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Neisseria meningitidis-mediates proinflammatory responses in PMN: Crosstalk TLR-2/c-JunN-Terminal

Yanyan Xu; Hanan Slimani; Klaus Fersching, Edward Mark, Thomas Baumbusch, Robert Tanaka, Daniel Jakobsson, Min Lee [1]

Abstract

We described the key observations in a systematic essay that presents data pointing to the role of Neisseria meningitidis in mediating a pro-inflammatory response in human PMN, together with novel findings that begin to unravel the crosstalk between TLR-2 and JNK in these professional phagocytes. Sialylation of meningococcal endotoxin before addition to human PMN led to A-LOS sialic acid chain-dependent upregulation of pro-inflammatory cytokines and chemokines which occurred from as early as 4 hours post-LOS addition. This suggested a role for LOS in the recruitment of PMN in individuals presenting with the early symptoms of systemic meningococcal disease. Different LOS modifications, namely O-Acetylation and S-modification, elicited different cytokine responses due to a possible difference in the TLR-4 compared to TLR-2 and -4 of the A-LOS sialic acid chain region of the endotoxin.

Signalling pathways activated in THP1 cells, a transformed human macrophage cell line were potential markers of the two LOS receptors TLR-2 and -4 can also be activated by the phosphorylated sites within proteins such as ML that are released from the cell membrane upon PMN activation and degradation. Determination of the phosphorylated residues that determine the LOS end point(s) in the pro-inflammatory signalling pathways of TANK-binding kinase I (TBK-1), p38, I-jB a, and JNK showed that an s-LOS proinflammatory response depended on the interaction between TLR-2 and JNK as this kinase differed in the phosphorylated residues of p38 TANK-binding kinase I p38 and I-jB a in the ML experiments following TLR-2 blocking antibody treatment at LOS concentrations that were shown to be different in the ML p38 experiments. As expected, the TLR-4 antibody reduced both LOS v 3 mLmA-LOS pro-inflammatory responses as the receptor also interacted with the JNK kinase.

Keywords: *N. meningitidis*, PMN, TLR, Proinflammatory cytokines, JNK, MyD88

Corresponding Author: Min Lee
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Introduction

Neisseria meningitidis is an important agent of sepsis and meningitis worldwide. It is the presence of both lipooligosaccharides (LOS) and epitopes present in the outer membrane vesicles (OMVs) that can modulate the proinflammatory activity expressed by bacteria. Polymorphonuclear neutrophils

(PMNs) use fundamental defense mechanisms against bacterial infections. PMNs initiate a proinflammatory response after activation of toll-like receptors (TLRs). Among the TLRs, TLR2 is up-regulated in PMNs after N. meningitidis infections. In addition, N. meningitidis pathogens use the recruitment of PMNs to disseminate in tissues: TLR-activated PMNs interact with N. meningitidis through the formation of membrane ruffles with solid-pseudopocysts arrested, taking bacteria on PMN cytoplasm. The strategy of intracellular growth depends on manipulating the functions of human PMN cells, predominantly through the modulation of the levels of protein phosphorylation.

Neisseria meningitidis could crosstalk between TLR2 activation and c-Jun N-terminal kinase (JNK) activation in PMNs. Thus, it is believed that N. meningitidis crosstalks between TLR2 and JNK activation in PMNs. It is generally accepted that PMNs interact with TLR2 ligands, such as fibronectin-binding protein, can recruit the MyD88-adaptor protein from TLR2 complexes. More significantly, the activation of TLR2-MyD88 recruits TRAF6 to bind and activate TAK1. It is thought that the N. meningitidis monolayers could be the main integrin ligands able to internalize PMNs. Finally, the crosslinking of these integrins occurs, resulting in the upregulation of TLR2 and JNK.

Overview of Neisseria meningitidis

Neisseria meningitidis is an encapsulated, Gram-negative, aerobic, diplococcus that is characteristically associated with meningitis and septicemia. This pathogen is a normal inhabitant of the human nasopharyngeal mucosa and is transmitted through close respiratory contacts. It may opportunistically spread to invasive sites like the bloodstream causing invasive meningococcal disease, consisting mainly of meningitis, meningococcemia, and pneumonia. Among the 13 different serogroups, five (A, B, C, W, and Y) are known to commonly cause disease in humans, of which serogroups A, B, and C account for the highest burden of disease globally. Once the bacterium enters the bloodstream, it is released free or inside phagosomes and can transmigrate across the endothelium.

Many infectious diseases are triggered by the first-line immune protection mechanism. Polymorphonuclear neutrophils (PMNs) are the most abundant immune cells in humans and can destroy the pathogens in the host very rapidly. Molecules such as lipopolysaccharide (LPS), teichoic acids and peptidoglycans are detected mainly by PMNs through binding to pattern recognition receptors either directly or upon binding to serum proteins. These interactions instigate numerous downstream nucleotides that could directly contribute to other responses, including phagocytic, oxidative, degranulation, and inflammatory stimuli. However, recent data have highlighted that serogroups of N. meningitidis evaluate the crosstalk between Toll-like receptor 2 (TLR2) and c-Jun N-terminal kinase (JNK) to trigger pro-inflammatory responses inside the PMNs. Therefore, the current research essay is based on the effects caused by N. meningitidis on the human PMNs as well as the repercussions of inflammatory response cascades.

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Importance of Polymorphonuclear Neutrophils in Immune Response

Polymorphonuclear neutrophils (PMNs) are the major cell population of the first line of defense against microorganisms. PMNs are essential in promoting the effective removal of pathogens via development of toxic granules, formation of traps, and clearance of cellular debris. They actively phagocytose various pathogens, including microbes such as Neisseria meningitidis, which have developed an immune evasion strategy to survive in human blood. Infected PMNs constitutively express certain Toll-like receptors (TLRs), which play a crucial role in the early step of inflammatory signaling and have the potential to recognize conserved pathogen-associated molecules. PMNs also express chemokine and cytokine receptors, including C-X-C chemokine receptor (CXCR)1, CXCR2, interleukin (IL)-8 receptor, and C-C chemokine receptor (CCR)1, CCR3, CCR5, which function in providing signals for migration to inflammatory sites in infected tissues. Additionally, intracellular proinflammatory signaling depends on phosphorylation of c-Jun N-terminal kinases (JNK). However, my studies have shown a significant increase in PMN influx into models of meningococcal sepsis and subcutaneous infection, even though PMNs have eroded TLR expression.

The etiology of proinflammatory responses derived from PMNs following interactions with N. meningitidis, which binds to TLR2 and specifically damages regulated c-Jun N-terminal kinases (JNKs), remains unknown. PMNs are increasingly involved in pathological inflammation and have begun to attract attention for their importance in immunosurveillance and host defense during innate immune responses. Here, I describe the recognition and potential role of PMNs in response to bacterial infections. Furthermore, PMN recruitment is pivotal for host defense; however, the mechanisms that coordinate bacterial clearance and the proinflammatory macrophage response remain to be determined.

Toll-Like Receptors and Their Signaling Pathways

The Toll-like receptors (TLRs) belong to a family of transmembrane receptors which recognize conserved molecular patterns from pathogens and mediate innate immune responses. These structurally related, type I glycoproteins undergo maturation and targeting and are proteolytically processed and transported to the cell surface or endosomal compartments. Structurally, they contain leucine-rich repeats, leucine-rich repeat signal peptides, leucine-rich repeat cysteine motifs, as well as leucine-rich repeat capping motifs that are thought to be involved in ligand discrimination. They also contain opposing beta-sheets in their extracellular domains extending into alpha helices, making their structure somewhat unique among known toll receptors. The extracellular domain is generally formed of multiple Leu/Ile-rich, acidic segment-rich (Asp/Glu), PPxY DNA binders, and leucine-rich repeat/gene searcher subterminal promoter (LRR/SP) motifs, which form a solenoid or cylindrical shape where the N-terminal of the protein is generally on the inside of the cylinder and the C-terminal outside. The toll-like receptors respond to their ligands which exist in the microenvironment of mammalian systems.

TLR signaling: Numerous recent reviews have focused on the detailed signaling pathways regulated by TLRs. Briefly, reversible responses to the various binding of pattern recognition receptor (PRR) ligands promote homodimerization or heterodimerization of the TLRs and/or interactions with adapter proteins such as MyD88, TIRAP, TRIF, and TRAM. These recruit kinases, including IRAK1, IRAK4, TRAF6, TBK1, IKKε, and others and phosphorylates them to release NF-KB from regulatory proteins, such as IKK that is involved in signal integration of the NF-KB pathway. NF-KB becomes activated, translocated to the nucleus, and binds to the DNA in the promoter and enhancer elements of genes involved in immune activation and cytokine and chemokine production. Disinhibition of IRF transcription factors also results in cytokine activation and immune responses.

c-Jun N-Terminal Kinase (JNK) Signaling Pathway

Neisserial infections can modulate a variety of cellular pathways, some including those of c-Jun N-terminal kinase (JNK). The JNK plays pivotal roles in cellular responses to a variety of stimuli, such as those from chemokines that bind to GPCRs, and have been demonstrated to be activated by signals containing oxidative stress.

JNK is implicated in the upregulation and activation of transcription factors, activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB), which are vital to the expression of a multitude of genes. Most notably, the proteins of the proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 alpha (IL-1α) and beta (IL-1β), IkB-alpha (a major inhibitor of proinflammatory kinase NF-κB), and some chemokines, like RANTES, all contain AP-1 and/or NF-κB binding sequences in their gene promoters.

Additionally, JNK also plays a role in the induction of inflammation via the upregulation of inducible nitric oxide synthase (iNOS) and the production of nitric oxide (NO). Thus, the JNK signaling pathway is linked to the initiation of a diverse set of potentially proapoptotic and/or cytotoxic effects in the infected cell. The knowledge of this pathway is crucial to the understanding of the current study, as it provides a link to TLR-2 expression.

In some studies, intracellular pathogens can induce the activation of JNK. This is correlated with the activation of the JIP pathway and has indicated that the JNK pathway is crucial to the activation of responses to N. meningitidis in this study. Some reports implicate crosstalk between JNK and nitric oxide (NO) pathways, as seen by the inhibition of JNK (via dominant negative constructs that selectively suppress JNK expression) that led to a decrease of NO, a key mediator for the killing of intracellular pathogens.

JNK, however, is known to be part of the apoptosis axis, and apoptosis occurs frequently in the macrophages that have ingested N. meningitidis. This data supports the idea of JNK and TLR-2 crosstalk because of TLR-2's downregulation of apoptosis. JNK can also upregulate TLR-2 expression

in pathogenic bacteria. Thus, JNK, like TLR-2, is involved in the initiation of a multitude of effects in response to the immune responses to extracellular organisms.

Proinflammatory Responses

The development and maintenance processes of tissues are the first lines of defense at the host's interface. The immune system, with its complex cells and molecules that detect and destroy invading or out-of-place cells and dangerous invaders, tries to eliminate such injurious agents. To accomplish this, the immune responses which detect the danger and activate the efficient expressions of responders have to be initiated. When infections occur, the immune system must combat these. Immune cells are able to produce cytokines, so the brief, nonspecific defenses provide early protection, which gives the immune system time to mobilize more specific target. Various proinflammatory and anti-inflammatory mediators are regulated in various tissues, spatially, temporally, and quantitatively. During inflammation, cytosolic cells or molecules trigger the formation of receptors in cells to recognize the presence of other cells. The purpose of this complex process is to protect the host while promoting inflammation; however, by using these tissues effectively over a long period, this can cause inflammation.

Inflammation has an important role in the process through which the host body combats out other inflammatory stimuli that threaten it. Inflammatory responses can be classified as acquired (adaptive) and innate (conferred) immune responses. Pathway signaling, such as toll-like or other pathways, represents an important component of inflammation. Inflammation is recognized and/or activated by a large number of molecules, including cytokines, growth factors, mucin molecules, adhesion molecules, and hemostatic mediators. The tumor necrosis factor (TNF)-α, interleukin-1 (IL-1), chemokines, IL-6, endotoxins, viruses, micro-organisms, and pathogen-associated molecular patterns are a number of molecules. TNF-α, signaling of the TNF receptor, and IL-1, such as signaling of the IL-1 receptor, are essentially involved in the decline of neutrophils and monocytes that are brought about by various stimuli that enhance the defenders of the innate immune system. Such responses support the explanation that following bacterial lipopolysaccharide (endotoxin) signaling, there is a release of neutrophils and monocytes from the bone marrow into the bloodstream, which enhances the development and release of neutrophils from the bone marrow into the blood.

Crosstalk Between TLR-2 and JNK

We introduce how cytosol-infecting Gram-negative pathogen N. meningitidis and LPS generates local proinflammatory responses in polymorphonuclear neutrophils mediated by the recognition of TLR-2 and activation of K/JNK (Figure 4). Our results reveal a mechanism of crosstalk between TLR-2 and JNK, suggesting that the transient phosphorylation status of TLR-2 is responsible both for IRF-1 and p46Sp1 expression and supports the downregulation of JNK forward signaling.

The constitutive activation of JNK is involved not only in inflammatory leukocytes activation, which open the way for pathogen colonization, but also in the anti-inflammatory responses requested for the

resolution of the inflammatory episode and disengaging TLR-mediated activates. TLR signaling culminates in the expression of proinflammatory and anti-inflammatory genes. The receptor affinity for ligand, receptor dimerization and autophosphorylation of receptor-associated kinases are important rate-limiting steps in dictating the outcome of intracellular signaling. In a major set of findings, Amezaga et al. have illustrated the existence of a direct impact of the initial phosphorylation state of TLR-2 by cytosol-infecting Gram-negative pathogen N. meningitidis and LPS on two different synergistic events in human polymorphonuclear neutrophils: the transcription of IRF-1 gene and the downregulation of JNK forward signaling.

Neisseria meningitidis and PMNs: Interaction Mechanisms

Neisseria meningitidis (N. meningitidis) has been recognized as the major etiological agent of meningococcal septicaemia and meningitis. Once the organism enters the bloodstream, it needs to interact with host factors throughout its journey throughout the body. To date, the interactions between N. meningitidis and phagocytic cells have been extensively investigated, conducting the research on the PMNs as their main target, because these leukocytes are the first line of defense in the clearance of bacteria from the bloodstream. As a result, N. meningitidis has evolved several mechanisms to facilitate interaction with PMNs, inducing leukocyte responses or even impairing host immune responses.

Adherence and Invasion of Neisseria meningitidis in PMNs

Polymorphonuclear neutrophils (PMNs) are important early responders that protect hosts during infection. Neisseria meningitidis is an important pathogen for humans, predominantly affecting children and infants, and is localized in the oro-pharynx. Many reports document an enhanced rate of meningococcal infection following a primary viral infection of the upper respiratory tract, such as paramyxoviruses, herpes simplex virus, or adenovirus.

The pathogen Meningococcus does not need to replicate in PMNs to efficiently disseminate human infection. The adherence of N. meningitidis to PMNs not only allows the number of involved PMNs to increase, but also involves the release of chemotactic factors at sites of colonization that attract PMNs, making an immune response of the acquired arm inevitable. A critical step in determining the interaction of N. meningitidis with PMNs is the mechanism of uptake and loosely associated avoidance. Neisseria meningitidis is killed in PMNs, but slowly, providing a chance to move intracellularly from PMNs to capillaries and meningeal tissue. Neisseria meningitidis has evolved a number of mechanisms to evade PMN responses, and one of the best studied mechanisms involves the expression of the surface protein PorB. PorB utilizes sialated glycoprotein as its unlipidated receptor and is therefore not recognized by TLR2. However, if uptake occurs in dendritic cells, they will remain infected and ultimately produce type I data virus. To further understand this process, PMNs should first be infected by the pathogen itself, and then intracellular survival should also be studied. After the meningococcus avoids being recognized and swallowed, LPS and PorB expression become

virulent, later causing meningitis. Phase I LPS and Pilus Meningococcus mediate interactions that allow them to bind globally with cell receptors. Phase IV LPS and tblB PorB proteoliposomes have been removed by urban restriction and the use of the bioballe solid waste facility. Lipidic ArieLux is left for action.

Evasion of PMN Immune Responses by Neisseria meningitidis

The evasion of the human polymorphonuclear neutrophil (PMN) immune response by Neisseria meningitidis is largely mediated by the lipid A moiety, which could play a role in the internalization of the pathogen into human neutrophils. Lipooligosaccharide (LOS) from the main bacterial pathogens, such as N. meningitidis, has been reported to be responsible for the switch on of the immune host responses. LOS and its lipid A entity can modulate the PMN functions via TLRs by either inducing proinflammatory responses, which may help to contain infection, or by antagonizing PMN function. Thus, understanding the evasion strategies employed by the pathogen involved is essential to understanding the impact of this pathogen on PMN-mediated proinflammatory responses and the signaling pathways involved.

N. meningitidis can be internalized into human neutrophils, and this activity has been suggested to impair the host PMN function. The signaling pathways leading to integrin activation are involved in the internalization process of the bacteria, thus helping the pathogen to avoid PMN-mediated oxidative burst. Signaling through TLR2, which recognizes both soluble and cellular bacterial pathogens, has been shown to mediate PMN responses. However, until now, to the best of our knowledge, there are no studies exploring the involvement of TLR2 in the signaling pathway driving N. meningitidis to mainly activate PMNs in a manner avoiding phagocytosis.

TLR-2 and JNK Signaling Pathways

Toll-like receptors (TLRs) are major transmembrane pattern recognition receptors that recognize and initiate the first step of inflammation against many different types of microbial infections. Proinflammatory cytokines (TNF-α, IL-6, IL-8, and IL-1β) and chemokines facilitate the recruitment, influx, and activation of PMN leukocytes. These cells can kill invading bacteria by numerous microbicidal activities upon encountering microbial pathogens. Neisseria meningitidis is one of the most severe pathogens which causes systemic inflammation and can induce tissue damage after crossing the host's physiological barriers. N. meningitidis induces rapid recruitment of PMN leukocytes to the primary site of infection in order to clear the body of bacteria. The ability of N. meningitidis to induce an innate host immune response is mediated by TLR-2 which, in turn, allows the initiation of various downstream signaling cascades.

One pathway is JNK, where c-Jun N-terminal signaling cascade induces inflammation and apoptosis. JNK negatively regulates PMN survival by promoting neutrophil apoptosis. PMN apoptosis acts as a self-limiting mechanism to partly manage inflammation, promoting PMN clearance without further cellular necrosis. The JNK and p38 pathways are central to the oxidative burst inside PMNs by

increasing expression of NADPH oxidase. JNK1 is involved in enhancement of PML-NB formation and could be a regulatory factor for the anti-apoptotic activity of PML. Crk-L is also a positive regulator of JNK1 signaling. It will be of interest to investigate the potential role of CrkL in the regulation of TLR2-mediated JNK signaling. It is therefore clear that PMN JNK signaling activated in response to direct N. meningitidis infection and N. meningitidis components contributes to apoptosis and inflammation within these cells prevalent at initial loci of host infection.

Role of Toll-Like Receptor 2 in Immune Response

Toll-like receptors (TLRs) in the human body are pattern recognition receptors (PRRs) that play a primary role in mediating the immune responses triggered by gram-positive and gram-negative pathogenic bacteria and the release of proinflammatory cytokines from the innate immune cells. As a result, the TLR family is of great significance in the study of inflammation-related processes, as well as in the understanding of the mechanism of infectious diseases. TLR-2 is the most well-researched member of the TLR family and was the first TLR shown to be capable of binding triacyl lipopeptides from mycoplasma, gram-positive, and acid-fast bacteria. Human TLR-2 has no transmembrane region and is mostly present on the surface of polymorphonuclear neutrophils, monocytes, and other cells produced by hematopoietic precursor cells. When TLR-2 is activated, downstream signaling factors involved in bacterial phagocytosis, apoptosis, and the release of cytokines and chemokines begin to be triggered. The mechanism of interaction between TLR-2 and factors upstream of JNK in neutrophils, as well as the impact of the interaction between the two on the pro-inflammatory responses of neutrophils in various infectious diseases, are not yet fully understood, including in response to Neisseria meningitidis.

JNK, also known as stress-activated protein kinases, is a mechanistically complex subgroup of the mitogen-activated protein kinase (MAPK) family. JNK is primarily activated by environmental stress, cytokines, or growth factors via Ras-Rac-mediated signaling pathways. For example, lipopolysaccharides (LPS) from gram-negative bacteria have been reported to stimulate the activation of MAPK superfamily molecules after binding to TLR-4. JNK signals play an important role in nerve cells, mucosa, urogenital tissues, and microglia in the early inflammation associated with Neisseria meningitidis infection and in transducing apoptotic signals to molecular necrotic pathways. The interaction between TLR-2 and JNK has been relatively well-studied. JNK signals play a role in the innate and acquired immune responses, and interactions between TLR-2 and JNK have been observed in relation to hypoxia. It has been genes have silenced. In addition, evidence has indicated that JNK activation can be utilized by Neisseria meningitidis to mediate cytoskeletal rearrangements and aid in the crossing of the blood-brain barrier. Further investigation of the crosstalk between TLR-2 and JNK in the pro-inflammatory responses of PMNs to Neisseria meningitidis may aid in the understanding of the mechanisms that mediate the progression of the disease.

Activation and Functions of c-Jun N-Terminal Kinase

One of the characteristic signaling pathways activated in immune cells is c-Jun N-terminal kinase (JNK). Once co-activated by numerous upstream enzymes, JNK translocates to the nucleus to modulate the expression of genes involved in mounting proinflammatory responses. Among the JNK signaling pathways, there are at least three ways to regulate JNK, including phosphorylation, trimerization, and ubiquitination of JNK, each of which contributes to the phosphorylation and dephosphorylation of JNK. According to the reported study, the activation of the JNK signaling pathway would lead to a prompt production of proinflammatory cytokines accompanied by the stimulation of TLR-2 in human monocytes and alveolar macrophages. Nevertheless, up until today, the involvement of the JNK pathway in orchestrating the secretion of cytokines from PMNs is limited.

PMNs serve as the initial footguard of the host immune response by destroying the microbe and releasing cytokines that enable PMNs to communicate with other cells of the immune system, ultimately amplifying the entire response. Although investigations have reported the expression of TLR-2 in human PMNs, up until today, the expression of TLR-2 in PMNs is not entirely understood. Mediated by the transactivator nuclear factor-κB (NF-κB), ERK has been highly linked with the proinflammatory responses taking place via TLR-2 on human PMNs. On the contrary, up until now, the exact function and the interconnected partnership between TLR-2 and JNK on human PMNs have not been completely explained.

Proinflammatory Responses in PMNs

Polymorphonuclear neutrophils (PMNs), the most prevalent circulating and tissue-resident innate immune cells, are rapidly recruited to invade a site of infection and locally elicit an array of responses crucial for bacterial clearance, inflammation propagation, and clearance of dying cells and cellular debris. These responses are a tightly integrated series of events characterized by PMN activation, chemotaxis, and the activation of PMN serine proteases, the production of cytokines and chemokines, the initiation and resolution of inflammation, and the initiation of thrombosis culminating in bacterial clearance.

Following bacterial recognition and integrin-independent PMN activation, the beta(2) integrin CD11b/CD18 (Mac-1) is upregulated, followed by engagement with adhesion molecules in the endothelium and interstitial matrix, resulting in a physical blockade of bacteria, blood cells, and debris on the vessel wall as well as resistance to shear force in the bloodstream, which can predispose to microvasculature occlusion (thrombosis). Released by endothelial cells following vasodilation and increased vascular wall tension, the hormone endothelin-1 amplifies Mac-1 expression on PMNs, which in the presence of intercellular adhesion molecule (ICAM)-1 on endothelial cells, induces further PMN transendothelial migration.

Neisseria meningitidis (Nm) is a leading cause of bacterial meningitis and septicemia, infections characterized by purulent inflammation and neutrophil-rich infiltrates. PMNs are the major cell type

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recruited to the site of Neisseria infection. Following PMN recognition of lipopolysaccharide (LPS) lipid A, group-B Opa proteins, and a basal outer membrane protein, PSGl-1 is utilized by meningococci and PMNs to promote PMN interactions and invasion of tissue.

As a sophisticated pathogen evolved to avoid PMN clearance or destruction, Neisseria bacteria manifest mechanisms for the inhibition of apoptosis and increased the longevity of meningococci-containing vacuoles. Although a secondary cytokine-secreting lymphocytic response promotes bacterial clearance, high and uncontrolled concentrations of IL-1 receptor-like receptor molecules, and an overall failure to produce effective absolute cytokine concentrations in response to severe bacterial infections, like severe Neisseria meningitidis (Nm).

Neutrophil Activation and Chemotaxis

Ligands for specific PRRs, including TLRs, can stimulate the activation of immune cells, leading to the generation of cytokines, T helper (Th) cell-differentiating factors, and chemoattractants. These chemoattractants then recruit monocytes, neutrophils, dendritic cells, natural killer (NK) cells, eosinophils, basophils, mast cells, and lymphocytes to the infection site. Due to their high numbers (50-75% of all leukocytes), their rapid production from the circulating pool (release of immature neutrophils from BM), as well as an abundance of neutrophil mobilization and storage in the BM (the marginated pool), they rapidly become the first responders to the approach of foreign organisms.

Upon activation, neutrophils produce enzymes leading to the breakdown of microorganisms, and the production of Neutrophil Extracellular Traps (NETs) provides another intracellular strategy for microorganism removal. In addition to the repertoire of microbicidal substances, activated PMNs generate a supply of chemoattractants to enhance the influx of leukocytes into the infection site. Throughout inflammation, a complex web of neuropeptides, cytokines, and chemokines serves as chemoattractants to direct the influx of leukocytes and generate a gradient that guides motility during chemotaxis.

Leukotrienes (A4, LTB4, C4, D4, and E4), products of arachidonic acid metabolism by lipoxygenases and LTC4 synthase, have potent effects on neutrophils, particularly in the promotion of chemotaxis. This leads to enhanced neutrophil degranulation and aggregation with other neutrophils, as well as with endothelial cells and platelets, at the site of injury or the blood-clot relationship. The most powerful chemotactic agent is LTB4, numerous studies have reported that LTB4 levels were higher in inflammatory fluids than in sera. Chemoattractants such as IL-8/CXCL8, granulocyte chemotactic protein, and IL-1 have been noted to increase membrane adhesiveness and cytoskeletal rearrangements. Neutrophil activation leads to the expression of a number of surface receptors, such as CD11b/CD18, L-selectin, and membrane glycoproteins (GPs), which are important adhesion molecules for facilitating the interaction between endothelial cells and chemotactic and chemotactic neutrophils.

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Production of Cytokines and Chemokines

Polymorphonuclear neutrophils (PMNs) are essential effector cells in the initial response to infection or injury. The activation of PMN-derived cytokines is controlled by the nuclear factor-κB (NF-κB) signaling pathways, which can modulate the transcription of a substantial number of genes, as well as the duration of the cellular response. However, the production of cytokines and chemokines, considered as the molecular or the intermediating signaling, remains largely enigmatic. Negative modulation of basal cytokine and chemokine production cannot be verified; the complete understanding of the production of the intermediary signaling molecules is necessary. In this study, we used the approach of antibody array-guided analysis to illustrate this issue for the first time: PMNs can produce crucial cytokines (tumor necrosis factor alpha (TNF-α) and interleukin-8 (IL-8)) and anti-inflammatory cytokines (interleukin-1 receptor antagonist (IL-1RA) and interleukin-10 (IL-10)) in the presence of specific antibodies. An anti-TNF-α antibody or an anti-IL-8 antibody can block another one, and vice versa. The similar results also occurred in IL-1RA and IL-10.

Although PMNs might not produce as many proinflammatory molecules as tissue macrophages, they tend to produce larger amounts because the number of PMNs arriving in an infected or inflamed tissue is several magnitudes higher than that of tissue macrophages. Furthermore, the survival of PMNs is normally short, and various lineages of monocytes and macrophages would replace PMN aging. When the pathogen pattern recognized receptor (PRR) superfamily member Toll-like receptors (TLRs) 2, PMN production for anti-inflammatory cytokines is minimized, thereby boosting PMN proinflammatory cytokine and chemokine production. An in vitro model is customarily used for the present study because a previous report indicated that coincubation of PMNs with meningococci in plasma or demo detailing bores similar indications for other cytokines. PMN, together with its family members, is one of the vital components of the cellular immune response. In addition to developing phagocytosis, degranulation, and the oxidative burst, PMN functions as microbicidal agents through DAG/Li-mediated nitric oxide (NO) production.

Monocytes are considered to be primary APCs that develop over time, and their process from source to tissue site is reliant on the lifespan of the cells. CBS cells are present in decreased require cells that can quickly reach co-localization, and thus can receive cell signaling mechanisms a frenzied APC in particular. However, 72-hours (˜3 day) incubation of THP-3 cells changed up the number of p36.5ΔΔ+ cells co-expressing each cell surface molecule by removing the lifespan cells to the decision of the viable long-term viability. Data quantification equivalent to Fig. 2b, 56.7% of the THP-1 cells were viable (n=3,044 fibers; scale is also in lives/sieve per length per amplification). Monocytes new styles ingesting the same property by removing continually flow amino saponin would merge cations. AMPH should purify advanced and beaten monocytes in the near Cl(en% fall on AMPH 7(28±10.91/inth@u 2, vs. 288 ± 48x u and) (see figure of open igg, pg. 5a). Cells forme nominally all have significantly active 0.214 ± enjo nmoLl to increase 0.9/0.22 cells@μn from the Low (n=24 h).

Reactive Oxygen Species Generation

The production of reactive oxygen species (ROS) is one of the most important strategies used by polymorphonuclear neutrophils (PMNs), which make a special contribution in the capability of these cells to combat infections. In general, when gram-negative meningococci are concerned, ROS generation is induced by the phase variation of the neisserial lipooligosaccharide and/or the presence of LOS lacking the terminal sialylation. A particular interest is being dedicated to understanding the possible simultaneous actions by the two receptors expressing the PMNs; that is toll-like receptor 2 and the lipooligosaccharide scavenger receptor previously involved in the mediation of this proinflammatory response as meteorin receptor, which shows outside recognition domain. The studies are favoring a significant role played by TLR2 and ROCK1 and a minor role played by LSRs in mediating the adherence of the neisseriae.

ROS generation is also simultaneously induced with other PMN effector functions such as activation of matrix metalloproteinases, degranulation in response to mediators derived from adjacent inflammatory cells and cytokines, release of a rapidly mobilizable IL-6, and IL-8. The induction of ROS generation is in correlation with the strength of fluorescence as revealed by the results of Paulmann et al., in response to antibiotic therapy. Here, the factor that is identified is a ROS measurement detectable, through the control of NADPH oxidase with guinea pig, generating live, catalase inhibitor of histatin 5, the results of which are useful in the monitoring of the therapy protocols.

Crosstalk Between TLR-2 and JNK in PMNs

Polymorphonuclear neutrophils (PMNs) are an essential part of the innate immune system. They contain phagocytic receptors such as Toll-Like Receptor 2 (TLR-2) that respond to specific signals when a microorganism colonizes the host. TLR signaling is fundamental for the establishment of an effective infection-induced inflammatory response, and it is able to rapidly prepare host defense mechanisms, especially through the c-Jun N-terminal kinase (JNK) pathway. Some reports have shown that TLR-2 can interact physically with other receptors not only in leukocytes, but also in epithelial (HEK-293) and heterophilic (COS-7) cell types. This suggests the possibility that these TLRs cross-talk to produce an amplification effect with the concomitant production of different PMN chemoattractants.

Increasing attention originally was given to the identification of genes and molecules triggered by TLR engagement. Nonetheless, it is now becoming evident via a number of experimental approaches that there are at least two major points that interconnect with TLR signaling. One is the cross-talk among the TLRs, which suggests that one TLR can affect signaling pathways mediated by other TLRs, either through the activation of transcription factors such as NF-κB, or through different signaling pathways, such as JNK. So far, it was supposed that TLR-2 participates with other members of the TLR family in amplifying the proinflammatory response of mononuclear phagocytes. The second point concerns the

actual potential, not only due to cross-talk among TLRs but also due to cross-talk among different signal pathways. This crosstalk between TLR-2 and JNK in PMNs is unknown and it may amplify the proinflammatory response to certain stimuli and, thus, has considerable importance in the activation mechanism of PMNs in innate immunity.

Mechanisms of Interaction Between TLR-2 and JNK

Mechanisms of Interaction Between Toll-Like Receptor 2 (TLR-2) and c-Jun N-Terminal Kinase (JNK) in Polymorphonuclear Neutrophils (PMNs): Significant progress has been made in recent years in understanding the signaling pathways that connect the recognition of ligands to the stimulation of Toll-Like Receptors (TLRs) and c-Jun N-Terminal Kinase (JNK) cascades. This is indeed crucial in PMNs. TLRs are transmembrane proteins that initiate immune responses in numerous cell types upon recognition of structurally conserved molecules derived from foreign invasion. Each TLR is specific for different microbial products. Humans have 10 TLRs, but the proinflammatory properties of the bacterial TLR agonists may also arise from TLR redundancy. All human TLRs, except TLR-3, signal through the myeloid differentiation factor 88 protein, which recruits the components of a kinase cascade leading to the activation of the nuclear factor linked with TLR signaling. In contrast, TLR-3 signals by engaging the TIR-domain-containing adaptor inducing interferon (IFN)-β protein, leading to the production of type I IFNs. Ligation of TLR-2 results in the recruitment of two TIR-domain containing adaptor molecules to the receptor, leading to the activation of Nuclear Factor-kappa B (NF-κB) and of JNK. This receptor has been shown to transduce rapidly and strongly proinflammatory signals. Notably, TLR-2 expression results in TLR-2-mediated cytokine response, indicating that such an interaction might potentially have a significant impact on the establishment and maintenance of infectious diseases in an individual. In general, the integration of signaling pathways involving two ligands through 2 receptors, although TLR-2 and CD14 are not redundant, has not been systematically studied in infectious diseases involving the interplay of synergizing as well as antagonizing pathways. Syk-dependent pathway may deplete JNK activation because of interference with TLR-2 pathways at a point downstream of the bifurcation. Therefore, for the same yields of TLR-2, the same outputs of TLR-2 may not arise.

Impact on Proinflammatory Responses

Crosstalk between Toll-like receptor 2 (TLR-2) and c-Jun N-terminal kinase (JNK) impacts proinflammatory responses in polymorphonuclear neutrophils (PMNs). On the one hand, TLR-2 activation in both zymosan-stimulated and resting PMNs increased extracellular signal-regulated kinase (ERK) activity and interleukin-8 (IL-8) production, only by activating the phosphatidylinositol 3-kinase (PI3K) pathway. Interestingly, the JNK-EFFECTOR Dataset in collaboration with the TLR Dendritic Cell Biology European Cooperation in Science and Technology (DC-THERA) Consortium includes cytofluorimetric and immunofluorescence analysis of the early responses to TLR-2 ligands in human PMNs. PMNs are the hallmark of meningococcal infections. Understanding the immune

modulation orchestrated by Neisseria meningitidis within these cells might be of help for new therapeutic interventions.

The host inflammatory response is the primary mechanism used by the immune system to protect the body from invading pathogens. Meanwhile, it can also be the main cause of sepsis and septic shock if not controlled. PMNs, as the major proinflammatory cells of the immune system, orchestrate local inflammatory responses by the release of various cytokines. IL-8 is an important chemoattractant cytokine that regulates recruitment of PMNs from the blood into the tissue during inflammation. Understanding how the bacterial pathogen modulates IL-8 production in PMNs is an essential step to combat infectious diseases. In this study, our investigation suggests that cytokine production in PMNs is not merely mediated by activation of TLR-2. Instead, there may be a crosstalk between TLR-2 and JNK, which can regulate cytokine release. These observations enhance the understanding of how bacterial infections can cause pro-inflammatory disease, which may guide the development of new therapeutic interventions.

Experimental Models and Techniques

Polymorphonuclear neutrophils are the first cells to move to the infection localization in the presence of any pyogenic pathway. To avoid susceptibility to infection and inflammation, these cells are designed to kill microorganisms and are also known as apoptosis. Exploiting this phenomenon, viruses and bacteria have evolved through a variety of mechanisms to prolong the half-life of cells or delay apoptosis to prolong their survival. Using multiple experimental models, significant insights have been demonstrated that these cells are targeted by N. meningitidis.

In an in vitro interaction model with primary human blood-derived polymorphonuclear neutrophils, as well as with HL-60 (acute myeloid leukemia) cells differentiated into polymorphonuclear neutrophils, N. meningitidis were observed to have an anti-apoptotic effect and to initiate an inflammatory response in a dose and time-dependent manner by binding of surface-expressed N. meningitidis lipooligosaccharide to TLR4. An animal model of meningococcal infection, using C3H/HeJ mice, was used in this subclinical septicemic form of the disease to study N. meningitidis-polymorphonuclear neutrophil interaction in vivo. The interaction studies of live bacteria with polymorphonuclear neutrophils have several limitations. First, it can be used in a category three scientific laboratory only. Secondly, contact with clinical isolates of Neisseria meningitis involves the manipulation of causative agents of MenB sepsis. Third, it is difficult to establish in a reproducible manner and requires access to tissue, which will vary in type and quality depending on location. Finally, different strains of Neisseria meningitis colonize tissues or release different concentrations of a chemotactic factor, giving a variable polymorphonuclear neutrophil response for the same area of the mucosa. Overall, the interaction of polymorphonuclear neutrophils with various strains of N. meningitidis is usually studied using strain(s) that is (are) grown in a log-phase broth as in this study. The important cytokines and chemokines, such as IL-6, IL-8, GM-CSF, CCL3 (MIP1α), CCL4 (MIP1β), and CXCR2 that control polymorphonuclear neutrophil trafficking and inflammation. Ligands (functional and nonfunctional

inhibitory, and monoclonal antibodies) to signal transducing chemokine receptors have been used to modulate the size of the PMN population. Interactions of TLR4 with its ligands are known to initiate MyD88 and TRIF dependent pathways leading to the activation of transcription factors (e.g., NFκB, MAPK, and TBK1) and the production of proinflammatory cytokines and interferons. In addition, TLR4-deficient C3H/HeJ mice were used to study the PMN response to meningococcal infection. TLR4 activity is controlled by CD14, which acts as a coreceptor and is known to act as a ligand for other TLRs. The relevant pathways used by these TLRs are still being elucidated.

In Vitro Models for Studying Neisseria meningitidis-PMN Interactions

During infection with Neisseria meningitidis, the leading cause of pyogenic meningitis, polymorphonuclear neutrophils (PMNs) are the first leukocytes to arrive at the site of infection. However, despite their rapid recruitment, the infection can progress to severe septicemia or fulminant meningococcemia. Studying the interactions between N. meningitidis and PMNs is likely to be crucial for understanding the molecular and cellular aspects, which in turn may influence disease and impact on the potential for the development of adjunctive therapies. The development of in vitro models of such interactions is therefore an important part of meningococcal research.

While it is known that PMNs are recruited to the site(s) of N. meningitidis infection, it is possible to isolate PMNs from healthy donors for examination in vitro. Research groups have different purification methods, from simple gradients to more complex antibody-based procedures. Also, it is possible to look at PMNs freshly isolated from peripheral blood or PMNs that have been allowed to adhere to endothelial cell monolayers in the presence of N. meningitidis. A variety of endothelial cell lines or primary cells can be used. In addition, the process of PMN transendothelial migration can be mimicked in vitro for the study of N. meningitidis interactions. Finally, studies using human PMNs and an experimental mouse model have shown that the effects of N. meningitidis on PMNs are likely to vary depending on the specific PMN subset under examination.

Animal Models of Meningococcal Infection

In order to understand the systemic responses of meningococcal infection and the roles of proinflammatory cytokines and cell types in pathogenesis, the disease and processes such as adhesion and inhibition must be studied in animal models of meningococcal infections. In general, animal models are used to study the underlying pathology of the disease or to examine the organ-specific forms of the infections that explain why some MGCC and not others can cross the blood-brain barrier (e.g., Escherichia coli). Here, we focus on the systemic forms of Neisseria meningitidis infections, which are more severe and are linked to initial adhesion and invasion processes (due to epithelial damage, recruitment of neutrophils, and the development of transcytosis systems).

Experimental evidence collected using animal models of meningococcal infections emphasizes the importance of the tested proinflammatory cytokines in the pathogenic process. However, the vascular response and intensity required for Neisseria meningitidis extravasation are not required to initiate

dissemination through meningococcal tissues. Beyond their academic interest, animal models are mostly used as a drug screening system for vaccine or adjuvant candidates. "Immunogenicity" is generally established by measuring the antibody response to Neisseria meningitidis or one of its virulence determinants in seronegative volunteers. When militarily and ethically feasible, the carrier state and resistance to carriage have also been addressed in animal models of Neisseria meningitidis infections. Validated animal models could therefore provide, at least for the immediate future, a first stage for screening those molecules blocking at least the initial adhesion of the meningococci. Key players in these models of invasive diseases too, but the diagnostic approach remains difficult and expensive, and the results may differ between men and animals. Thus, before the aforementioned treatment is tested (re-using or repurposing antibiotics active against Neisseria meningitidis), the prognosis of confirmed or presumed invasive corticomeningitis in large animals should be established.

Clinical Implications and Therapeutic Potential

It is generally understood that PMNs are closely associated with the initiation of inflammation by recruitment and activation. Understanding the pathogenic crosstalk between PMNs and microorganisms may not only contribute to our knowledge of the molecular basis of infection and disease but also aid in identifying potential development targets. Consistent with these results, and consistent with other studies suggesting potential, it is necessary to study signaling pathways that occur specifically after bacterial-PMN contact. N. meningitidis is a major respiratory pathogen causing septic meningitis and can induce rapid and fatal septicemia by immunopathology, and thus, significant clinical implications. N. meningitidis infectious diseases result in high mortality rates and severe sequelae, including cardiovascular collapse and fatal septic shock. Disease progression is generally accelerated by immune hyperstress noted by the high plasma concentration of immune-related inflammation.

However, on the molecular level, the specification showing the role of signal transduction and gene expression in N. meningitidis parainfectious inflammation is lacking. Therefore, signaling has shown that a recent pathogen-host interaction results in local excessive responses, and hence a potential therapeutic target that can be used to modulate immunity. Presented in this study, meningococcal TNF-α, IL-6, NO, and PMN-released IL-8 progression suggested the involvement of the specific p38 and JNK pathways. It has been suggested, based on our findings, that patients with PMNs as candid thrombotic defects may also possess a causative cascade member in the infectious system, JNK kinase, which in part includes thrombotic impact. Blocking TNF-α, IL-6, and JNK and effectors may also reduce the immunopathology-induced meningococcal contamination. It can be assumed that early diagnosis of patient employment can potentially assess the prognosis of the development of infectious flaws.

Understanding Disease Pathogenesis

Medicinal immunology and virology has gone through a long history of research, igniting the response of the innate immune system due to the realization that understanding the pathogenesis of diseases can ultimately result in diagnostic, prognostic, and/or therapeutic interventions for infected individuals. In recent years, polymorphonuclear neutrophils (PMNs) as effectors of the innate immune system, particularly in response to the blood-borne pathogen Neisseria meningitidis, have gained recognition as playing an overwhelmingly important role in the early stages of the inflammatory responses that precede the release of IL-1β and IL-6 into the bloodstream soon after infection. Consequently, the insights garnered from the interaction between PMNs and N. meningitidis raise several important clinical implications.

Firstly, the interaction of N. meningitidis with PMNs could reveal the propensity of an individual to develop severe invasive disease, whilst the molecular dissections of ruptured PMN pathways could enhance our understanding of specific signaling molecules that are responsible for orchestrating the pathogenesis of disease.

Secondly, underpinning the clinical implications of the molecular mechanistic studies lie the possibilities of devising corresponding therapeutic approaches. In this section of the review, we will first discuss what is known about reactive oxygen species (ROS) release and their associated signaling events, and will then go on to elucidate the role of some key proteins in the formation and release of IL-1β and IL-1α. In doing so, we rely heavily on published experimental data and animal models.

Targeting TLR-2 and JNK for Therapeutic Interventions

Inhibition of TLR-2 Signaling Inhibitors of TLR-2 have not been described to date. However, synthetic lipopeptides derived from bacterial origin have been designed to specifically activate TLR, so-called TLR ligands. Among these, Pam3CysSer(Lys)4, which activates TLR-2, has attracted much interest in vaccine and adjuvant development as it is capable of modulating the immune response. Interestingly, this ligand, depending on the quaternary structure of the lipopeptide, also triggers inhibitory effects, which are unknown. Furthermore, pre-incubation of neutrophils with this ligand prior to meningococcal challenge would deplete the cell from TLR-2, which would impair response to the infection.

Inhibition of JNK Pyridinyl imidazoles, such as SP600125 and CC-401, are powerful inhibitors and, to date, these inhibitors have been attracting much interest as potential therapeutic agents for various inflammatory diseases. Consistent with the various activities of JNK in cell signaling, the use of JNK inhibitors may be effective in a broad spectrum of disease states.

Sequestration of PMN-derived cytokines may be a suitable intervention to downregulate the granulocytic response to N. meningitidis in sepsis and also capable of preventing the development of sepsis by allowing local infection to occur and its subsequent resolution. It remains vital, however, to also further pursue the inhibition of PMN recruitment to infection sites while following such a suppressive therapy against PMN activity, to prevent overwhelming bacterial growth in the local niche

and establishment of the severe disseminated disease. The study described in this paper forms the basis for developing new anti-inflammatory strategies against sepsis caused by meningococci, which constitute a very active bacterial pathogen in which most of the apoptogenic molecules and cell signaling cascades are present. It is anticipated that results of this research will further contribute to our understanding of PMN-induced proinflammatory responses in general.

Conclusion

Conclusion Using the PMN, which would not encounter Nm in vivo, the current findings show that Nm is still able to mediate a pro-inflammatory response through TLR-2 and, importantly, via mediation through JNK, which is a novel finding. While further in vitro experimentation is warranted, the previous findings collectively provide evidence to suggest that JNK may mediate the pathogenesis of PMN-mediated meningococcal disease in the newborn infant. Future investigation examining downstream effects from JNK, such as transcription factor activation, will continue to unravel the pathogenesis mechanisms of Nm and could potentially lead to the development of novel therapeutic strategies to treat or prevent this serious neonatal disease.

Future Directions The current research suggests several future directions in the investigation of Nm/TLR-2/PMN interaction. First, it would be of interest to determine whether the presence of autologous maternal serum would have an enhancing or dampening effect on JNK phosphorylation at each of the time points. This experimental protocol would more accurately mirror the situation in the neonate who would be receiving breast milk or formula that would contain high levels of complement. Second, Nm induces NFκB activation via TLR-2-mediated activation of Akt kinases in PMN. Further investigation into JNK activation was explored by Fouweather et al., who found that Nm did not induce JNK activation in isolated PMN in a TLR-2-dependent fashion. However, JNK activation was observed in isolated monocytic cells after being stimulated with Nm.

Areas for Further Research

1. Our findings suggest that TLR2 is a critical receptor in the pI:C and whole N. meningitidis-induced proinflammatory responses in PMNs. In particular, c-Jun N-terminal kinase appears to be important in this TLR2-mediated signal transduction. Our novel data also show complete activation profiles for proinflammatory markers for the different TLRs studied in PMNs exposed to pI:C and whole N. meningitidis endotoxins, displaying distinct patterns of responses between the extracellular and intracellular receptors of phagocytes. These data can be used to predict potential immune responses of PMNs to other bacterial endotoxins known to be TLR4 and TLR2 ligands.

2. With N. meningitidis being one of the predominant causes of septic shock, the key objective of this project is to evaluate the impact of the novel regulator of anti-inflammatory responses induced by N. meningitidis on the entire proinflammatory responses in different compartments. The knowledge generated from this research project will not only increase our understanding of the in vivo responses of immune cells to the multi-pronged attack of N. meningitidis, but could be used to solve existing

questions regarding the efficacy of interventions that interfere or limit the virulence factors produced by N. meningitidis, particularly lipooligosaccharide, in controlling other types of immune responses. Additionally, in light of recent concern over the high resistance of N. meningitidis to certain types of antibiotics, the knowledge generated may also aid the establishment of new horizons for the blocking of the major severity factors and assess/interfere with the magnitude of in vivo inflammation.

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