An Insight into the Host-Pathogen Interactions between Macrophage and Different Microbial Species: A Detailed Approach

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ABSTRACT

The macrophage phagolysosome possesses a highly acidic environment which explains its antimicrobial properties. Many strains of bacterial strains which are Gram-positive such as *Staphylococcus aureus* replicates within the niche and possesses the GraXRS regulatory system to counter the acidic pH within macrophages. On the other hand, *Mycobacterium tuberculosis* (Mtb) expresses Hsp 16.3 to counteract the inflammatory cytokine response, phagocytosis or pathogen escape by M1 or M2 macrophages respectively. In Gram-negative Bacterial cells such as *E. coli* mediate the SLAMF1-induced trafficking of TRAM in macrophages which is stimulated particularly against the bacterial Lipopolysaccharides (LPS).Virulent factors such MgtC and OprF are defensive mechanisms, used by *Pseudomonas aeruginosa* for survival at the acidic pH in macrophages. Interaction between SARS-CoV-2a strain of the coronavirus and macrophage results in cytokine secretion by macrophages. It gives rise to a cytokine storm which is combatted by spike protein S. A new variant of Coronavirus, i.e. omicron's binding affinity to Abs was found to be reduced and its structure elucidated using various computational tools. Future research on more such interactions would help to decipher the detailed molecular mechanisms of such variants and their role in targeted drug delivery

Keywords: Bacterial cell, Gram-positive, Gram-negative, Macrophage, Protein interactions, Viral cell, SARS-CoV-2, Omicron.

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INTRODUCTION

Macrophages are vital for innate immunity because of their ability to phagocytose bacterial cells. Two different types of bacterial strains, namely Gram positive and Gram negative may infect any particular site of body, which may include the macrophage microbicidal phagolysosome. One such example is *Staphylococcus aureus*, a Gram-positive bacterium which display enhanced virulence and able to colonize every body tissue.[1] Some virulence factors include toxins such as leucocidins which may poison macrophages and neutrophils. Macrophages are vital for providing host defense against infection by *S. aureus*. The presence of a regulatory system, i.e. GraXRS in the bacterial cell aids it to perceive phagolysosomal acidification and elicit adaptive responses. Such responses are able to resist in killing and replication.[1,2]

Gram negative bacterial cells interact with macrophages and may involve changes not only in the core genome but in the pan-genome too. An example is *E. coli* which is commensal but also responsible for causing several diseases such as urinary tract infection, gastroenteritis or neonatal meningitis. It may be evident that some pathogenic strains might have evolved from commensal

strains of E. coli but it is difficult to distinguish the specific genes which are particularly responsible for conferring pathogenicity to the bacterial strain.[3,4] It was observed that certain pathoadaptive mutations may be involved in such 'switches', even in naturally-occuring pathogens too. For instance, loss of mucA in *Pseudomonas aeruginosa* has an increased ability to evade phagocytosis, while the loss of oprD by P. aeruginosa along with an associated carbapenem-resistance phenotype leads to an increased cytotoxicity towards macrophages. Allelic variations in FimH, the type 1 adhesion of *E. coli* may change the ability of uropathogenic strains to colonise and invade the tissue of the bladder.[3,5] Macrophages recruit certain defensive mechanisms against multiple pathogens via recognition of specific pathogen-associated molecular patterns. For instance some Toll-like receptors such as TLR4 recognises Lipopolysaccharides (LPS) from Gram-negative bacteria in association with co-receptors such as myeloid differentiation factor 2 and CD14. Thereafter, they recruit signalling adapters myeloid differentiation primary response gene88 (MyD88) and MyD88 adapter-like (Mal) which leads to an immediate activation of nuclear factor κB (NF-κB) and the production of proinflammatory cytokines.[6] At the same time, the signalling adapter Toll Receptor-Associated Molecule (TRAM) is recruited to the particular site on phagosomes and macrophages where TLR4 is present.[6,7] TRAM is responsible for the correspondingrecruitment of Toll/Interleukin(IL)-1 Receptor(TIR) domain-containing adapter-inducing IFN-β(TRIF) or other downstream molecules, thus leading to IFNβ secretion.[8-10] An assembly of TLR4-TRAM-TRIF complex ,which isfollowed by activation of TANK-Binding Kinase 1(TBK1) results in the induction of type I IFNs. The latter is required for host resistance to group B streptococci, pneumococci, *E. coli*.[11]

The presence of twin proteins, PE and PPE proteins in M. tuberculosis and M. smegmatis belonging to two unique but related protein families, are associated with an increased virulence[12] Their role in host-pathogen interactions may be characterised by modulation of the inflammatory response via Nuclear Factor Kappa B (NF-κB) signalling through the RelB pathway.

Interactions of a SARS-CoV-2 viral protein with human macrophages initiated signals which lead to the production of pro-inflammatory cytokines such IL6, TNFα, IL8, CXCL5 etc.[12,48] Recent studies performed on SARS-CoV-2 viral genome revealed that SARS-CoV-2 signalling was mediated by IRAK4, where IRAK-M a negative regulator of IRAK4 signalling may be inhibited. Therefore macrophages were more prone

to increased responsiveness to TLR signals, which in turn lead to the development of cytokine storm observed in COVID patients. Protein interactions between the virus and macrophage proteins are not easy to be studied experimentally thus the use of various computational tools such as AlphaFold, RosTTAFold or HADDOCK may be used to determine the three dimensional structure of a new variant of the Coronavirus, Omicron[13,53] Unleashing the structures and interactions with antibodies would thus help to determine the detailed molecular mechanisms of such interactions or even more new rising variants.
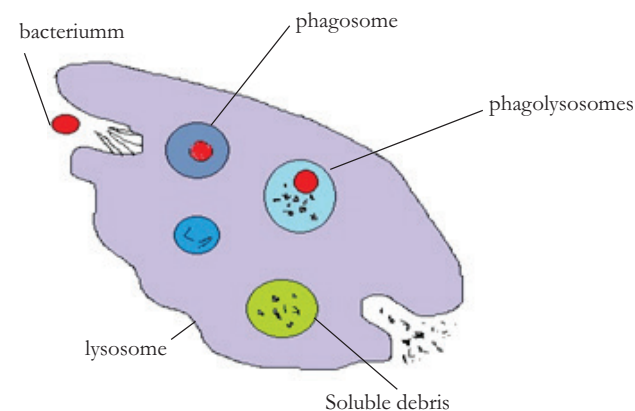

Figure 1: Macrophage- *S. aureus* Interactions Require GraXS Regulatory System

Staphylococcus aureus, a Gram-positive bacterium is a versatile pathogen and seemingly capable of infection and survival in any body niche. Thus it has a remarkable ability to distract the immune mechanisms of the host. In the extracellular environment, *Staphylococcus aureus* produces toxins that target and harm immune cells, particularly macrophages (Figure 1)[13] Studies utilizing pulse-chase experiments with fluorescein isothiocyanate-dextran have revealed that *S. aureus* is able to replicate within mature phagolysosomes[14,15] Further research has demonstrated the crucial role of the GraXS regulatory system in enabling S. aureus survival at acidic pH levels within macrophages..

GraXRS plays a crucial role in the growth of bacteria within the macrophage phagolysosome. This is due to the upregulation of genes, such as mprF, which enhance bacterial survival within the antimicrobial environment[12,13] Previous research has shown that *S. aureus* employs toxins, including Hla and PSMα peptides, to facilitate escape from the bacterium-containing phagosome.

Macrophages have their own defensive mechanism: the alkalinisation of macrophage lysosomes was found to impair the intracellular growth of wild-type *S. aureus* in wild-type macrophages.[16] The GraS sensor was found to possess a periplasmic loop and several critical

residues contained within it were essential for its signal transduction.

The regulation of several genes, including mprF, dltABCD and vraFG, is controlled by GraXRS in order to confer antimicrobial peptide resistance. Studies have shown that bacterial cells lacking GraS are unable to replicate within the macrophage phagolysosome. This sets *Staphylococcus aureus* apart from other bacterial cells such as *Listeria monocytogenes* and *Mycobacterium tuberculosis*, as these bacteria typically disrupt phagosome maturation in order to avoid fusion with the phagolysosome.[16-19]

S. aureus is distinct from other bacterial cells e.g. *Listeria monocytogenes* and *Mycobacterium tuberculosis* inits ability to reside within mature phagolysosomes despite their attempts to disrupt phagosome maturation and evade phagolysosome fusion.[20] Various studies about GraS response and role of acidic pH of macrophages as a means of invasion against the bacterial cell represent an important understanding at the molecular about such host-pathogen molecule interactions.

Macrophage-Mtb Interactions Leads To Hsp 16.3 Expression in Mtb

Mycobacterium tuberculosis, a common causative agent of Tuberculosis (TB), has the ability to survive within host macrophages and trigger them to adopt an M2 phenotype, contributing to the development of latent MTB infection. This intracellular pathogen is responsible for both acute and latent forms of TB. As a Gram-positive bacterium, it typically targets the lungs where alveolar macrophages ingest it and migrate to the pulmonary interstitial space. Here, they may attract other immune cells, including mononuclear-derived macrophages, to form tuberculous granulomas[21,22] Macrophages are generally divided into M1 and M2 by function. M1 macrophages has an increased phagocytosis, inflammatory cytokine secretion, antigen presentation abilities while M2 macrophages can promote wound repair, fibrosis, mediate the escape of tumours or pathogens and participate in Th2-type immune responses[23,24] Interactions with Mtb increase the expression of inflammatory cytokines such as IL-12, TNF-α, IL-6, inducible Nitric Oxide Synthase (iNOS), CD86 by polarized M1 macrophages and thus the ability to kill pathogens and promotion of Th-1 immune response. On the other hand, M2 macrophages can be divided into three subpopulations, M2a, M2b and M2c, respectively. M2a type expresses mannose receptor, scavenger receptor, arginase-1 (Arg-1), M2b macrophages increase IL-10 production and promote Th2 response, while M2c type responds to pentraxin 3

and chitinase 3 secretion as 3 and play a role in wound healing.[24,25]

Mtb too has developed several strategies to colonise macrophages and survive for longer periods of time through the secretion of various protein components and virulence factors.[26] Some of them include inhibition of phagosome-lysosome fusion and phagolysosomal maturation and induction of M2 macrophage polarisation.[27,28] Recent studies have investigated the role of Heat-shock proteins (sHsps) in MTB which contribute towards the development of tuberculosis. One of them, MTB heat-shock protein (Hsp 16.3). Is is a member of the α-crystal superfamily encoded by the HspX gene and called Acr1, antigen of 16kDa and Rv2031c. To survive in the macrophage, expression of the DosR gene is activated in macrophages, which in turn leads to increased expression of many bacterial proteins, where Hsp 16.3 dominates. A recent treatment study showed that MTB Hsp16.3 treatment increased the production of two specific chemokine receptors, CCRL2 and CX3CR1, in BMDMs. Although CCRL2 is critical for macrophage Th2 responses and M2 polarization, CX3CR1, the fractalkine receptor, is another important macrophage receptor. CX3CR1 expression even contributes to maintaining the balance of the inflammatory response in the intestine by producing the anti-inflammatory cytokine IL-10. At the same time, the involvement of the AKT/ERK/p38-MAPK signaling pathway played an important role in the expression of various inflammatory factors, phagocytosis and resistance to pathogenic bacteria.[29-34] Thus the role of protein interactions between the macrophage polarisation and MTB might help to explore more about Hsp 16.3 in MTB; an in-depth understanding regarding such interactions would help to improve tuberculosis control and prevention.

Macrophage-*E. coli* Interactions mediate the TLR4-TRIF-signalling in human macrophages.

E. coli is a genus of Gram-negative bacteria which is commonly found in human intestines. Though most strains are not pathogenic yet some are pathogenic and cause various gastrointestinal disorders. Thus Toll-Like Receptors (TLRs) are recruited by macrophages for defense against such multiple pathogens by recognising pathogen-associated molecular patterns[3,35] Lymphocyte activation molecule family 1 (SLAMF1) has been shown to be required for TLR-4-mediated IFNβ induction and even killing of such Gram-negative bacteria by human macrophages. SLAMF1 is responsible for controlling the transport of Toll Receptor-Associated Molecules (TRAM) from the Endocytic Recycling Compartment

(ERC) to *E. coli* phagosomes... Endogenous Immuno Precipitation experiments (IPs) using anti-SLAMF1 antibodies and anti-TRAM antibodies showed a physical association between them which further confirmed by Lipopolysaccharide Stimulation (LPS). Subsequent stimulation of E. coli SLAMF1 was observed to be transferred from ERCs to E. coli-containing phagosomes; endogenous SLAMF1 was already associated with TRAM before stimulation and thus both proteins were recruited to phagosomes by Rab11 GTPases.[36,37] The surface TLR4 interacts with the LPS on *E. coli* outer membrane and induces rapid intracellular complex formation and multiple posttranslational modifications of signalling molecules.[37] It was evident that SLAMF1 is a critical TRAM regulator, as its silencing resulted in a significant reduction in TRAM accumulation around *E. coli* phagosomes. The role of TBK1-IKKε-kinase and Akt-kinase was important, as their inactivation during SLAMF1 silencing was found to affect the antibacterial functions of TBK1-IKKε[38,39] TBK-IKKε kinase activates Akt kinase. The kinase has been implicated in the activation of NADPH oxidase by phosphorylation of p47 subunit which may result in the generation of Reactive Oxygen Species (ROS) that are required for bacterial killing[40] Meanwhile, Akt S473 phosphorylation was found to be TRAM- and SLAMF1-dependent; Akt phosphorylation is mainly induced by pure TLR2 and TLR4 receptors. Thus, SLAMF1-regulated transport of TRAM to the TLR4 signaling complex enhances IFNβ secretion. Targeting human SLAMF1 may be a potential target for the regulation of TLR4-mediated cytokine production, which is one of the effective methods for killing bacterial cells by the phagosome.

Interactions between *Pseudomonas aeruginosa* OprF and macrophages initiate resistance to macrophage clearance during an acute infection.

Pseudomonas aeruginosa was considered as an extracellular pathogen in addition to being a type of Gram-negative bacterium. It has been reported to be engulfed by macrophages of both cellular and animal models[41] Once it enters the host cells, an intracellular residence phase occurs, which can be important in addition to a classic extracellular infection. The importance of macrophages was highlighted using macrophage-depleted zebrafish embryos, which were found to be highly susceptible to *P. aeruginosa* infection, as an experimental vehicle.[42,43] The zebrafish embryo was used primarily because it offers powerful tools to address the macrophage-pathogen interactions. MgtC and OprF have been shown to be bacterial factors involved in macrophage survival. OprF is an important outer membrane porin involved in cell structure maintenance, environmental sensing, outer membrane permeability, adhesion, biofilm formation, virulence and is even responsible for the production of quorum sensing-dependent virulence factors such as pyocyanin, elastase, lectin PA-1L. and exotoxin A[42,44]

OprF plays an important role in the regulation of bacterial virulence factors. Use of the OprF mutant was found to show reduced expression of T3SS genes and secretion of ExoT and ExoS toxins.[45,46] Therefore the ability to escape from phagosomes may be associated with the downregulation of T3SS expression in macrophages, as both T3SS and ExoS have been found to play specific roles in bacterial phagosomal escape[47] Together with the test results of *P. aeruginosa* was found to use ExoS to avoid acidified compartments in epithelial cells; Whether ExoS prevents vacuolar acidification in epithelial cells or by directing bacteria to other cellular compartments has not yet been determined. Therefore, the discovery of the effect of OprF in the bacterial cell on macrophage clearance during acute infection, preventing its destruction in phagolysosomes, was important. Previous studies have also suggested that such an effect probably correlates with an intracellular effect of OprF on the expression of ExoS, a T3SS effector described for its role in phagosomal escape.[47,48]

The different modes of action utilised by different bacterial species to combat the action of the macrophage within a living system are highlighted in Table 1. Future research on such macrophage-bacterial protein interactions would help to unleash more details into the molecular level of such interaction studies.

Table 1: Different modes of action utilised by macrophages and bacterial/viral species for interaction.

Bacterial/Viral Species	Type	Macrophage Mode of Action	Bacterial Mode of Action
S. aureus	Gram positive	acidic pH	GraXS regulatory system.
Mycobacterium Tuberculosis (Mtb)	Gram positive	M1 type - enhanced-phagocytosis, cytokine secretion, Th1- immune response.	Hsp 16.3
E. coli	Gram negative	SLAMF1 regulated transport of TRAM, TLR4-TRIF signalling.	Bacterial LPS.

P. aeruginosa	Gram negative	Acidic pH.	MgtC, OprF which leads to ExoT, ExoS toxin production.
SARS-CoV-2	-	Cytokine, chemokine secretion such as IL-6,MIP1a, TNFα, TLR2/TLR4 signalling.	Spike protein(S) on viral surface displays resistance.

Interactions between SARS-CoV-2 and macrophages promotes pro-inflammatory cytokine production in Macrophages

Severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) which is a positive-sense single-stranded RNA virus is the main cause of the Coronavirus Disease 2019(COVID-19). The severity of COVID-19 is associated with an enhanced level of inflammatory mediators which include cytokines, chemokines and typically characterised by a cytokine release syndrome (CRS) (Figure 2)[48] When THP-1 macrophages were stimulated with SARS-CoV-2 spike protein (S) as a model to study these interactions, increased expression of IL-6, MIP1α and TNFα was observed. This suggested that the virus/ACE2 interaction initiated signals that induced macrophage activation. At the same time, PAM3csk4-stimulated THP-1 macrophages were found to express more IL-6 in the presence of SARS-CoV-2 S protein and showed reduced expression of the inactive IRAK kinase and the negative regulator IRAK-M of TLR signaling. Thus, it has been suggested that the virus modulates TLR signaling (TLR2, TLR4) and sensitizes macrophages to TLR ligands, leading to the hyperinflammatory state of COVID-19[49,50] Thus, a strong protein-protein interaction between TLR4 and SARS-CoV-2 S protein signifies that TLR signalling is responsible for the viral mediated lung injury and inflammation. IRAK-M expression may thus provide a potential biomarker that predicted macrophage response to cytokine storm development and infection. In a recent study, neural pathways such as the inflammatory reflex, indirect activation of the α7-nicotinic acetylcholine receptor (α7-nAChR) by acetylcholine produced by splenic T cells, inhibited the expression of certain proinflammatory cytokines, especially M2-type cytokines, on splenic macrophages. Thus, the direct interaction of the virus with macrophages may indicate the involvement of α7-nAChR in the pathogenesis of COVID-19, which may explain the dysfunction observed in the viral innate response.[51,52]

The affinity of Omicron's receptor-binding domain for neutralizing antibodies was found to be reduced compared to the reference RBD constructs. Although there were many mutations in the omicron RBD, the predicted structure of AlphaFold2 did not appear to cause large conformational changes that would completely prevent Ab production. The RoseTTAFold results revealed a conformational change in the Omicron RBD that can promote antibody turnover or significantly reduce antibody binding affinity. Predicting the actual structure of a protein is time-consuming and therefore protein-protein interaction (spike-Ab) is difficult to perform experimentally *in vitro*. More computational tools such as AlphaFold, RoseTTAFold and HADDOCK would help to infer the epidemiological effects of such variants.[53]
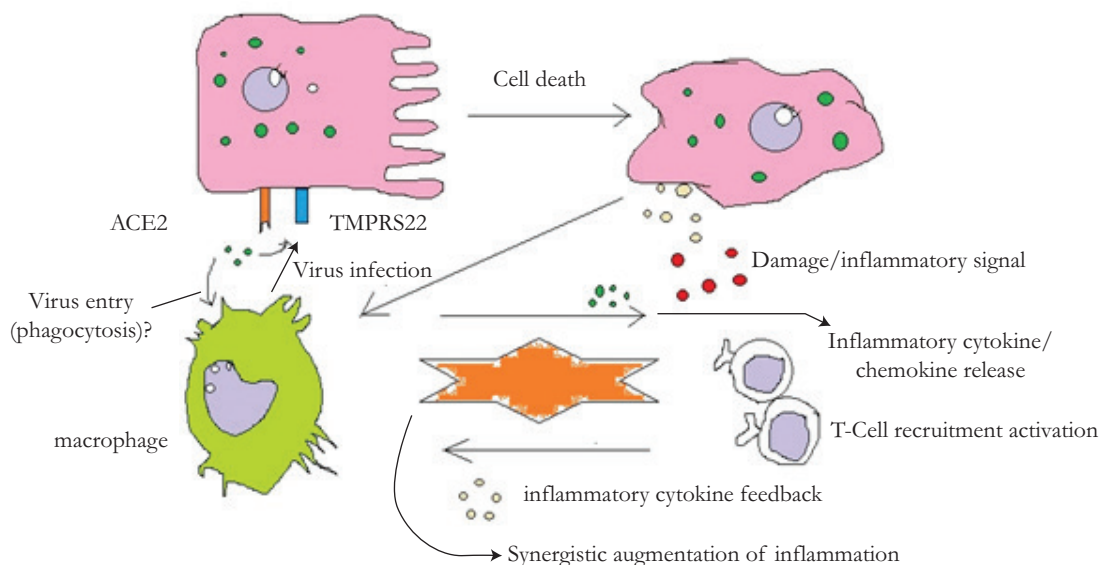

Figure 2: Interaction of SARS-CoV-2 ACE2 receptor with a macrophage recruits cytokine/chemokine production

CONCLUSION

The macrophage phagolysosome possesses an acidic pH and known for their ability to phagocytose bacterial cells. Two main strains of bacterial cells, Gram-positive and Gram negative bacteria have evolved various strategies to combat the mode of action by macrophages. For instance, *S. aureus*, a Gram-positive bacterium utilises the GraXS regulatory system to enable it for survival within the macrophage phagosome; whereas another species *Mycobacterium tuberculosis* expresses DosR against the various inflammatory cytokines such IL-6, IL-12, TNF-α secreted by macrophages which is responsible for upregulation of proteins such as Hsp 16.3. The AKT/ERK/p38-MAPK signalling pathway was found to be equally important for bacterial resistance. On the other hand, *E. coli* which is a Gram negative species possessed LPS which interacted with SLAMF1 expressed by macrophage, which in turn transported TRAM to TLR4 signalling complex and resulted in IFN production in macrophages, a mode of targeting and effective killing of the bacterial cell. The Presence of OprF and Mgt bacterial factors had been used as survival mechanisms by *Pseudomonas aeruginosa* which even uses ExoS to escape the acidic pH within the phagosome compartment.

Interaction of SARS-CoV-2 virus or its variants, including Omicron with a macrophage may lead to the production of either a cytokine storm in COVID patients or unleash insights into the interactions of Abs with the viral proteins respectively by revealing details regarding the structural implications of the viral variant. Future research on such host-pathogen interactions would help to unleash the underlying molecular mechanisms and help to develop targeted drugs or vaccines in the upcoming days.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Mtb: Mycobacterium; **LPS:** Lipopolysaccharides; **TLR:** Toll-like receptor; **TNF:** Tumour Necrosis Factor; IL**:** Interleukin; **IFN:** Interferon; **SARS-CoV-2:** Severe acute respiratory syndrome coronavirus 2; **NF-αB:** Nuclear factor αB; **MIP:** Macrophage Inflammatory Protein; **SLAMF1:** Lymphocyte activation molecule family 1; **α7-nAChR:** α7-nicotinic acetylcholine receptor; **Mal:** MyD88 adapter-like Summary; **TRAM:** Toll receptor-associated molecules; **iNOS:** Nitric oxide synthase; **BMDM:** Bone marrow-derived macrophages; **MAPK:** Mitogen-activated protein kinase; **ERK:** Extracellular signal-regulated kinase; **RBD:** Receptor binding Domain; **TMPRSS2:** Transmembrane protease serine 2; **IRAK:** Interleukin-1 receptor-associated kinase; **ACE:** Angiotensin-converting enzyme; **TRIF:** Toll/interleukin(IL)-1 receptor(TIR) domain-containing adapter-inducing IFN-β; **TBK1:** TANK-binding kinase 1; **IKK:** Inhibitor of nuclear factor- κB kinase; **ERC:** Endocytic recycling compartment; **Arg-1:** Arginase 1; **Hsp:** Heat Shock Protein.

SUMMARY

- The macrophage phagolysosome possesses a highly acidic environment which has antimicrobial property.
- Many strains of bacterial strains which are Gram-positive such as Staphylococcus aureus possess the GraXRS regulatory system to counter the acidic pH within macrophages.
- Mycobacterium tuberculosis (Mtb) expresses Hsp 16.3 to counteract the inflammatory cytokine response, phagocytosis or pathogen escape by M1 or M2 macrophages respectively.
- Gram-negative Bacterial cells such as E. coli mediate the SLAMF1-induced trafficking of TRAM in macrophages stimulated particularly against the bacterial Lipopolysaccharides(LPS).
- Virulent factors such MgtC and OprF are defensive mechanisms, used by Pseudomonas aeruginosa for survival at the acidic pH in macrophages.
- Interaction between SARS-CoV-2a strain of the coronavirus and macrophage results in cytokine secretion by macrophages.It gives rise to a cytokine storm which is combatted by spike protein S. A new variant of Coronavirus, i.e. omicron's binding affinity to Abs was found to be reduced and its structure elucidated using various computational tools.

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