Journal of Advanced Zoology

ISSN: 0253-7214

Volume 45 Issue 2 Year 2024 Page 1564-1573

Abrogation Of Gram Negative Bacteria Induced Inflammation

Manojit Bysack[1*], Rajen Dey[2]

[1*,2] *Department of Medical Laboratory Technology, School of Allied Health Sciences, Swami Vivekananda University, Telinipara, Barasat-Barrackpore Rd, Bara Kanthalia, West Bengal-700121, India*

***Corresponding author:** Mr. Manojit Bysack*
**Department of Medical Laboratory Technology,School of Allied Health Sciences, Swami Vivekananda University,Telinipara, Barasat-Barrackpore Rd, Bara Kanthalia, West Bengal-700121, India E-mail: bysackmanojit@gmail.com*

Abstract:

Lipopolysaccharide (LPS) is a prototypical exogenous endotoxicpyrogen as it binds with TLR4, and CD14 receptors of especially expressed on monocytes, macrophages, dendritic cells thereby secreting pro-inflammatory cytokines, nitric oxide, eicosanoids superoxide etc. As a result, LPS generates a variety of mediators, such as TNF-α, which triggers NF-κB, which results in cellular damage and fatal tissue injury, such as multiple organ dysfunction syndrome (MODS) and systemic inflammatory response syndrome (SIRS). The TNF-α being soluble diffuses through the membrane and interacts with the trimeric mTNFR1. This in turn causes the activation of STAT1 and STAT5 resulting in production of NF-κB induced P_{65}-P_{50} heterodimer that transcribe mRNA. This results in the production of pro-inflammatory cytokines such as IL-1, IL-6, and IL-12, which in turn regulate inflammation. When the cell is at rest, the PIP2 on its surface facilitates the recruitment of an adaptor called MyD88-adapter-like (MAL/TIRAP). When MAL and MyD88 are recruited during the initial phases of Microbes-associated molecular pattern (MAMP) recognition, TLR4 activation occurs after stimulation. causes the MAP kinases JNK, p38, and NF-κβ to become activated as a result, which in turn causes the pro-inflammatory cytokine TNF-α to be produced. When cells release TNF-α, they bind with TNFR1, which releases the transcription factor domain death (SODD). Additionally, they recruit receptor interacting protein-1 (RIP-1), which in turn recruits mitogen-activated protein kinase kinasekinase 3, transforming growth factor β-activated kinase (TAK-1), and finally, they activate inhibitor of NF-κβ kinase (IKK) complex. The IKK complex then phosphorylates IκBα and thereby releasing NF-κB subunit, bound to unstimulatedIκBα by ubiquitination and degradation of IκBα. This soluble NF-κBtranslocate into the nucleus and thereafter evaluate gene transcription that is a central mediator of pro-inflammatory effects. The signalling takes place via activation of STAT1 and triggering the expression of IL-12, NOS-2 and Suppressor of cytokine signalling (SOCS), characterized by the expression of TNF-α as a major and iNOS as well as high nitric oxide and intermediate ROS production. By convention blocking of production of pro-inflammatory cytokines may be resulting in production

	of anti-inflammatory causes the regulation of LPS induced sepsis by anti-inflammatory mediators. This may be an alternative regulating option to diminish the septic shock via activation and translocation of STAT6 by JAK1 and JAK3 signalling. In addition to that IL-10 is produced by these cytokines causes activation of STAT3 via up-regulating the expression of SOCS3, which mediates the suppression of pro-inflammatory cytokine signalling pathways. Thus TNFRSF1A blocking will surely help society to find out future treatment strategies of acute and chronic diseases such as sepsis, disorientation of vital body functions, certain antibiotic resistant, Capillary leak syndrome, disseminated Intravascular Coagulation, purpura, ecchymoses, gangrene, multiple sclerosis etc. via the wound healing sub one secrete IL-4 and remodelling of the degrading tissues.
CC License CC-BY-NC-SA 4.0	***Keywords: Inflammation, Interleukins (IL), LPS, STAT, TLR4, TRNF1***

Introduction

Macrophages are the most numerous first line immune cells present in different parts of body under homeostatic conditions and are ideally positioned to dictate the innate defence mechanism of the body. Macrophage populations originate from blood monocytes are heterogeneous and demonstrate wide range of plasticity, based on variations in origin, tissue residency, environmental influences etc. According to the anatomical location, there are major following types of macrophages in the body viz, splenic macrophages, peritoneal macrophages, glial macrophages and bone marrow macrophages. They are obviously different in their maturation state as well as in morphological characteristicsand functions. The heterogeneity is reflected in their morphology i.e. the types of foreign invaders they can recognize as well as the levels of inflammatory mediators e.g. IL-1, IL-6, TNF-α they produce. Thus macrophages possess wide range of pro-inflammatory, anti-inflammatory, destructive, scavenging, remodelling potentialsthat contribute towards the pathogenesis of inflammatory and degenerative diseases.

Gram-negative bacteria's outer membrane contains lipopolysaccharide (LPS), a big endotoxin molecule made up of lipid-A and a polysaccharide consisting of O-antigen, outer core, and inner core connected by a covalent connection. By boosting negative charge and shielding the membrane from some chemicals, it serves the primary purpose of preserving the structural integrity of the bacterium. LPS is a prototypical exogenous endotoxicpyrogen as it binds with TLR4,and CD14 receptors of especially expressed on monocytes, macrophages, dendritic cells thereby secreting pro-inflammatory cytokines, nitric oxide, eicosanoids superoxide etc. Therefore, LPS generates a variety of mediators, including TNF-α, which contributes to septic shock, a form of endotoxemia. This is one of the potent initiator of high inflammatory responses. Endotoxic shock syndrome and systemic inflammatory response syndrome (SIRS) are two examples of the deadly tissue injury and cellular damage brought on by the elevated level of TNF-α caused by LPS-induced NF-κB and sepsis. Severe sepsis that leads to multiple organ dysfunction syndrome (MODS), which is characterized by loss of consciousness, obesity, insulin and some antibiotic resistance, blood vessel damage, irregular heartbeat, Capillary leak syndrome, dilation of blood vessels, disseminated intravascular coagulation, kidney, lung, adrenal gland, purpura, ecchymoses, and gangrene. Certain auto-immune diseases like multiple sclerosis may also be highly fatal, developed in extreme sepsis.

Tumor Necrosis Factor Receptor 1 (TNFR1), alternatively referred to as TNFR Superfamily Member 1A, is a widely distributed membrane receptor that binds TNF-α. The TNF-α produced by the LPS mediated TLR4 interaction cascade, being soluble diffuses through the membrane and there after interacts with the trimeric mTNFR1 to activate that. This in turn causes the activation of STAT1 and STAT5 resulting in production of NF-κB induced P_{65}-P_{50} heterodimer that transcribe mRNA. This produces pro-inflammatory cytokines like Interleukin-1 (IL-1), Interleukin (IL-6), Interleukin-12 (IL-12) and thus resulting in the maturation of the macrophage to pro-inflammatory M1 phenotype. It functions as a regulator of inflammation.

Activated macrophages are routinely classified into mainly 2 different types according to the paradigm of macrophage activation: M1 macrophages (classic activation) and M2 macrophages (alternative activation). M1 macrophages are pro-inflammatory and have roles in host defence mechanism against infections. On the other hand M2 macrophages are related to the anti-inflammatory response reactions including tissue remodelling. These 2 phenotypes polarization are regulated by specific sets of signalling pathways, transcriptional and post-

transcriptional regulatory mechanisms. The PIP2 binding domain on the cell surface during the resting state facilitates the recruitment of an adaptor called MyD88-adapter-like (MAL/TIRAP). When LPS stimulation occurs, TLR4 activation triggers the recruitment of MAL and MyD88 in the early phases of MAMP recognition, which is related with microbes. results in the activation of the transcription factor NF-κβ, the MAP kinases JNK and p38, and the generation of the pro-inflammatory cytokine TNF-α.The release of TNF-α, a soluble ligand, from cells binds to TNFR1, which releases the Silencer of Domain Death (SODD). Additionally, RIP-1, a receptor interacting protein, attracts TAK-1, TAK-activated kinase, and mitogen-activated protein kinase kinase 3, which in turn activates the inhibitor of NF-κβ kinase complex. After that, the IKK complex phosphorylates IκBα, releasing the NF-κB subunit that had been linked to unstimulatedIκBα through ubiquitination and IκBα destruction. Soluble NF-κBtranslocates into the nucleus, where it assesses gene transcription, a key pro-inflammatory impact mediator.

The M1 phenotype of macrophages, also known as classically activated macrophages, are triggered by pro-inflammatory cytokines (like interferon-ϒ or IFN-ϒ) or microbial products (such lipopolysaccharides, which are found in the outer membrane of Gram-negative bacteria) either alone or in combination. The signalling takes place via JAK1 and JAK3 regulating theactivation of STAT1 and triggering the expression of interleukin-12(IL-12), nitric oxide synthase-2(NOS-2) and Suppressor of cytokine signalling(SOCS). The M1 phenotype is defined by the expression of high quantities of pro-inflammatory cytokines, including iNOS, nitric oxide, and intermediate ROS (superoxide radical) generation. Major pro-inflammatory cytokines include tumour necrosis factor-α (TNF-α).

In comparison to the M1 phenotypes, the alternative activated macrophages or M2 phenotypes of macrophages which are anti-inflammatory.Pro-inflammatory cytokines such as TNF-α are induced by LPS, leading to the polarization of macrophages to the M1 subtype, which is observed in cases of severe sepsis. Conventionally, suppressing the generation of pro-inflammatory cytokines may cause the production of anti-inflammatory cytokines, which in turn causes macrophages to adopt an anti-inflammatory M2 phenotype. This might be causes the regulation of LPS induced sepsis by anti-inflammatory mediators. Thus, M2 polarization may be an alternative regulating option to diminish the severity of septic shock via blocking of mTNFR1. These M2 are promoted via activation and translocation of STAT6 by JAK1 and JAK3 signalling. In addition to that IL-10 is produced by these cytokines causes activation of STAT3 via up-regulating the expression of SOCS3, which mediates the suppression of pro-inflammatory cytokine signalling pathways. The 2 subtypes of M2 macrophages i.e. regulatory macrophages produce immunosuppressive cytokine IL-10, while, the wound healing sub one secrete IL-4. Thus, remodelling the devastating tissues.

Macrophage polarization is a dynamic process whereby macrophages respond to cues from cellular microenvironments by adopting distinct functional programs. Whether peritoneal macrophages can be polarized to M1 and M2 phenotypes after stimulation with LPS and there after neutralization of TNFR1 receptor has not yet been fully understood from any other study. Therefore, by examining the impact of TNFR1 neutralization in those macrophages following LPS stimulation, we hope to determine whether peritoneal macrophages derived from Swiss Albino Mice become pro-inflammatory (M1 polarized) or anti-inflammatory (M2 polarized) in vitro mice model. The M1/M2 macrophages polarization may have a therapeutic potential to treat different inflammatory diseases and we have tried to evaluate the same in this experiment.

Review of literature

LPS Induced Sepsis:

Present in the outer membrane of Gram-negative bacteria, lipopolysaccharide (LPS) is a big endotoxin molecule made up of lipid-A and a polysaccharide comprised of O-antigen, outer core, and inner core connected by a covalent connection [1]. By boosting negative charge and shielding the membrane from some chemicals, it serves the primary purpose of preserving the structural integrity of the bacterium. Due to its ability to bind to TLR4 and CD14 receptors, which are highly expressed on monocytes, macrophages, and dendritic cells, LPS is a quintessential exogenous endotoxicpyrogen. This binding causes the release of pro-inflammatory cytokines, nitric oxide, eicosanoids, superoxide, and other chemicals. Therefore, LPS generates a variety of mediators, including TNF-α, which contributes to septic shock, a form of endotoxemia. This is one of the potent initiator of high inflammatory responses. The increased level of TNF-α causes by LPS induced NF-κB resulting in sepsis causes cellular damage, lethal tissue injury including endotoxic shock syndrome or systemic inflammatory response syndrome (SIRS) [2]. Severe sepsis that leads to multiple organ dysfunction syndrome (MODS), which is characterized by loss of consciousness, obesity, insulin and some antibiotic resistance, blood vessel damage, irregular heartbeat, Capillary leak syndrome, dilation of blood vessels, disseminated intravascular coagulation, kidney, lung, adrenal gland, purpura, ecchymoses, and gangrene.

Multiple sclerosis is one of the extremely deadly auto-immune disorders that can develop in severe sepsis. TLR4 on animal immune cells is responsible for identifying the mechanism of action of LPS as a sepsis inducer. The two accessory proteins CD14 and MD-2 contribute in recognition. The response between stimulation and the adaptor molecule MyD88 results in The NF-κB is activated by a signaling pathway that is dependent on MyD88 and controls the expression of target genes that encode pro-inflammatory mediators leading septicaemia [3].

Properties of Peritoneal Macrophage:

Macrophages represent heterogeneous phenotypes as they are distributed in different tissues and organs after birth. It is found that their distinctive morphological features according to their anatomical localization may be attributed to their heterogeneity. This makes macrophages flexible in their immune function. Peritoneal macrophages (PMs) are most mature and commonly used for experimental purposes possibly due to their low organ tension in the peritoneal cavity. Some remarkable features such as higher expression of iNOS, Superoxide, TNF-α and IL-12[4]. PMs display larger cell size and higher lysosomal content compared to other types of macrophages.

Structure of TNFR:

TNF and its receptors combine to produce a well-known and significant pro-inflammatory cytokine system that is involved in many different biological processes. It was the original and initial member of the TNF–TNF-receptor superfamily, which at this point has about 40 members with identical structural makeup. These ligands and receptors, which are mostly expressed on immune system cells, are all self-assembling trimerictransmembrane proteins. The TNF–TNF-receptor superfamily plays a significant role in host defense, organogenesis, apoptosis, inflammation, septic shock, and autoimmune, among other biological processes.Proteases can cleave TNF, a homo-trimeric cytokine, to release the soluble form. TNF is produced as a membrane-bound protein. The soluble cytokine and the transmembrane are both physiologically active. In addition to adipocytes, keratinocytes, mammary and colon epithelium, osteoblasts, mast cells, and many other cell types, monocytes and macrophages, dendritic cells, B cells, T cells, and fibroblasts also produce TNF.Two different receptors are known to be used by TNF (also known as TNFSF2) to signal: TNFR1 (also known as p55 or TNFRSF1A) and TNFR2 (also known as p75 or TNFRSF1B). Both of its receptors, like those of the cytokine, have the ability to cleave off the cell surface and circulate in soluble forms, where they can function as TNF "decoy" (i.e., non-signalling) receptors. The fact that TNF receptors, like the majority of superfamily receptors, interact with several ligands within their respective superfamily adds to the complexity. Accordingly, it has also been demonstrated that lymphotoxin a (LT-a, also referred to as TNFSF1) activates both TNF receptors [5].

TNF initiates two different signaling pathways through TNFR1. A pro-inflammatory pathway that includes MAP3K, TRADD, RIP-1, TRAF2, and cIAPs activates the IKK-complex, which in turn activates NF-κB and causes the transcription of pro-inflammatory mediators. The MAPK pathway is triggered by an alternative MAP3K. The activation of caspases by FADD initiates the pro-apoptotic pathway. TRAF1, TRAF2, and cIAPs are involved in TNF signaling via TNFR2, which also activates NF-κB. TNF receptor-associated death domain (TRADD), receptor-interacting protein-1 (RIP-1), TNFR-associated factor 2 (TRAF2), TNFR-associated factor 1 (TRAF1), Fas-associated protein with death domain (FADD), NF-κB essential modulator (NEMO), inhibitor of NF-jB kinase subunit a (IKKa), inhibitor of NF-κB kinase subunit b (IKKb), cellular inhibitor of apoptosis (c-IAP), mitogen activated protein kinase (MAP-kinase), MAP kinase kinase (MAP3K), mitogen-activated protein (MAP) kinase, and NF-κB (nuclear factor kappa light chain enhancer of activated B-cells)[5].

Biological Immune Function of TNFR1 via Signalling:

Tumor Necrosis Factor Receptor 1 (TNFR1), alternatively referred to as TNFR Superfamily Member 1A, is a widely distributed membrane receptor that binds TNF-α. The TNF-α produced by the LPS mediated TLR4 interaction cascade, being soluble diffuses through the membrane and there after interacts with the trimeric mTNFR1 to activate that. This in turn causes the activation of STAT1 and STAT5 resulting in production of NF-κB induced P_{65}-P_{50} heterodimer that transcribe mRNA. In turn, this results in the production of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin (IL-6), and interleukin-12 (IL-12), which mature macrophages into pro-inflammatory M1 phenotypes. It controls the level of inflammation. Tumor Necrosis Factor Receptor 1 (TNFR1), alternatively referred to as TNFR Superfamily Member 1A, is a widely distributed membrane receptor that binds TNF-α. The transcription factor NF-κβ is triggered by this receptor. It controls the level of inflammation. When the cell is at rest, the majority of the phosphatidylinositol on its surface is present as PIP2, which facilitates the recruitment of an adaptor called MyD88-adapter-like

(MAL/TIRAP), which has a PIP2 binding domain. When LPS stimulation occurs, TLR4 activation triggers the recruitment of MAL and MyD88 in the early phases of MAMP recognition, which is related with microbes. Consequently, it triggers the activation of the transcription factor NF-κβ, MAP kinases JNK and p38, and produces pro-inflammatory cytokine TNF-α [6]. The soluble TNF-α release from cells, binds with trimeric TNFR1. This sets off a pro-inflammatory cascade and activates irreversible intracellular signaling. Silencer of domain death (SODD) is released when a ligand binds to TNFR1, which then induces the TNFR-associated death domain (TRADD) domain to interact with TNFR1. The activation of the inhibitor of NF-κβ kinase (IKK) complex is subsequently caused by the recruitment of receptor interacting protein—1 (RIP-1), which in turn recruits mitogen-activated protein kinase kinasekinase 3 and transforming growth factor β-activated kinase (TAK-1). The IKK complex then phosphorylates IκBα and thereby releasing NF-κB subunit, bound to unstimulatedIκBα by ubiquitination and degradation of IκBα. As a key modulator of pro-inflammatory effects such endotoxic shock or sepsis, this soluble NF-κBtranslocates into the nucleus and then assesses gene transcription [6][7]. Extensive everity also leads to obesity and insulin resistance (Huifang Liang et al., 2008) and development of certain auto-immune diseases like multiple sclerosis, Guillain-Barre Syndrome, Miller-Fisher Syndrome [8].

Macrophage Activation and Polarization by LPS:

Activated macrophages are routinely classified into mainly 2 different types according to the paradigm of macrophage activation: M1 macrophages (classic activation) and M2 macrophages (alternative activation). M1 macrophages are pro-inflammatory and have roles in host defence mechanism against infections. On the other hand M2 macrophages are related to the anti-inflammatory response reactions including tissue remodelling. These 2 phenotypes polarization are regulated by specific sets of signalling pathways, transcriptional and post-transcriptional regulatory mechanisms[9].The M1 phenotype of macrophages, also known as classically activated macrophages, are triggered by pro-inflammatory cytokines, such as interferon-ϒ or IFN-ϒ, or by microbial products, such as lipopolysaccharides or lipopolysaccharides found in the outer membrane of Gram-negative bacteria, either alone or in combination [10]. Generated by Th1 cells, IFN-ϒ polarizes macrophages toward the M1 phenotype. JAK1 and JAK3 are involved in the signaling process, which controls STAT1 activation and initiates the production of IL-12, NOS-2, and SOCS (suppressor of cytokine signaling). The M1 phenotype is defined by the expression of high levels of pro-inflammatory cytokines, including nitric oxide and intermediate ROS (superoxide radical) production, as well as pro-inflammatory cytokines such as interleukin-12 (IL-12), interleukin-6 (IL-6), interleukin-23 (IL-23) and tumour necrosis factor-α (TNF-α).The M2 phenotypes of macrophages, which are anti-inflammatory in nature and alternative to M1 phenotypes, are stimulated by the activation and translocation of STAT6 by JAK1 and JAK3 signalling, as well as the association of interleukin-4 (IL-4) and interleukin-13 (IL-13) to receptor IL4Rα. Additionally regulated are transcription factors such as STAT6, IRF4, PPARϒ, KLF4, IRF4,HIF-2α, BMP-7, FABP4, LXRα linked with M2 macrophages, resistin-like-α (Retnla), macrophage mannose receptor 1 (Mmc-1), and others. Furthermore, by up-regulating the expression of SOCS3, which regulates the inhibition of pro-inflammatory cytokine signaling pathways, the cytokines' production of IL-10 activates STAT3.IL-10 and IL-1 receptor antagonist (IL-1Ra) production are characteristic of macrophages with the M2 phenotype. Scavenger, mannose, glucocorticoid, and galactose receptors are also highly expressed in these cells. The two subtypes of M2 macrophages—regulatory macrophages and wound healing subtype—secrete IL-4 and increase arginase activity, respectively, and create the immunosuppressive cytokine IL-10. Restructuring the ravaged tissues as a result [12]. Therefore, macrophage polarization is a dynamic process in which they take on various functional programs in response to cues from their cellular microenvironments [13].

Role of TNF and TNFR1 in Control of Sepsis:

Sepsis brought on by an unchecked infection that triggers a systemic inflammatory response. This continues to be a significant contributing factor to morbidity and death in the intensive care unit. Severe pneumonia and surgical device-induced intra-abdominal infections, including peritonitis and bacteremia, are the most frequent causes. Sepsis may be caused by one of two main types of systemic dysfunctions that arise from bacterial infection or exposure to a wide variety of mycotoxins. On the one hand, an excessive amount of inflammation can seriously weaken the immune system.However, sepsis can also trigger a strong and overwhelming immune response that results in an overproduction of mediators. These mediators can harm the host by causing fever, cardiovascular problems, and multiple organ failure [14]. TNF was regarded as a key mediator in these intricate pathophysiological disorders' research. In fact, by causing hypotension, cardiac failure, and vascular leakage, an in vivo injection of TNF largely mimics the symptoms of septic shock.The function of TNF and its closest relative, lymphotoxin (LT), 3 has been thoroughly investigated in this regard. Numerous cell types produce

TNF in vivo, and by its action on certain receptors, it performs a variety of physiological activities [15]. TNF is first expressed on the cellular membrane during cell activation, and it is then converted to soluble TNF trimer by the protease TNF-converting enzyme. Homo-trimeric membranes and soluble TNF or LT interact with two receptors, TNFR1 (CD120) and TNFR2, to exert comparable but different activities [16]. TNF affects an infection in both positive and harmful ways. TNF promotes angiogenesis and leukocyte recruitment while quickening the pathogens' removal, including Mycobacterium [17].TNF, on the other hand, increases mortality in septic shock and sepsis. TNF inhibitors shield mice against bacterial or LPS-induced sepsis. Genetically engineered mice or soluble TNFR1 have been used to demonstrate that TNFR1 deficiency protects mice against LPS/D-galactosamine shock [18]. Mice lacking TNFR1 in the model have the same death rate as the control group [19]. Mortality is higher in TNFR1-deficient animals or following TNF Ab neutralization in the cecal ligation and puncture scenario. However, TNFR2 loss appears to enhance vulnerability to sepsis, according to a recent publication [20]. Few studies, on the other hand, have examined the involvement of TNFR2 in the pathophysiology of sepsis. These two receptors can be proteolytically cleaved into two soluble forms, sTNFR1 and sTNFR2, following cell stimulation by a variety of stimuli, including TNF itself. These soluble forms can be found in the circulation of patients suffering from a variety of inflammatory diseases, including sepsis brought on by lipopolysaccharide (LPS) for extended periods of time and at high concentrations. By preventing cytokines from binding to their membrane receptors and inducing a biological response, these soluble receptors play a role in the regulation of cytokine activity. For the first time, researchers used a microbial model of abdominal infection to examine the immune and inflammatory responses in TNFR1/R2 double-deficient mice in an effort to better understand the role of the TNF-TNFR axis in polymicrobial sepsis and the reasons why neutralizing TNF therapy has proven to be relatively ineffective clinically.According to reports, TNFR1 deficiency is linked to protection as shown by increased survival, peritoneal cavity bacterial clearance, and neutrophil recruitment. A diminished hyperinflammatory response linked to an abdominal infection is another effect of TNFR deficiency that keeps multiple organ failure and death from occurring. Because they prevent bacteria from growing, neutrophils are essential for the management of infection. When bacteria are present, TNFR1 signaling pathways can affect neutrophil homeostasis by first increasing chemotaxis and later lowering apoptosis. Lastly, neutralization using an antibody that inhibits mouse TNF [21], in contrast to results with TNFR1 deletion, may demonstrate enhanced protection against polymicrobial-induced sepsis [20].

Functional Aspect via TNFR1 Neutralization:

1.In severe sepsis, the induction of pro-inflammatory cytokines such as TNF-α by LPS leads to the polarization of macrophages to the M1 subtype. Conventionally, blocking the production of pro-inflammatory cytokines may result in the production of anti-inflammatory cytokines, which in turn converts macrophages to the anti-inflammatory M2 phenotype. This might be causes the regulation of LPS induced sepsis by anti-inflammatory mediators. Thus, M2 polarization may be an alternative regulating option to diminish the severity of septic shock via blocking of mTNFR1. Whether peritoneal macrophages can be polarized to M1 and M2 phenotypes after stimulation with LPS and there after neutralization of TNFR1 receptor has not yet been fully understood from any other study. Therefore, by examining the impact of TNFR1 neutralization in those macrophages following LPS stimulation, we hope to determine whether stimulated peritoneal macrophages from Swiss Albino Mice become pro-inflammatory (M1 polarized) or anti-inflammatory (M2 polarized) in vitro mice model. Here, we'd like to look into how inhibiting the lipopolysaccharide (LPS) sensor's cell surface Tumor Necrosis Factor Receptor 1 (TNFR1) affects the repolarization of LPS-induced macrophages into an alternative activated anti-inflammatory macrophage phenotype, or M2 macrophages, which is followed by the release of cytokines and reactive oxygen species (ROS). We have attempted to assess the therapeutic potential of M1/M2 macrophage polarization in treating several inflammatory disorders in this investigation. The major aim of this review is to look into the consequences of inhibiting Tumor Necrosis Factor Receptor 1 (TNFR1) on the cell surface and to assess & interpret the benefical aspect towards polarization to M2 macrophages followed by release of cytokines, reactive oxygen species (ROS) that may be gradually help in human welfare.

Performed Experiments

Male Swiss Albino Mice with body weight 18±2 g were taken for the experiment from the local registered breeder. The mice were placed in plastic cages in the departmental animal house facility. Normal rodent diet was provided to the mice.Isolation of Murine Peritoneal Macrophages using protocol [38].Murine peritoneal macrophage, stimulated with Gram negative *E. Coli*LPS for 1 hour incubation. Then the cells were treated with primary antibody of anti-mouse TNFR1 [8].Analysis and sorting of M1 and M2 phenotype of macrophages were done after TNFR1 neutralization of LPS stimulated peritoneal macrophages [22]. Following

FACSanalysis and sorting the M1 and M2 macrophages were separated in different Eppendorf tubes and were centrifuged separately. After centrifugation, release of cytokines, reactive oxygen species (ROS) were determined from the cultured supernatant and the expression of TNFR1 was done from the lysate of the M1 and M2 macrophage.Nitric oxide generation was determined by the Griess assay [24]. Superoxide anion generation was determined by a standard assay with slight modification [40].Using sandwich ELISA, the cytokine concentrations in cell culture supernatants were determined. Recombinant cytokines in mice were employed as reference points. The Lowry method [23] was used to standardize cell culture supernatants from various groups to the protein content, and the manufacturer's instructions were followed to assess the amounts of TNF-α and IL-1β. After sorting and FACS analysis, M1 and M2 macrophages were centrifuged independently. The cell pellets from the various groups were then suspended in ice-cold RIPA-NP40 buffer that contained a protease inhibitor cocktail. The Lowry technique was used to measure the total protein levels of the supernatants obtained from the cell extracts. To investigate the expression of TLR4 from the lysate of M1 & M2 macrophages, samples with similar protein concentrations were separated by SDS-PAGE and transferred to a nitrocellulose membrane for Western blot analysis.From the immunoblot assay of TNFR1 receptor expression in murine peritoneal macrophage it was seen that there was no such changes in expression in control group of M1 and M2 phenotype; high expression was observed in LPS administered both M1 and M2 macrophages and TNFR1 Ab neutralized LPS treated macrophages group showed a bit lesser expression compared to LPS stimulated macrophages in both M1 and M2 subgroup [41].

Summery and Conclusion

Bacterial LPS is considered as a major causative agent of different life threatening diseases occurs in human. Pathogenic LPS ensures intracellular survival of pro-inflammatory M1 subtype of macrophages and there by causes inflammation. In this study, we want to investigate whether stimulated peritoneal macrophages obtained from Swiss Albino Mice become pro-inflammatory (M1 polarized) or anti-inflammatory (M2 polarized) in vitro mice model by investigating the effect of TNFR1 neutralization in those macrophages after LPS stimulation i.e. to investigate the effects of blocking of cell surface Tumor Necrosis Factor Receptor 1 (TNFR1) on LPS induced macrophages towards repolarization to M2 macrophages followed by release of cytokines, reactive oxygen species (ROS). To investigate the effects of blocking of cell surface Tumor Necrosis Factor Receptor 1 (TNFR1) on LPS induced macrophages towards repolarization to M2 macrophages followed by release of cytokines, reactive oxygen species (ROS). It was observed from the flowcytometric analysis that the amount of anti-inflammatory M2 phenotype of macrophages was persist in greater percentage than that of the pro-inflammatory M1 phenotype of macrophages after blocking the LPS treated macrophage with anti-TNFR1 antibody i.e. it was found that in normal untreated control macrophages as well as control macrophages treated with the anti-TNFR1 antibody causes the existence of pro-inflammatory subtypes of macrophages in greater extent. After treated the LPS sensored macrophages neutralized with anti-TNFR1 antibody there causes a profound shifting or polarization of the macrophages to anti-inflammatory M2 subtypes. There also caused some amount of M1 to shift into M2 phenotypes via paradigm. From present study, it is clear that TNFR1 neutralization causes marked reduction in O_2^- and NO release from murine peritoneal macrophage cells means lower O_2^- and NO resulting in reducible presence of pro-inflammatory M1 phenotype of macrophages and at the same time increasing anti-inflammatory M2 sub-population of macrophages [as shown in the Table given below]. These studies and observation implies that TNFR1 blocking has a potential role in pathogenesis of LPS induced sepsis via M2 macrophage polarization process of murine peritoneal macrophage.

Therefore, it is evident from the above study that TNFR1 neutralisation is an effective measure for bacterial LPS induced tissue damage to host via active polarization process of pro-inflammatory M1 phenotype of macrophages to the anti-inflammatory M2 sub-population of macrophages. Thus for therapeutic purpose TNFR1 blocking should be fully beneficial if anti-TNFR1 antibody is used. It will surely help society to find out future treatment strategies of acute bacterial LPS induced acute and chronic diseases such as sepsis. Though, further investigations might be required to find out the most effective antibiotic combination along with anti-TNFR1 antibody for more accurate, tremendous worthwhile recovery of the inflammatory disease.

The proposed phenomenon has been summarized in the schematic representation given below.

Hence, TNFR1 neutralisation might be an effective measure for bacterial LPS induced tissue damage to host via active polarization process of pro-inflammatory M1 phenotype of macrophages to the anti-inflammatory M2 sub-population of macrophages. Thus for therapeutic purpose TNFR1 blocking might be fully fruitful if anti-TNFR1 antibody is used. It will surely help society to find out future treatment strategies of acute bacterial LPS induced infection such as sepsis.

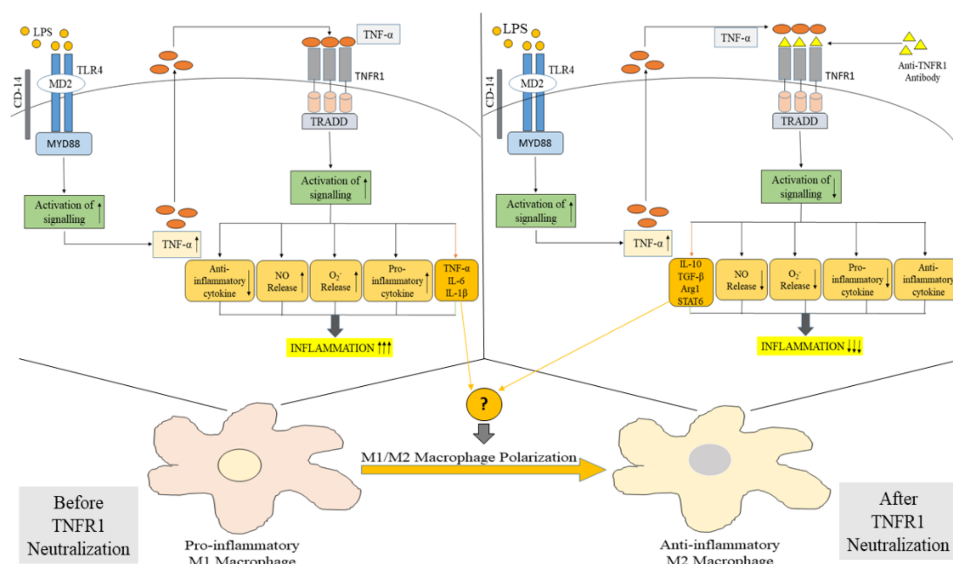

Figure No. 1: Possible Schematic representation of M1/M2 macrophage sub-population polarization after TNFR1 neutralization in LPS stimulated murine peritoneal macrophages during abrogation of inflammation

Acknowledgments

The authors are highly thankful to the Department of Medical Laboratory Technology, Swami Vivekananda University, Barrackpore. The authors also declare that no funds or grants were received during the preparation of this article.

Conflict of interest

All authors declare that there are no conflicts of interest.

Data availability statement

No data was used for the research described in the article.

Author's contribution

ManojitBysack (MB) participated in the conception of the study. RajenDey (RD) and MB participated in literature searches and extraction. MB and RD wrote the manuscript for submission to this journal.

References

1. Huber Mayer, U. RamadasBhat, Hussein Masoud, Joanna Radziejewska-Lebrecht, Christiane Widemann and Jurgen H. Krauss, Bacterial lipopolysaccharides, *Pure & Appl. Chem.* (1989), Vol. 61, No. 7, pp. 1271-1282.
2. Raetz, C. R. (1990). Biochemistry of endotoxins. *Annual review of biochemistry*, *59*(1), 129-170.
3. Solov'eva, T., Davydova, V., Krasikova, I., &Yermak, I. (2013). Marine compounds with therapeutic potential in gram-negative sepsis. *Marine drugs*, *11*(6), 2216-2229.
4. Zhu, C. B., Blakely, R. D., & Hewlett, W. A. (2006). The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. *Neuropsychopharmacology*, *31*(10), 2121-2131.
5. Blüml, S., Scheinecker, C., Smolen, J. S., &Redlich, K. (2012). Targeting TNF receptors in rheumatoid arthritis. *International immunology*, *24*(5), 275-281.
6. Rogero, M. M., & Calder, P. C. (2018). Obesity, inflammation, toll-like receptor 4 and fatty acids. *Nutrients*, *10*(4), 432.
7. Liang, H., Yin, B., Zhang, H., Zhang, S., Zeng, Q., Wang, J., ...& Li, Z. (2008). Blockade of tumor necrosis factor (TNF) receptor type 1-mediated TNF-α signaling protected Wistar rats from diet-induced obesity and insulin resistance. *Endocrinology*, *149*(6), 2943-2951..

8. Williams, S. K., Maier, O., Fischer, R., Fairless, R., Hochmeister, S., Stojic, A., ...& Diem, R. (2014). Antibody-mediated inhibition of TNFR1 attenuates disease in a mouse model of multiple sclerosis. *PloS one*, *9*(2), e90117.
9. Martinez, F. O., & Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000prime reports*, *6*.
10. Jakobi, V., &Petry, F. (2008). Humoral immune response in IL-12 and IFN-γ deficient mice after infection with Cryptosporidium parvum. *Parasite immunology*, *30*(3), 151-161.
11. Doyle, A. G., Herbein, G., Montaner, L. J., Minty, A. J., Caput, D., Ferrara, P., & Gordon, S. (1994). Interleukin-13 alters the activation state of murine macrophages in vitro: Comparison with interleukin-4 and interferon-γ. *European journal of immunology*, *24*(6), 1441-1445.
12. Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J., & Hill, A. M. (2000). M-1/M-2 macrophages and the Th1/Th2 paradigm. *The Journal of immunology*, *164*(12), 6166-6173.
13. Jenkins, S. J., & Allen, J. E. (2010). Similarity and diversity in macrophage activation by nematodes, trematodes, and cestodes. *Journal of Biomedicine and Biotechnology*, *2010*.
14. Hotchkiss, R. S., & Karl, I. E. (2003). The pathophysiology and treatment of sepsis. *New England journal of medicine*, *348*(2), 138-150.
15. Vassalli, P. (1992). The pathophysiology of tumor necrosis factors. *Annual review of immunology*, *10*(1), 411-452.
16. Ruuls, S. R., Hoek, R. M., Ngo, V. N., McNeil, T., Lucian, L. A., Janatpour, M. J., ...& Sedgwick, J. D. (2001). Membrane-bound TNF supports secondary lymphoid organ structure but is subservient to secreted TNF in driving autoimmune inflammation. *Immunity*, *15*(4), 533-543.
17. Flynn, J. L., Goldstein, M. M., Chan, J., Triebold, K. J., Pfeffer, K., Lowenstein, C. J., ... & Bloom, B. R. (1995). Tumor necrosis factor-α is required in the protective immune response against Mycobacterium tuberculosis in mice. *Immunity*, *2*(6), 561-572.
18. Garcia, I., Miyazaki, Y., Araki, K., Araki, M., Lucas, R., Grau, G. E., ...&Vassalli, P. (1995). Transgenic mice expressing high levels of soluble tnf-r1 fusion protein are protected from lethal septic shock and cerebral malaria, and are highly sensitive to Listeria monocytogenes and Leishmania major infections. *European journal of immunology*, *25*(8), 2401-2407.
19. Zantl, N., Uebe, A., Neumann, B., Wagner, H., Siewert, J. R., Holzmann, B., ...&Pfeffer, K. (1998). Essential role of gamma interferon in survival of colon ascendens stent peritonitis, a novel murine model of abdominal sepsis. *Infection and immunity*, *66*(5), 2300-2309.
20. Secher, T., Vasseur, V., Poisson, D. M., Mitchell, J. A., Cunha, F. Q., Alves-Filho, J. C., &Ryffel, B. (2009). Crucial role of TNF receptors 1 and 2 in the control of polymicrobial sepsis. *The Journal of Immunology*, *182*(12), 7855-7864.
21. Waterston, A. M., Salway, F., Andreakos, E., Butler, D. M., Feldmann, M., & Coombes, R. C. (2004). TNF autovaccination induces self anti-TNF antibodies and inhibits metastasis in a murine melanoma model. *British journal of cancer*, *90*(6), 1279-1284.
22. Maitra, U., Deng, H., Glaros, T., Baker, B., Capelluto, D. G., Li, Z., & Li, L. (2012). Molecular mechanisms responsible for the selective and low-grade induction of proinflammatory mediators in murine macrophages by lipopolysaccharide. *The Journal of Immunology*, *189*(2), 1014-1023.
23. Lowry, O., Rosebrough, N., Farr, A. L., & Randall, R. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, *193*(1), 265-275.
24. Ghasemi, A., Hedayati, M., &Biabani, H. (2007). Protein precipitation methods evaluated for determination of serum nitric oxide end products by the Griess assay. *Jmsr*, *2*(15), 29-32.
25. Tracey, K. J., Beutler, B., Lowry, S. F., Merryweather, J., Wolpe, S., Milsark, I. W., ...&Cerami, A. (1986). Shock and tissue injury induced by recombinant human cachectin. *Science*, *234*(4775), 470-474.
26. Arteaga Figueroa, L., Abarca-Vargas, R., García Alanis, C., &Petricevich, V. L. (2017). Comparison between peritoneal macrophage activation by Bougainvillea xbuttiana extract and LPS and/or interleukins. *BioMed Research International*, *2017*.
27. Itoh, K., Udagawa, N., Kobayashi, K., Suda, K., Li, X., Takami, M., ...& Takahashi, N. (2003). Lipopolysaccharide promotes the survival of osteoclasts via Toll-like receptor 4, but cytokine production of osteoclasts in response to lipopolysaccharide is different from that of macrophages. *The Journal of Immunology*, *170*(7), 3688-3695.
28. Zakharova, M., & Ziegler, H. K. (2005). Paradoxical anti-inflammatory actions of TNF-α: inhibition of IL-12 and IL-23 via TNF receptor 1 in macrophages and dendritic cells. *The Journal of Immunology*, *175*(8), 5024-5033.

29. Wang, L. X., Zhang, S. X., Wu, H. J., Rong, X. L., &Guo, J. (2019). M2b macrophage polarization and its roles in diseases. *Journal of leukocyte biology*, *106*(2), 345-358.
30. Tan, H. Y., Wang, N., Li, S., Hong, M., Wang, X., &Feng, Y. (2016). The reactive oxygen species in macrophage polarization: reflecting its dual role in progression and treatment of human diseases. *Oxidative medicine and cellular longevity*, *2016*.
31. Elizur, A., Adair-Kirk, T. L., Kelley, D. G., Griffin, G. L., Demello, D. E., & Senior, R. M. (2008). Tumor necrosis factor-α from macrophages enhances LPS-induced Clara cell expression of keratinocyte-derived chemokine. *American journal of respiratory cell and molecular biology*, *38*(1), 8-15.
32. Sedger, L. M., & McDermott, M. F. (2014). TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants–past, present and future. *Cytokine & growth factor reviews*, *25*(4), 453-472.
33. Fischer, R., Kontermann, R. E., & Maier, O. (2015). Targeting sTNF/TNFR1 signaling as a new therapeutic strategy. *Antibodies*, *4*(1), 48-70.
34. Gane, J. M., Stockley, R. A., &Sapey, E. (2016). TNF-α autocrine feedback loops in human monocytes: the pro-and anti-inflammatory roles of the TNF-α receptors support the concept of selective TNFR1 blockade in vivo. *Journal of immunology research*, *2016*.
35. Tamura, K., Noyama, T., Ishizawa, Y. H., Takamatsu, N., Shiba, T., & Ito, M. (2004). Xenopus death receptor-M1 and-M2, new members of the tumor necrosis factor receptor superfamily, trigger apoptotic signaling by differential mechanisms. *Journal of Biological Chemistry*, *279*(9), 7629-7635.
36. Kratochvill, F., Neale, G., Haverkamp, J. M., Van de Velde, L. A., Smith, A. M., Kawauchi, D., ...& Murray, P. J. (2015). TNF counterbalances the emergence of M2 tumor macrophages. *Cell reports*, *12*(11), 1902-1914.
37. Chang, Y. T. (1964). Long-term cultivation of mouse peritoneal macrophages. *Journal of the National Cancer Institute*, *32*(1), 19-35.
38. Guo, M., Xiao, J., Sheng, X., Zhang, X., Tie, Y., Wang, L., ...&Ji, X. (2018). Ginsenoside Rg3 mitigates atherosclerosis progression in diabetic apoE–/–mice by skewing macrophages to the M2 phenotype. *Frontiers in Pharmacology*, *9*, 464.
39. Ghasemi, A., Hedayati, M., &Biabani, H. (2007). Protein precipitation methods evaluated for determination of serum nitric oxide end products by the Griess assay. *Jmsr*, *2*(15), 29-32.
40. Nyberg, P., &Klockars, M. (1990). Measurement of reactive oxygen metabolites produced by human monocyte-derived macrophages exposed to mineral dusts. *International Journal of Experimental Pathology*, *71*(4), 537.
41. Sawoo, R., Dey, R., Ghosh, R., &Bishayi, B. (2021). TLR4 and TNFR1 blockade dampen M1 macrophage activation and shifts them towards an M2 phenotype. *Immunologic Research*, *69*, 334-351.