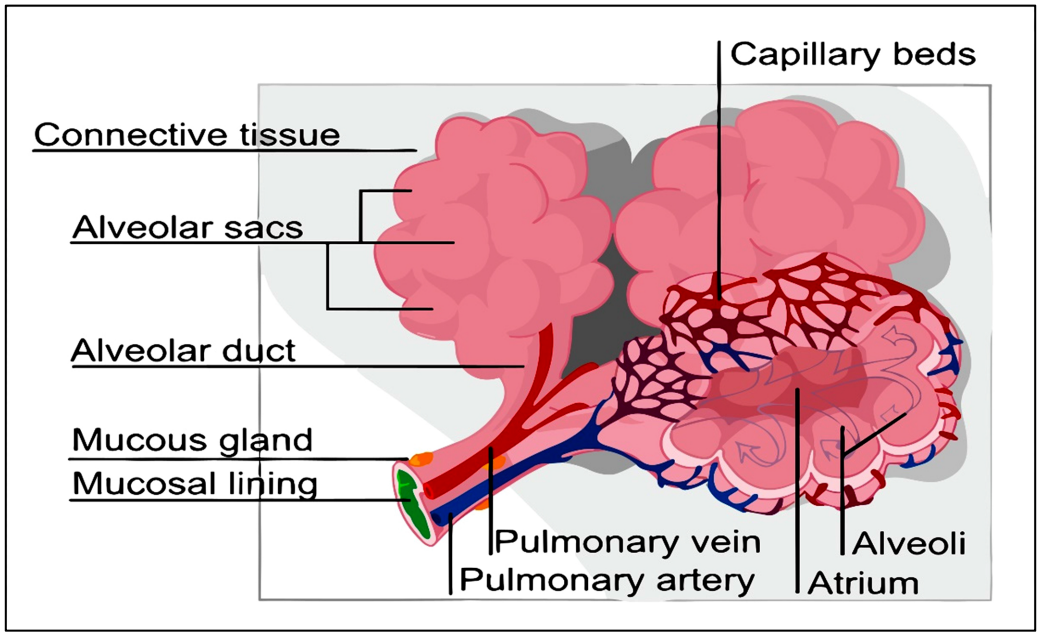

Fig. (1). Structure of lungs showing internal anatomy. Lungs are divided into smaller subunits *i.e.*, alveolar sacs confined with fine capillaries (www.wikipedia.org).

INNATE IMMUNE SYSTEM AND PATTERN RECOGNITION RECEPTORS (PPRs)

Apart from acting as the first line of protection, innate immunity is also essential for the initiation of adaptive responses, which guard against recurrent infections caused by the same pathogen [1]. The cellular elements of innate immunity also include T lymphocytes, cytotoxic natural killer (NK) cells, phagocytic

macrophages and granulocytes, and DCs that deliver antigens. By using autophagy, phagocytosis, complement activation, and immunological stimulation by several families of PRRs, the innate immune system is initially able to identify and restrict microorganisms in an infection [2]. To identify the evolutionarily conserved features of pathogens, referred to as pathogen-associated molecular patterns (PAMPs), the innate immune reactions require a small number of pattern recognition receptors (PRRs), which are encoded in the germ line [3]. In addition, PRRs identify host elements as "danger" signals if they are found in atypical biological macromolecules or on areas of either infection, inflammation or any kind of cellular stress. Several different categories of PRRs were identified by a particular pathogen *via* diverse PAMPs. Host PRRs recognize microbes with extremely diverse biochemical compositions by nearly identical mechanisms. A transmembrane protein called TLRs and cytosolic NLRs are the two PRR families with the best-described members. The induction of specific genes and the formation of a wide variety of chemicals, such as cytokines, cell adhesion molecules, chemokines, and immunoreceptors, are the final effects of PRR-induced signal transduction processes. These molecules work with each other to synchronize the initial infection-related response from the host in addition to serving as a crucial link to the adaptive immune response. The two most important PAMPs are thought to be viral RNA and bacterial endotoxins. One of the strongest PAMPs ever discovered is LPS, a bacterial endotoxin that causes inflammation.

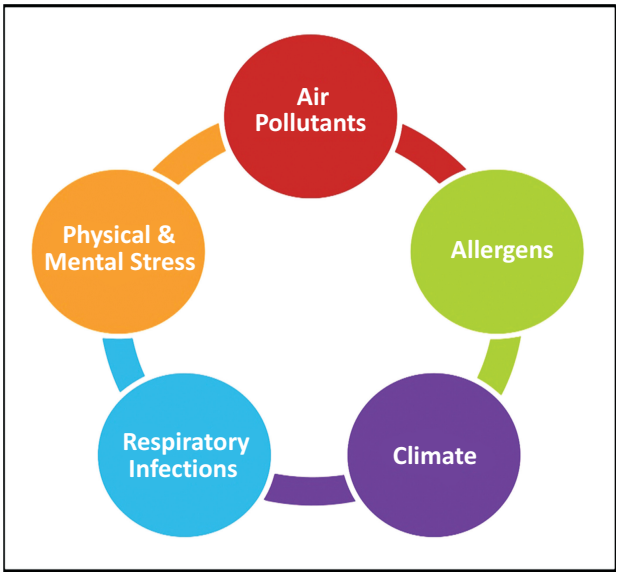

Fig. (2). Common inducers of respiratory diseases among thousands of known factors (www.wikipedia.org).

CHAPTER 2

NLRP3 Inflammasome: A Novel Mediator in Pulmonary Hypertension

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Abstract: Pulmonary hypertension (PH) is marked by elevated mean pulmonary arterial pressure, unfavorable vascular remodeling and right ventricular failure. Current enormous amounts of clinical and preclinical data suggest the role of inflammation as a crucial factor for PH onset and development by modulating both innate and adaptive immune responses. In this context, NLRP3 inflammasome appears as a key step in the signaling cascade that negatively regulates various PH-associated conditions by inducing inflammatory outbursts. The activation of NLRP3 by pathogen-associated molecular pattern molecules/damage-associated molecular pattern molecules and caspase-1 mediated release of proinflammatory cytokines IL-1β and IL-18 are the key molecular events associated with NLRP3 inflammasomal pathway. Released IL-1β and IL-18 bring about adverse consequences on the pulmonary vasculature and the resulting onset of PH. Within this section, we will provide an in-depth understanding of present pulmonary hypertension (PH) treatments and their shortcomings. We will also discuss the contribution of NLRP3 inflammasomes in promoting inflammation within the context of PH pathobiology, as well as explore potential therapeutic approaches to target them.

Keywords: Inflammasome, Interleukin, Lung, NLRP3, Pulmonary hypertension.

INTRODUCTION

The main purpose of the immune system of our body is to safeguard against bacterial infections, eliminate carcinogenic cells, and arouse immune responses during cellular damage. During innate immunity, germline-encoded signaling receptors [pattern recognition receptors (PRRs)] recognize the microbial agents [pathogen-associated molecular patterns (PAMPs)] or agents released from injured host cells [damage-associated molecular patterns (DAMPs)]. Their interactions evoke the inflammatory responses against microbial agents or injured

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cells and reinstate cellular homeostasis. Interestingly, as various commensal microbes are present in different organs (like gut and lung), a specific mechanism in the tissue selectively discriminates such local microflora from the foreign pathogens and hence helps to sustain a balanced immune response.

Several infectious agents, as well as exogenous particulates, are inhaled into our respiratory tract during inspiration. Innate immunity protects our respiratory machinery from these unpleasant substances,. In the lungs, specialized PRRs such as NOD-like receptors (NLRs), Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and Toll-like receptors (TLRs) stimulate the inflammatory response and induce immune cells to secret different chemokines like Monocyte chemoattractant protein-1 (MCP-1) or cytokines like interleukin-8 (IL-8), Tumor necrosis factor (TNF) and promote the involvement of various immune cells (like lymphocytes, neutrophils, *etc*.). Various cytokines, including IL-1β as well as IL-18, can trigger lung inflammation. Both of them are capable of exhibiting proteolytic activity, regulated by various innate immune receptors that assemble into a multiprotein composite called inflammasome [1]. Inflammasome activation is not only pathogen dependent but also dysregulated metabolism or damaged tissue triggers the onset of inflammasome-induced immune response. Evidence suggest that the NLRP3 inflammasome activation is required for viral or bacterial infections. Prolonged stimulation of this signaling factor causes the onset of various respiratory disorders like idiopathic pulmonary fibrosis (IPF), asthma, pulmonary hypertension (PH), and so on.

PH is a severely ruinous disorder where the mean arterial pressure of the pulmonary system remains more than 25 mm Hg at resting condition [2]. According to the World Health Organization, pulmonary arterial hypertension (PAH) is a Group 1 PH, which refers to increased pulmonary vascular resistance along with vascular remodeling of lung tissue [3]. The remodeling occurs in all the 3 layers of the vessel —the intima, media and adventitia, which cause enhanced resistance of pulmonary vasculature, functional impairment of the right ventricle and resulting mortality [4]. Recently, the role of inflammation on PH onset has received greater attention. Immune cells like macrophages, neutrophils, B-lymphocytes as well as T-lymphocytes inside the vessels have been found to be associated with both animal and human PH. In addition to the existence of autoantibodies, higher amounts of cytokines related to the inflammasomal pathway have been detected in the blood of PAH patients [5]. The presence of inflammasomal effectors and blockade of cytokines related to the inflammasomal pathway suggest the possible therapeutic approach to target inflammasome in PAH therapy. In this context, our initial focus is directed towards comprehending

the activation of the NLR family pyrin domain-containing 3 (NLRP3) inflammasome, subsequently delving into its role in regulating pulmonary hypertension (PH).

Overview of NLRP3 Inflammasome

The NLRP3 inflammasome is composed of a multiprotein complex that stimulates the activation and maturation of inflammatory cytokines IL-1β and IL-18 [6]. NLRP3 belongs to the NOD-like receptor family, having three domains: a carboxy-terminal leucine-rich repeat domain (LRR), a middle nucleotide-binding domain (NACHT), and an amino-terminal pyrin domain (PYD). When the LRR domain senses a PAMP or a DAMP, the NLRP3 NACHT domain leads the process of oligomerization, and then the PYD domain interacts with the PYD domain of the adaptor molecule apoptosis-associated speck-like protein containing a CARD (ASC). Subsequently, the ASC CARD domain interacts with the pro-caspase-1 CARD domain that ultimately causes caspase-1 cleavage. The resulting activation of caspase-1 induces pro-IL-1β and pro-IL-18 cleavage to active IL-1β and IL-18 form and subsequent inflammation. Moreover, functional caspase-1 mediated gasdermin D activation triggers the onset of programmed cell death, *i.e.*, pyroptosis [7].

Effectors of NLRP3 Inflammasome Activation

The formation of NLRP3 inflammasome involves the requirement of a broad range of inducers like exogenous as well as endogenous activators. Exogenous activators are generally environmental particulates (silica crystals, asbestos fibers, *etc*.). The accumulation of endogenous activator molecules during metabolic disbalance or alteration of cellular homeostasis triggers the formation of NLRP3 inflammasome. It has been found that uric acid presents as a nontoxic soluble form under normal conditions. However, a high concentration of uric acid causes the formation of monosodium urate crystals that trigger the formation of NLRP3 inflammasome and induce IL-1β-mediated chronic inflammation. Likewise, intracellular ATP plays an important role in normal cellular homeostasis. During tissue damage, extracellular ATP interacts with P2X purinoreceptor 7 (P2X7) and causes NLRP3 activation [8]. As the formation of the NLRP3 inflammasome relies on a diverse range of signals, these stimuli do not engage with receptors. Alternatively, NLRP3 interacts with general upstream activators. Various types of intracellular events can lead to NLRP3 activation, like alteration of the redox system, ion concentration, and lysosomal stability.

CHAPTER 3

Modulatory Mechanism of NLRP3 Inflammasome in Heart Diseases: "An Enigma Wrapped in a Riddle"

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Abstract: Despite breakthroughs in therapy over the prior two decades, heart failure is considered the foremost cause of mortality globally. The inflammasome plays a pivotal role in the advancement of heart failure, abdominal aortic aneurysm, atherosclerosis, diabetic cardiomyopathy, hypertension, dilated cardiomyopathy, cardiac remodeling and calcific aortic valve disease. The NLRP3 inflammasome is a crucial multi-protein signaling platform that tightly regulates inflammatory responses. It regulates antimicrobial host defense, which causes pyroptosis through caspase-1 activation by the eventual production of pro-inflammatory cytokines. The investigation of the NLRP3 inflammasome in various cardiovascular diseases may reveal critical disease triggers and endogenous modulators, leading to the development of new therapeutic interventions in the future. The target of this chapter is to summarise the recent literature describing the activation mechanism of the NLRP3 inflammasome by implicating different inflammatory pathways in the pathophysiology of heart failure.

Keywords: Caspase-1, Heart failure, IL-1β, Inflammation, NLRP3 inflammasome.

INTRODUCTION

Heart failure (HF) is a leading cause of morbidity and mortality in the United States, with rising prevalence of obesity, diabetes, hypertension, *etc*., throughout the world. In the United States, about 6 million people have been officially diagnosed with heart failure. Additionally, many others have a condition called

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asymptomatic left ventricular insufficiency, which means their hearts are not working quite right, and they are at risk of developing heart failure. Unfortunately, heart failure causes over half a million deaths every year in the United States. For people with severe heart failure, more than 50% of them do not survive past one year. This information is extracted from the American Heart Association 2003 guidelines. Heart failure presents with symptoms such as dyspnea, peripheral edema, orthopnea, paroxysmal nocturnal dyspnea, and bendopnea [1]. Nevertheless, significant breakthroughs in the insight of HF at the body system and cellular-molecular levels have prompted significant progress in heart failure medication that has changed clinical practice. Although symptomatic relief and reliability of life improvement are still essential goals, it is now feasible to start therapy with the assumption that disease progression can be slowed and, in many cases, survival can be extended.

The main components of this disease, volume overload (congestion) and myocardial dysfunction (heart failure), have historically been the focus of pharmacological therapy. The use of diuretics and cardiac glycosides has traditionally been highlighted in treatment plans, with research efforts focused on the creation of novel medicines that enhance contractile function. Such treatments have not been shown to increase survival while being successful in alleviating symptoms and stabilizing patients with hemodynamic decompensation. Further recent research has shed more light on the origins and progression of CHF, offering a conceptual framework that views the condition as the result of disturbed circulatory dynamics and pathologic cardiac remodeling. The way blood moves in the body and the changes that take place in the heart when it is not healthy have improved a lot. These improvements have helped doctors to take care of heart failure better. Before we talk about how a thing called the NLRP3 inflammasome is connected to heart failure, first make sure we understand how NLRP3 is linked to treating heart failure [2].

Innate immunity and adaptive immunity are key components in mammals to provide protection against internal and external dangers in the host. Endogenous and exogenous pathogens are sensed by the utilization of pattern recognition receptors or sensor proteins (PRRs) of the innate immune response. In 2002, the novel PRR, *i.e*., inflammasome, was reported for the first time. PRR is a protein complex with a high molecular weight that triggers pro-interleukin-1 (pro-IL-1) to be processed as well as the stimulation of inflammatory caspase. Inflammasomes are the critical components of innate immunity because they function as signaling platforms exceedingly proficient in tackling a wide variety of harmful microorganisms, pathogens and cell-related products linked to stress and damage [3]. Five distinct members of the nucleotide-binding oligomerization domain (NOD) family, which includes NLRP1, NLRP3, and NLRC4, along with absent-

in-melanoma 2 (AIM2) and pyrin, have been demonstrated to play a role in inflammasome formation [4]. Additionally, findings of the formation of the inflammasome by other PRR members comprising NLRP2, NLRP6, NLRP7, NLRP12, and IFI16 (interferon-γ inducible nuclear gene) have been documented in the literature [5, 6]. Through recognition receptors (PRRs) such NLRP1, NLRP3, AIM2 and pyrin, an apoptosis-associated speck-like protein with a caspase-recruitment domain (ASC) is essential for engaging pro-caspase-1 in the inflammasome complex. The LRR (leucine-rich repeat) domain senses the danger signal and causes NLRP3 monomer oligomerization *via* the NACHT domains and is accompanied by the link between the PYD domains of NLRP3 and ASC. Eventually, ASC, which functions as an adapter protein, enlists procaspase-1 into the assembly through its CARD domain [7].

The structure of the recombinant complex of the mitotic Ser/Thr kinase NEK7 and the NLRP3 protein without the pyrin domain was recently identified by Sharif *et al.* An earring-shaped structure made up of curved LRR and spherical NACHT domains was visible on the cryo-EM map. The C-terminal lobe of NEK7 interacts with the NBD (nucleotide-binding domain), LRR and HD2 (helical domain 2) domains of NEK7 (NIMA-related kinase 7). This structure implies that NEK7 may form bipartite contacts with neighboring NLRP3 subunits to activate the NLRP3 inflammasome [8]. The NLRP3 inflammasome-mediated increase in IL-1 and IL-18 production is linked to the beginning of atherosclerotic plaque formation in both atherosclerotic patients and animal models [9]. Both experimental autoimmune encephalomyelitis (EAE) in animal models and multiple sclerosis (MS) in humans have been linked to the NLRP3 inflammasome as their cause [10].

The activation of the NLRP3 inflammasome is also associated with various other conditions, including inflammatory bowel disease (IBD), ulcerative colitis, and Crohn's disease [11]. Malignancies related to the NLRP3 inflammasome include gastrointestinal cancers, melanoma, hepatitis C virus-associated hepatocellular carcinoma, breast cancer, and colon cancer [12]. Over and above NLRP3 activation anomalies, cryopyrin-associated periodic disorders (CAPS) are the genetic NLRP3 abnormalities [13]. Mutations within the NLRP3 gene lead to a gain-of-function outcome, which causes CAPS diseases, which are characterized by increased IL-1β secretion and other symptoms that are unique to CAPS [14].

TRIGGERING OF NLRP3 INFLAMMASOME

A number of triggers, including pathogen-associated RNA, adenosine triphosphate (ATP), heme, potassium ionophores (K^+), particulate matter, and bacterial and fungal toxins, can stimulate NLRP3. Since NLRP3 is yet to be seen

CHAPTER 4

TB and Inflammasome: A Complex Relationship

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Abstract: The reputation of *Mycobacterium tuberculosis* (*Mtb*) as one of the most successful human pathogens has been corroborated bysignificant experimental and clinical evidence. It infects the human host for long enough to co-evolve with the host, developing a robust repertoire of effectors to evade the immune response of the host. It has the capability to survive and multiply inside the very tools of the host immune system that are employed to eradicate it. Granuloma is a classical structure formed as a compensatory step in which both the host and the pathogen benefit partially. While a lot of mycobacterial virulence factors like cell wall envelope components, secreted proteins and dormancy regulon have been researched extensively, the comparatively newer concepts of inflammasomes need much attention. This chapter is an attempt to understand the complex relationship between the inflammasomes and *Mtb* in light of recent studies. With the emerging problems of drug resistance in the treatment of Tb, understanding the relationship between inflammasome and *Mtb* may present newer avenues in the development of host-directed therapy (HDT) strategies for combating Tb .

Keywords: Caspase-1, Interleukin-1beta, Innate immunity, Inflammasome, *Mtb*, Pattern recognition receptors, Pyroptosis, Virulence.

INTRODUCTION

Tuberculosis (TB) is a communicable disease that has been prevalent for thousands of years, as evidenced by scientific studies on skeletons of humans as well as literature. Scientific investigations of the archaeological pieces of evidence by various groups across the world have shown ancient TB prevalence. An important evidence in this contextis Pott's disease, as demonstrated in Peruvian mummies and *Mycobacterium tuberculosis (Mtb)* DNA recovered from Egyptian mummified tissues [1]. TB is known by several other names in historical literature such as phthisis and consumption. Notably, despite its considerable

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prevalence in human history, the disease was romanticized in European literature. The infectious nature of TB was demonstrated by a French military surgeon Jean-Antoine Villemin in 1865 [2]. However, the pathbreaking discovery and elucidation of the causative agent bacillus were done by Dr Robert Koch in 1882 for which he was awarded the Noble Prize in Medicine or Physiology in 1905. His elucidation of the aetiology of tuberculosis changed the course of events and its subsequent nomenclature as *Mycobacterium tuberculosis*. On the basis of the tissues affected, TB is described as pulmonary (lungs) and extrapulmonary (tissues other than lungs). Though TB is a contagious disease that spreads *via* the respiratory route and typically affects lungs (Pulmonary TB), it can also affect other sites (extrapulmonary TB). *Mtb* has co-evolved with its human host for millennia and has consequently adapted itself exquisitely to navigate the host immune system. It has developed a repertoire of effectors to not only evade the innate immune response but also to exploit it for survival and persistence. A remarkable feature of the pathogen is its ability to survive in different diverse intracellular microenvironments of different cell types of the host [3]. In the tug-of-war like situation between the pathogen and the host during infection, the outcome is determined by the genetic determinants of the host as well as the pathogen, co-morbidities, environmental factors and others.

Understanding the advancements in the innate host response to *Mtb* has promising potential in the developmentof host-directed therapy to counter TB. The concept of inflammasome activation is one such area that requires special attention [4].

The Causative Organism, *Mycobacterium Tuberculosis* (Mtb)

Mtb is a Gram-positive, facultative intracellular bacterium that has a doubling time of 12-24 hours under optimal conditions. It infects the mammalian host cells and not only can survive but also replicates within the host macrophages. Its capability to avoid macrophage-killing repertoire makes it a major reason for causing infectious diseases and a malady for mankind. Some of the main effectors that play a fundamental role in its virulence are as follows [5].

Peculiar Cell Wall Structure

The cell wall of *Mtb* is a strong, impermeable barrier consisting of outer membrane, periplasmic space and inner membrane. The outer membrane is further composed of peptidoglycan and arabinogalactan and is rich in mycolic acids that form an exceptionally thick, waxy, impermeable barrier and significantly contribute to drug resistance. It is also known as the mycolyl-arabinogalacta--peptidoglycan (mAGP) complex and represents the core cell wall structure of *Mycobacterium*.

Protein Secretion Systems

These are complex secretion systems that are used to secrete proteins across the cell envelope of Mycobacteria. *Mtb* has five secretion systems: ESX-1 [early secreted antigen 6 kilodaltons (ESAT-6) system 1] to ESX-5. These systems have been reported to have various functions in *Mtb* virulence. To describe briefly, ESX-1 secretes antigens that cause phagosomal membrane rupture. ESX-2 has an important role in survivability in slow-growing mycobacterial species within immune cells. ESX-3 is considered to be essential in regulating the metal ion homeostasis. It is reported to have a role in Fe^{2+} and Zn^{2+} ion uptake as well as in hindering phagosome maturation. ESX-4 is phylogenetically ancient in origin and is required in conjugation. ESX-5 releases proline-glutamate (PE) /proline-proline-glutamate (PPE) and PE- polymorphic GC-rich repetitive sequences (PGRS) proteins, which play a role in immunomodulation.

The Dormancy Survival Eegulon (Dos)

Mtb has the ability to sense unfavourable hypoxic, nutrient-depleted microenvironmental conditions inside macrophages and granulomas. In such conditions, it undergoes dormancy by activating its Dormancy Survival Regulator (DosR) regulon that regulates a myriad of genes, ultimately resulting in a dormant state of the bacillus wherein it stops multiplying, metabolises anaerobically and stays latent until the host becomes immunocompromised.

TB Pathogenesis

Typically, *Mtb* enters the human host through aerosol inhalation and reaches the alveoli in the lungs. The pathogen encounters the mucosa and alveolar epithelial cells, macrophages, dendritic cells and neutrophils as the first line of defence [5 - 7]. These cells have various receptors to recognize the pathogen as a threat and phagocytose it for elimination and to mount an immune response *via* antigen presentation. If the bacilli are all killed, the pathogen is cleared. However, if the pathogen manages to survive within the phagocytes, it replicates exponentially and results in a high bacterial burden. After the initial uptake, primarily by the alveolar resident macrophages, other cells like dendritic cells and monocyte-derived macrophages also take part in phagocytic processes. These phagocytic cells play a crucial role in mounting and directing adaptive T-cell immunity of the host by mycobacterial antigen presentation and expression of costimulatory signals and cytokines. Also, *Mtb* can diffuse to other sites *via* hematogenous and lymphatic routes. This leads to the mounting of immune response and initiation of the adaptive immune response to tackle the infection. Neutrophils, lymphocytes and other immune cells infiltrate the site of infection that culminates in granuloma formation, representing a stalemate between the bacterium and the host. It is a

CHAPTER 5

Mechanism of NLRP3 Activation, Associated Cardiovascular Complications and Update on its Inhibitors Acting as Cardioprotective Agents

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Abstract: Cardiovascular disorders (CVDs) are a major healthcare issue worldwide and are accountable for significant mortality and morbidity. Despite advancements in cellular, molecular, physiological and pathological understanding, a comprehensive understanding of CVDs is still lacking. Hence, a better understanding of pathological changes is needed to develop a potential cardioprotective agent. In recent times, NLRP3 inflammasome has been extensively studied in various disease conditions, including CVDs. The activation of NLRP3 inflammasome has been found to be positively correlated with various CVDs, such as hypertension, angina, arrhythmia, cardiac fibrosis, myocardial infarction, heart failure, *etc*. Moreover, a number of NLRP3 inflammasome activators have been explored for their role in CVDs, and the outcomes of these studies are found to be promising. Therefore, in the present manuscript, we have discussed the structural component of NLRP3 inflammasome, its molecular mechanism of activation, and the outcome of various NLRP3 inflammasome inhibitors in CVDs. We found that NLRP3 inflammasome is an indispensable player of pathogenesis in CVDs, and thus, targeting this inflammasome can be an effective approach for managing and treating these diseases.

Keywords: Myocardial infarction and MCC950, NLRP3 Inflammasome, Nuclear factor kappa B (NF-kB), Oxidative stress.

INTRODUCTION

Cardiovascular disorders (CVDs) are one of the major causes of mortality and morbidity globally and significantly affect patients' quality of life [1]. Moreover, the management and treatment of CVDs have an enormous socioeconomic impact. As per the published evidence, more than 130 million cases of CVDs will be reported by the end of 2035 [2]. Undoubtedly, there has been a significant

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increase in the understanding of CVDs; still, there is a lack of information on molecular pathogenesis and precise target-based therapeutic approach. The inflammasome is considered a macromolecular and multiprotein complex that plays a pivotal role in the production and maturation of various proinflammatory cytokines, such as interleukin-1beta (IL-1β). The produced cytokines play a vital role in generating systemic inflammation and various other conditions, including CVDs [3]. NOD-, LRR- and Pyrin domain-containing protein 3 (NLRP3) is an extensively studied inflammasome having a potential pathogenic role in various CVDs, such as hypertension, angina pectoris, arrhythmia, myocardial fibrosis, heart failure, *etc*. Initially, under normal physiological conditions, NLRP3 inflammasome remains inactivated, but in response to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), it becomes activated and releases IL-1β and IL-18, which play a crucial role in CVD pathogenesis [4]. NLRP3 inflammasomes have been reported to promote atherosclerosis, coronary heart disease, hypertensive disorders, and other CVDs [5]. No doubt, the role of NLRP3 and IL-1β is well established in CVDs, but the molecular mechanism of pathogenesis is unclear. Moreover, clinical findings have also shown that IL-1β inhibitors are effective cardioprotective agents. Hence, a more detailed cellular and molecular mechanism of NLRP3 activation in CVDs is needed to design and develop potent NLRP3 inhibitors for managing and treating cardiovascular disorders.

Structure of NLRP3 Inflammasome

Inflammation is the body's physiological reaction to the incursion of external microorganisms [6]. Inflammasome plays a crucial role in this process [7, 8]. There are five primary Nucleotide-binding oligomerization domain, Leucine-rich Repeat, and Pyrin domain containing inflammasomes (NLRP) known as NLRP10, NLRP1, NLRP3, ice protease-activating factor (IPAF) and absent in melanoma 2 (AIM2). Among them, NLRP3 has received the utmost attention [9]. Inflammasomes are complexes of multi proteins of the Nucleotide-binding oligomerization domain (NOD)-like receptors (NLR) family. The complex of proteins (NLRP3, ASC, and pro-caspase-1 effector) forms the NLRP3 inflammasome. Cytosolic protein NLRP3, previously identified as a novel inflammatory gene, is now considered the primary component of the NLRP3 inflammasome and has C-terminal and N-terminal function structural domains [10]. The N-terminal contains the protein pyrin domain (PYD), the nucleotide-binding oligomerization domain (NOD), and the caspase-associated recruitment domain (CARD), whereas the C-terminal contains the leucine-rich repeat (LRR), which serves as a cap to recognize different patterns associated with the pathogen and other ligands. Rearrangement of the NOD structure domain occurs after the recognition of ligands by LRR, triggering the biological effects [11]. NOD is

centrally located and surrounded by the N-terminal protein PYD, CARD, and C-terminal protein LLR. NLRP3 activation and processing of IL-1β are mediated by the NOD domain having an ATP-binding site. NLRP3 detects pathogen and body's signals, attaches to pro-caspase-1 and converts it to caspase-1 *via* ASC protein, followed by caspase-1 autocatalytic activation. Consequently, caspase-1 processes pro-IL-1β and pro-IL-18 into its active forms, thereby arbitrating the consequent responses [12].

Activation of NLRP3 Inflammasome

Pattern recognition receptors (PRRs) are genetically coded receptors present in the innate immune system. Agonists with binding properties to the same PRR are identified as PAMPs. PAMPs are associated with NLRP3 inflammasome activation. PRRs with membrane-bound toll-like receptors (TLRs) and C-type lectins (CTLs) are used by the body's innate immune system to recognize PAMPs. DAMPs are referred to as self-molecules generated by injured cells. These may trigger PRR activation in the absence of an active contagion and are accountable for the activation of NLRP3 inflammasome [13, 14]. PAMPs and DAMPs are both recognized by NLRs and thus play a pivotal role in activating NLRP3 inflammasome [15]. Specifically, the NLRP3 inflammasome is activated in two phases. One is through PAMPs and DAMPs, which form the NLRP3 inflammasome protein complex of NLRP3, ASC, and pro-caspase-1 [16]. The second is *via* TLR and Nuclear factor kappa B (NF-kB) signaling pathways, which cause the synthesis of pro-cytokine, such as pro-IL-1β, leading to pyroptosis, as shown in Fig. (**1**) [17].

The Lysosomal Damage Mediating NLRP3 Activation

When macrophage cells swallow up the PAMPs, they damage lysosomes by destabilizing phagocytes and activating caspase-1 for processing pro-cytokines like pro-IL-1β and pro-IL-18 [18]. Particulate matters like urea, calcium, cholesterol crystals, and silica are engulfed by the macrophages that damage the integrity of the lysosomal membrane. Furthermore, cathepsin B is released in the cytosol after rupturing the lysosome and is accountable for triggering the NLRP3 inflammasome activation [4, 18]. The mechanistic understanding of lysosome destabilization-mediated NLRP3 activation is unknown. However, based on previous studies, it is found that monosodium urate releases an enormous amount of sodium ions that reduce the concentration of intracellular K^+ efflux [19]. Lysosome destruction causes K^+ outflow *via* pore formation and is responsible for activating the NLRP3 inflammasome [20]. Lysosomal damage can also be due to oxidative stress induced by intracellular Ca^{2+} ion, macrophage, and dysfunctional

CHAPTER 6

Role of NLRP3 in Protozoan Parasitic Infections

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Abstract: Nod-like receptors (NLRs) and the inflammasome complex have significant roles in regulating the innate immune system against bacterial and viral pathogens and have attracted significant attention to their role in protozoan infections. Several parasitic protozoan pathogens are the most prevalent that cause severe morbidity and pose a significant health burden. In the present article, we discussed the most common protozoan parasites and the roles of NLRs and inflammasomes against these parasites. *G. duodenalis*, *E. histolytica*, *T. vaginalis*, Plasmodium parasite, *T. cruzi*, *Schistosomes* parasite, *T. gondii*, and *Leishmania spp.* activate the NLRP3 inflammasome. The NLRP3 inflammasome protects the host in *Giardia, T. cruzi,* and *E. histolytica* infections. Also, its protective role in the case of *Trichomonas* infection has been suggested, but more studies are needed. However, NLRP3 induces pathology during *Schistosomes* and *Malaria* parasite infection. In *T. gondii* infection, NLRP3 causes inflammation and limits the parasite load burden and propagation. This provides a new dimension in the research on the role and exact mechanism of NLRP3 during *T. gondii* infection. The NLRP3 inflammasome protects the host by clearing the parasitic load; NLRP3 provides resistance toward some *Leishmania spp.* It alleviates the host's parasitic burden of *L. amazonensis* and *L. major*. However, *L. major* or *L. donovani* induces chronic non-healing infection-promoting lesion development. These contrary reports warrant more research on *Leishmaniasis.* For developing new treatment strategies, studying the role of NLRP3 in the host defense and inflammatory pathology is crucial in parasitic protozoan infection.

Keywords: IL-1β, IL-18, NLRP3, Protozoan infection.

INTRODUCTION

Protozoa are free-living or parasitic single-cell microorganisms. They propagate in humans and animals, leading to serious infection development. Protozoa

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infecting humans are classified into four groups: Sarcodina, Mastigophora, Ciliophora, and Sporozoa. Many protozoa have been poorly studied and come under the neglected tropical disease category.

Pathogen recognition receptors (PRRs) of the host play a role in the recognition of pathogens, the first initiating step of the host immune response and subsequent generation of an adaptive immune response [1]. These receptors recognize specific pathogen-associated molecular patterns (PAMPs) like Lipopolyglycan, flagellin protein, and many other pathogens and danger/damage-associated molecular patterns (DAMPs) like extracellular ATP, hemozoin and many other molecules [2, 3]. The four classes of PRRs are Toll-like receptors (TLRs), the Nucleotide-binding oligomerization domain (NOD)-Leucine-rich repeats (LRR)-containing receptors (NLR), the retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLR; RIG-I-like helicases—RLH) and the C-type lectin receptors (CLRs) [1]. NLRs are central regulators of inflammation and immunity; they recognize pathogens and regulate TLR-mediated immune system pathways, mainly expressed by non-hematopoietic cells and immune cells [4].

Structure and Function of NOD-like Receptor 3 (NLRP3)

The NLR family consists of 22 mammalian genes with diverse functions in innate immune response and inflammation. They share common structural motifs such as a variable N-terminal effector domain, either a caspase recruitment domain (CARD) or pyrin domain (PYD), a C-terminal leucine-rich repeat (LRR) domain for ligand recognition, and a central NOD (nucleotide-binding and oligomerization (NACHT) domain) required for self-oligomerization [5]. Based on phylogenetic relationships, NLRs are mainly divided into three subfamilies: NODs, NLRPs (also known as NALPs), and IPAF. NLRP3 is the best-characterized intracellular sensor, consisting of NOD-, LRR-, and a pyrin domain-containing protein 3. NLRP3 is known to form the assembly of an inflammasome complex. It plays the role of a scaffold with the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD), leading to the cleavage of pro-caspase-1 and activation and secretion of interleukin-1beta (IL-1β) and IL-18 [6]. Viral, parasitic, bacterial, and fungal pathogens lead to NLRP3 inflammasome activation, and their diverse functions depend on the infection type [7]. The NLRP3 may play an essential role in innate immunity and regulating inflammatory reactions during parasitic infection by killing the pathogen or initiating the destructive pathology. NLRP3 plays a varied role as it can identify mitochondrial DNA and ATP and regulates signaling cascades of TLRs and NOD. It is also involved in chronic immune diseases, tissue homeostasis, embryonic development, cancer, and autophagy [1].

Activation of NLRP3 and its Signaling Cascade

NLRP3 has a vast and varied number of agonists; many cause cation flux K^+ efflux and increased cytosolic Ca^{2+} concentration) or chloride ions (Cl^-), causing mitochondrial disruption, lysosomal disruption, mitochondrial dysfunction, metabolic changes, and trans-Golgi, disassembly Pathogen-derived products, sterile crystalline molecules, and ATP [8]. NLRP3 activation is mediated *via* a canonical and non-canonical pathway. Canonical activation is a two-step process that requires priming/transcription and activation. Priming occurs by recognizing PAMPs and DAMPs *via* PRRs, resulting in Nuclear factor kappa B (NF-κB) activation and upregulation of NLRP3 and pro-IL-1β transcription. Activation is caused by a pathogen (PAMPs and DAMPs) causing conformational change (post-translational modifications), causing the maturation of caspase 1 to induce the cleavage of GSDMD leading to pyroptosis and IL-1β and IL-18 release [1]. The non-canonical pathway directly activates the inflammasome (*via* caspase 4/5/11). It induces cell death and cleavage of Gasdermin D (GSDMD), causing N-terminal release and its assembly at the plasma membrane, resulting in membrane damage, potassium efflux, pyroptosis, and increased activation of NLRP3 inflammasome [1]. Caspase 8 and Fas-associated death domain (FADD) regulate the activation of NLRP3 inflammasome and maturation of IL-1β [9, 10].

Inflammasome Activation and Pyroptosis

Upon activation, NLRP3 couples with ASC and procaspase-1(inactive) and cleaves to convert it into caspase-1 (active form) that further cleaves the proIL-1β and proIL-18 into mature IL-1β and IL-18 (secreted forms) [11]. These cytokines further initiate inflammatory processes in the host. Activated NLRP3 inflammasome also leads to pyroptosis, a rapid, inflammatory-lytic type, programmed cell death. GSDMD has an amino-terminal cell death domain (GSDMDN term), a central short linker region, and a carboxy-terminal autoinhibition domain. Caspase 1 cleaves GSDMD, removing its autoinhibitory carboxyl terminus and releasing the GSDMDN term (amino-terminal), which binds, oligomerizes, and inserts into the plasma membrane, forming a pore, thus killing the cell [12 - 14].

The role of NLRs and inflammasomes in the detection of PRRs of the pathogen and their involvement in the host response are well defined, but their role in parasitic infection has been poorly studied. However, recently some studies reported the role of NLRs and inflammasomes in protozoan parasites. In this chapter, we have tried to discuss the role of NLRP3 in the parasitic protozoan infections in humans.

CHAPTER 7

The NLRP3 Inflammasome as a Target for Anti-inflammatory Drugs

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Abstract: The Nod-like receptor protein 3 (NLRP3) inflammasome plays a vital role in the nonspecific immune response to inflammatory triggers such as cellular infections, injury, or stressors, and it has also been associated with several inflammation-related diseases. NLRP3 inflammasome activation results in the production of proinflammatory cytokines, contributing to an increased risk of inflammatory conditions, such as cardiovascular, metabolic, infectious, and neurodegenerative diseases. Several signaling pathways and cellular events involved in the NLRP3 inflammasome assembly and activation have been studied, and inhibitory mechanisms have been identified. NLRP3 inflammasome inhibition decreases inflammation and inflammasome-mediated cell death. In prospecting for novel anti-inflammatory therapeutics, signaling molecules upstream or downstream on the NLRP3 inflammasome pathway can serve as viable drug targets. Effective inhibition of these molecules culminates in the downregulation of the expression of proinflammatory cytokines like interleukin-1beta (IL-1β) and IL-18. This chapter elucidates the various

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classes of NLRP3 inflammasome inhibitors, their resultant anti-inflammatory effects, and various mechanisms of action.

Keywords: Cytokines, Inflammasome, Inflammation, Inhibition, NLRP3.

INTRODUCTION

Inflammasomes are protein complexes that can trigger the activation of powerful inflammatory mediators, which are essential components of the innate immune system. Upon cellular infections, injury, or stressors, inflammasome components aggregate and oligomerize, forming a stable complex. This activation of inflammasome results in the splitting of procaspase-1 to caspase-1 (the active form). This consequently promotes the production and secretion of several proinflammatory cytokines, precipitating a series of inflammatory reactions [1, 2].

Aim2 (absent in melanoma 2), NLRP1, NLRP3, and NLRP4 inflammasomes are the four known inflammasomes, and among these, NLRP3 inflammasome is crucial in determining immune responses and also modulating the integrity of intestinal homeostasis in many prevalent inflammatory disorders [3].

The NLRP3 inflammasome, which is named after the NLRP3 protein in the complex, a member of the NLR family, is currently the most well-characterized inflammasome [4]. NLRP3 is a cytosolic protein of 115 kDa that is expressed in epithelial cells, dendritic cells, neutrophils, monocytes, osteoblasts, and lymphocytes, where they regulate the activation of proteolytic enzyme caspase-1 [5, 6]. It has three domains, which are PYD (N- terminal pyrin domain), NACHT (central nucleotide- binding and oligomerization domain), and LRR (C-terminal leucine-rich repeat) [7]. The NLRP3 inflammasome is made up of the NLRP3 scaffold, procaspase-1, and the adapter protein apoptosis-associated Speck-like protein containing a Caspase recruitment domain (ASC) [8, 9]. The NLRP3 inflammasome can either be primed or activated by two independent pathways [10].

NLRP3 Inflammasome Activation

The priming signal pathway is mediated by Pathogen-Associated Molecular Patterns (PAMPs) from bacteria or viruses or sterile Damage-Associated Molecular Patterns (DAMPs), which bind with and trigger the Toll-like receptors (TLRs) on cell surfaces [10]. In response to activation, TLR triggers the intracellular domains of Toll-interleukin 1 receptor (TIR) [11, 12] by two adapter molecules, namely TIR domain-containing adaptor protein (TIRAP) and MyD88. The interaction of these two adapter molecules activates the TNF receptor-associated factor 6 (TRAF6) [13] and IL-1 receptor-associated kinases (IRAKs)

[14]. TRAF6 then activates the MAPK [15] pathway and IκB kinase [16, 17]. An activated IκB kinase leads to the phosphorylation, ubiquitination, and dissociation of IκBα from the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [10]. The NF-κB translocates to the nucleus after the dissociation and destruction of the IκB protein, attaches to particular DNA sequences known as response elements, and increases the cytosolic expression of the inactive NLRP3 molecule and cytokine precursors like pro-IL-1 and pro-IL-18. [10, 18].

The activation signal pathway can be triggered by numerous PAMP- or DAMP-mediated mechanisms culminating in the development of the NLRP3 inflammasome complex. The most well-characterized and extensively researched PAMP is lipopolysaccharide (LPS), which is found in the outer layer of gram-negative bacteria's cell membrane [19]. Some of the various DAMP-associated molecular mechanisms that trigger the formation of the NLRP3 inflammasome complex include the production of reactive oxygen species (ROS) due to mitochondrial oxidative stress [20 - 22], pore formation and efflux of ATP potassium ions through ATP-gated P2X7 channel [23, 24] and lysosomal membrane damage which causes the release of cathepsin B [20, 25]. Upon the activation of the LRR domain of NLRP3, oligomerization of its monomers takes place through the NACHT domain with the subsequent triggering of the PYD domain to bind to the ASC. This facilitates the recruitment of the procaspase-1 protein through the Caspase recruitment domain (CARD) of the ASC [26]. Procaspase-1 becomes active caspase-1 after being activated by the NLRP3 inflammasome, which causes the intracellular maturation of pro-IL-1 and pro-I--18 cytokine precursors. When mature IL-1 and IL-18 are transported outside the cell, a series of inflammatory responses and pyroptotic cell death are triggered [17]. The activated caspase-1 cleaves gasdermin D (GSDMD), a pyroptosis substrate, at Asp276 by proteolytic processing to form a cytotoxic GSDMD-N domain and consequently creates GSDMD pore on the cell membrane where the cytokines can be released [27, 28].

The NLRP3 inflammasome is seen as a viable target in the development of innovative anti-inflammatory drugs for the treatment of various disorders related to inflammation, as it plays an essential role in modulating pathological processes [29]. The development of NLRP3-specific inhibitors can be made possible by understanding the intricate mechanisms of NLRP3 activation and can be used to treat a variety of NLRP3-related disorders [30].

The aberrant activation of the NLRP3 inflammasome has been associated with a number of disorders, including Type 2 diabetes (T2D), prion disease, Alzheimer’s disease, atherosclerosis, metabolic syndrome, cardiovascular diseases, neurodegenerative diseases, and some infectious diseases, sparking intense

CHAPTER 8

The Potential Value of Sputum Level Interleukin-38 and NLRP3 Inflammasome in Severe Childhood Asthma

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Abstract: Asthma in children is associated with serious exacerbations that are modulated by inflammation. The expression of inflammatory cytokines varies according to the severity of the disease. The transition from the state of exacerbation of the disease to the state of cure always passes through a relationship between inflammatory and anti-inflammatory mediators. This study looks at the expression of IL-38 and NLRP3 inflammasome in severe childhood asthma. NLRP3 inflammasome is upregulated in severe asthma, contrasting with low levels of IL-38. The inflammatory pattern of severe asthma in children is characterized by the expression of IL-17, IL-32, IL-1β, and NLRP3 inflammasome.

Keywords: ELISA, Gene expression, Induced sputum, Immunity, Severe asthma.

INTRODUCTION

Any immune response in the body begins with the pro-inflammatory phase. The regulatory process that follows restores immune homeostasis. Acute inflammation depends mainly on tissue immunity involving macrophages and dendritic cells. Inflammation is limited in time as resolution occurs with the removal of the initial stimulus. Pathogen-associated molecular patterns (PAMPs) interact with pattern recognition receptors (PRR) and danger-associated molecular patterns (DAMPs) receptors to trigger the inflammatory process. Toll-like receptors (TLRs), nucleotide-binding oligomerisation domain (NOD)-like receptors (NLR) and mannose-binding lectin (MBL) [1, 2] constitute the PRRs family. Activation signals include viral, bacterial (*e.g.*, LPS) [3] and cellular products (*e.g.*, nucleic

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acids) [4]. The inflammasome represents another innate system of immune activation [5, 6]. The release of DAMPs may also be due to several other metabolic triggers, chemical or physical [7]. However, chronic diseases are associated with persistent inflammation that implies continuously recruiting leuco- cytes to the target organ. Significant physiological and structural changes result in tissue remodelling and disease exacerbation. The main cytokines involved in this chronic inflammatory pathway of chronic inflammation are interleukin (IL)-1α, IL-1β, IL-6 and tumour necrosis factor-α (TNF-α). All these pro-inflammatory mediators are confronted with inhibitory mediators such as IL-37, IL-38, and IL-35.

We investigated the mechanisms of asthma. Activated inflammatory responses are major causes and common features of numerous disorders. Nucleotide-binding oligomerization domain (NOD) and leucine-rich repeat (LRR)-containing receptors or NOD-like receptors (NLRs) are inflammasomes that are critical in the initiation of innate immune responses to host-derived danger signals [8]. The activation of many prototypic NLRs, including NLR with a pyrin domain (NLRP) containing NLRP1, NLRP3, and NLRP4, results in the maturation and release of different pro-inflammatory cytokines (IL-1 β, and IL-18) [9]. The process has been suggested to be of great importance in the occurrence of programmed cell death, which is called pyroptosis [10]. As a part of the innate immune system, the NLRP inflammasome regulates the host's defence against harmful threats. Its activation is implicated in inflammatory sites (bronchoalveolar lavage induces sputum).

The NLRP inflammasome is a multi-protein complex that mainly consists of the nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain. All the NLRP (1, 3, 4) AIM2 inflammasomes contain caspase-1. The NLRP3 inflammasome has been deeply studied. Recent results describe the contribution of caspase-4/5 and caspase-11 in the activation of NLRP3 inflammasome in humans [11, 12]. In bronchi, NLRP3 inflammasome is activated [13] in circulating neutrophils, inducing IL-1b and IL-18 production [14]. NLRP3 inflammasome activation has been studied in several pulmonary diseases, including obstructive conditions (COPD and asthma), interstitial lung diseases and neoplasms [15, 16]. The activation of NLRP3 could be inappropriate, leading to persistent inflammation [17]. Despite these observations, the precise role of NLRP3 inflammasome in asthma is not clearly described. The objective of our work is to investigate the NLRP3 inflammasome in asthmatic patients.

Activation of the NLRP3 Inflammasome

NLRP3 inflammasome activation requires two steps: the first signal and a second signal. The first signal is also called the priming signal, which is usually PAMP. Some studies have shown that IL-1 β and TNF-α can also be used as priming signals. They initiate the gene transcription of NLRP3, CASP1, IL-1β and IL-18 through the NF- κB pathway and produce more pro-IL-1 β and pro-IL-18. DAMP and PAMP represent the second signal, which is the activation mechanism, mainly including K^+ efflux and Ca^+ influx, which alter the cellular composition of K^+ and Ca^{2+}. K^+ efflux is triggered by binding the cell membrane of extracellular ATP to the ATP-ligand-gated channel P2X7. The lowering of intracellular K^+ results in Pannexin-1 opening, allowing microbial molecules to penetrate the cytoplasm. These molecules recognized by the LRR are able to activate NLRP3. In addition, perforin causes an outflow of K^+ from the cell, forming pores in the plasma membrane, further promoting the passage of microorganisms into the cell [18]. Other molecules act in a similar way on K^+ efflux, such as membrane attack complex (MAC), silica, alum, and calcium pyrophosphate crystals [19, 20]. The cellular afflux of extracellular Ca^2 also activates NLRP3 [20]. Secondary to lysosome damage, cathepsin E is released into the cytoplasm and activates NLRP3 through LRR [3]. In the same way, DNA released by mitochondrial damage triggers NLRP3 activation [21, 22]. The NADPH oxidase, xanthine oxidase, cytochrome P450, cyclooxygenase and lipoxygenase can also generate ROS to induce NLRP3 activation [23].

Regulation of NLRP3 Inflammasome

The NLRP3 inflammasome includes transcriptional and post-transcriptional regulation and post-translational modification. Transcriptional regulation in cells is under the control of NF-κB, ensuring the functionality of the receptor protein NLRP3 before its activation. NF-κB regulation is linked to the presence of Toll-like receptor (TLR) or tumour necrosis factor (TNF). TNF receptors (TNFRs) suppress the inflammasome activation by blocking TNF. Moreover, type I interferons favour the release of interleukin 10 (IL-10), controlling the production of pro-IL-1β, thus blocking the activation of the inflammasome. miRNAs are responsible for post-transcriptional regulation. When miR-233 binds to the NLRP3 3' untranslated region, NLRP3 receptor proteins are downregulated, thereby blocking inflammasome activation [24, 25]. The inhibition of post-translational modification corresponds to the expression of the iNOS gene induced by type I interferon, leading to the release of ROS by the production of NO. Studies report that the LRR domain of NLRP3 can be ubiquitinated by the membrane-associated protein RING CH VII (WALK-7). Phosphorylation of its Ser291 residue may also negatively regulate NLRP3 activation [26].

CHAPTER 9

Inflammasomes, Inflammation and Neuropathic Pain

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Abstract: Inflammasomes such as NOD-like receptor protein 1 (NLRP1), NLRP3, NLR family CARD domain-containing protein 4 (NLRC4) and absent in melanoma 2 (AIM2) are the primary mediators of inflammation and its associated neuropathic pain. These inflammasomes are activated leading to various autoimmune & metabolic disorders, cancer, and other inflammatory diseases. The activation of inflammasomes occurs due to molecular alterations like mitochondrial dysfunction, neuroinflammation, lysosomal damage, oxidative stress, sensitization, and disinhibition, which lead to proinflammatory pathways causing inflammasome-related neuropathic pain. Among these inflammasomes, NLRP3 has been widely studied and proven to be the key player in the development of neuropathy. In this chapter, we have summarized the role of inflammasome and how NLRP3 is involved in neuropathic pain. Therefore, based on the facts available, it has been suggested that focusing on inflammasome activity may be a cutting-edge and successful treatment approach for neuropathic pain.

Keywords: Inflammasomes, Inflammation, Neuropathy, Neuropathic Pain models, NLRP3.

INTRODUCTION

Inflammation is a pivotal physiological reaction/immune response to harmful substances. Inflammation can occur locally or systemically by activating the innate immune system to provide a protective response in the presence of disease-causing pathogens and sterile intrusions, such as trauma, cancer, ischemia and metabolic perturbations. Pathogen-associated molecular patterns (PAMPs), the infectious pathogens, and damage-associated molecular patterns (DAMPs), the

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Puneetpal Singh (Ed.)

indicators of host cellular distress, are two different categories of innate inflammatory response. An increasing number of highly conserved sensors, called pattern recognition receptors (PRRs), detect PAMPs and DAMPs to eradicate disease-causing or harmful substances [1].

Inflammasomes are the cytosolic multiprotein complex of basic processing units for sensing PAMPs and DAMPs and actively integrating their downstream signalling. Inflammasomes are basically composed of a sensor molecule called the pattern recognition receptor, an adaptor protein and an effector known as inflammatory caspases. A few sensor molecules recognized that take part in the assembly are nucleotide-binding oligomerization domain (NOD), leucine-rich repeat (LLR), NOD-like receptors (NLRs) family, absent in melanoma 2-(AIM2-) like receptors (ALRs) family, interferon-inducible protein 16 (IFI-16), and retinoic acid-inducible gene I (RIG-I). The inflammatory signal is attained by the assembly of hetero-oligomeric complex. After the activation of the sensor molecule, they promote the involvement of apoptosis-associated spec like protein containing caspase activation and recruitment domain (CARD). They trigger the proteolytic activity of proinflammatory caspases, which leads to the activation of proinflammatory mediators that stimulate systemic immunological responses and inflammation [2, 3].

The most important function of the inflammasome is the detection of stimuli and to respond against those stimuli to induce cellular responses and host defense. This control system for the inflammatory processes is inherent to effectively regulating inflammasomes. Inflammatory caspase activation is the principal mechanism of inflammasome signalling. This activity requires the formation of hetero-oligomeric complexes, such as the AIM2 protein and the NOD-like receptor-pyrin-containing proteins (NLRP). The activation of caspases due to inflammasomes causes the activation of interleukin-1β (IL-1β) and interleukin-18 (IL-18) proteolytically, causing an inflammatory response. In particular, IL-1β is a guardian for cytokine that plays a crucial role in several processes that activate and control inflammation [4, 5].

Currently, there are six inflammasomes that have been identified which include NLR protein family and AIM2. NLR (Leucine-rich repeat and pyrin domain-containing protein) protein family includes NLRP1, NLRP3, NLRP6, NLRP12 and NLRC4/IPAF inflammasome (NLR family CARD domain-containing protein). These NLR inflammasomes have a C-terminus LRR for ligand recognition and a Nucleotide-Binding Domain (NBD) for self-oligomerization. NLR family, being a crucial component of the innate immune system, is able to sense a variety of bacterial and viral stimuli. Among all the NLR inflammasomes, NLRP3 inflammasomes are widely present in the immune cells, such as

macrophages, neutrophils and to some lesser extent in dendritic cells, microglial cells and dorsal root ganglia. Thus, the activation of NLRP3 leads to the release of proinflammatory mediators and contributes to the pathogenesis of several painful inflammatory conditions. Recently, AIM2 has also shown a significant connection to inflammation and pain-related pathways [6, 7] (Fig. **1**).

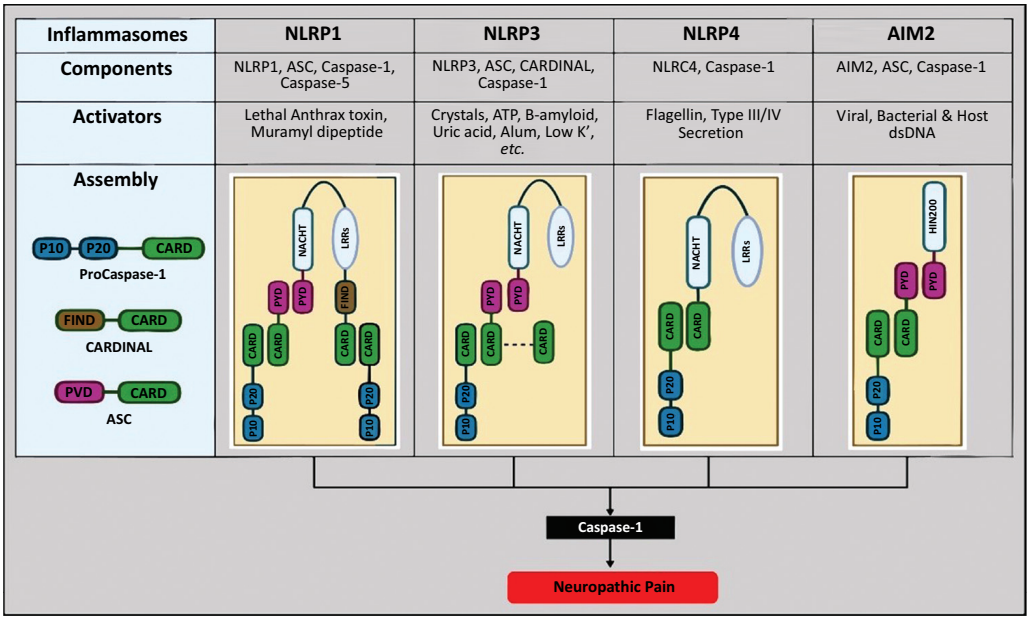

Fig. (1). NLRP1, leucine-rich repeat (LRR) and pyrin domain (PYD) containing protein 1; NLRP3, leucine-rich repeat (LRR) and pyrin domain (PYD) containing protein 3; NLRC4, NLR family CARD domain-containing protein 4; AIM2, absent in melanoma 2. All these Inflammasomes lead to the formation of caspase-1, followed by neuropathic Pain.

NLRP1 Inflammasomes

NLRP1 is the first cytosolic sensor discovered to construct a caspase-1-activated inflammasome response for the toxins produced by *Bacillus anthracis* [2]. NLRP1 connects to ASC *via* the PYD domain and caspase-1 *via* the CARD domain. Microbial ligands/bacterial toxins activate NLRP1, and these toxins, after entering the cytosol, play a pivotal role in the induction, assembly and activation of NLRP1 inflammasome [3, 4]. Numerous illnesses, including Vitiligo, Inflammatory Bowel Disease (IBD), Systemic Lupus Erythematosus (SLE), Human Mendelian Monogenic Disease, and Celiac disease are connected to NLRP1 inflammasomes [5, 6]. Recent studies have identified that the anti-apoptotic proteins Bcl-2 and Bcl-xL bind to NLRP1 and inhibit the activation. Apart from that, inhibiting the binding of ATP to NLRP1 has also been demonstrated to reduce NLRP1 oligomerization. As a result, it prevents caspase-1

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