

Leprosy and Immune System: An Insight into the Innate Immune System

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Innate immunity is a host immune mechanism to defend itself promptly. It includes physical and chemical barriers, cells in the circulation and tissues, several plasma proteins, and immune cells constituting phagocytic cells (monocytes/macrophages and neutrophils), dendritic cells, natural killer (NK) cells, blood proteins and cytokines. Leprosy is a chronic granulomatous disease caused by *Mycobacterium leprae*. Innate immune response in leprosy includes the role of pattern recognition receptor (PRR) in recognizing *Mycobacterium leprae* pathogen-associated molecular patterns (PAMPs), such as the Toll-like receptor (TLR)1, TLR2 and TLR6, nucleotide-binding oligomerization domain 2 (NOD2), cytokine release, macrophage and dendritic cells differentiation, and antimicrobial effector pathway. Recognition of microbial pathogen is followed by phagocytosis. In addition to phagocytosis, macrophages act as scavenger element to remove extracellular material such as oxidized lipid, vital for host lipid metabolism. Anti-microbial activity induced by vitamin D may also contribute to the disease outcome. The innate immune system's ability to instruct adaptive T cell response, mediated by dendritic cells, is part of the effective host defence in combating intracellular pathogens including leprosy. Additionally, host genetics and nutritional status still account for a substantial amount of disease susceptibility in leprosy requiring further studies to understand leprosy, specifically the immune system, comprehensively.

Keywords : Innate Immunity, *Mycobacterium leprae*, Toll-like Receptor 2, Vitamin D

Introduction

Host defence mechanisms comprise of two arms, innate (natural, pre-existing) immunity and adaptive (acquired) immunity. The former is a non-specific, weak, and ineffective immune mechanism directly protecting the host (Modlin et al 2012). However, it is an initial defence capable of controlling and eradicating infection before the

more potent adaptive immunity gets stimulated (Abbas et al 2020). The innate immune system consists of i) physical barrier, ii) cells in the circulation and tissue, iii) blood proteins, and iv) cytokines, with each component having a specific role in mounting immunity (Modlin et al 2012, Abbas et al 2020).

Leprosy is caused by *Mycobacterium leprae*

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(*M. leprae*), an acid-resistant bacillus that grows in temperatures below the human body temperature (37°C). It makes the predilection lesion in the cooler sites of the body (James et al 2011, Bryceson & Pfaltzgraff 1990). In 1971, researchers in the United States of America reported that armadillos reproduce neurocutaneous and systemic forms of leprosy when inoculated with *M. leprae* (Storrs 1971). The clinical manifestation of leprosy presents a spectrum with varying degrees of immunological response (Lockwood 2008).

The pattern recognition receptors (PRRs), such as the Toll-like receptors (TLRs) 2 and nucleotide-binding oligomerization domain-containing protein (NOD2), act as receptors for the ligand lipoproteins within the cell wall, one of the immunogenic proteins. Therefore, recognition of this protein by the PRRs initiates the host response to *M. leprae* (Lee et al 2012). One of the early responses after the recognition of immunogenic proteins is phagocytosis by phagocytes. These cells produce cytokines, such as interleukins (IL), tumour necrosis factor (TNF), transforming growth factor (TGF) and others (Wood 2006). These cytokines trigger inflammation and activate the NK cells, which trigger the DCs and macrophages to produce signals for adaptive immune responses (Abbas et al 2020). The other role of cytokines is to regulate cellular proliferation, differentiation, function, and the migration of white blood cells (WBCs) (Wood 2006).

This review article aims to provide a comprehensive understanding of protection conferred by the innate immune system, including the role played by the TLRs and other recognition receptors, phagocytosis and antimicrobial activity against *M. leprae*.

Mycobacterium leprae

Mycobacterium leprae, the causative organism of leprosy (also called Hansen's disease or Morbus

Hansen), is an obligate intracellular, non-motile, non-spore-forming, slow-growing (generation time 12-14 days), Gram-positive non-cultivable bacterium, meaning that it can live only in cells, specifically the Schwann cells and macrophages. The Genus *Mycobacterium*, contains mycolic acid and a glycolipid compound called mycoside. Mycolic acid has a role in acid-resistant staining when this particular bacillus is stained with carbol fuchsin (Bryceson et al 1990, Silva & De Castro 2008, Gautam et al 2021). Humans are the natural host, while armadillos are the reservoirs for *M. leprae* infection in humans, posing a potential endemic focus (Murray et al 2016). The main affected locations are the peripheral nerves, nose, ear lobe, bones, and viscera (testes and liver) (Bryceson et al 1990, Silva & De Castro 2008). The infection is transmitted through prolonged contact with lepromatous leprosy patients harbouring large amounts of *M. leprae* in their nasal secretions and skin lesions (Murray et al 2016, Sekar 2017).

Microscopically, *M. leprae* has a length of 1-8 µm and a diameter of 0.3 µm. The cell wall is a covalently linked peptidoglycan-arabinogalactan-mycolic acid complex, similar in composition to all mycobacterial cell walls. The cell wall consists of two layers; the inner leaflet, forming a pseudo-lipid bilayer, is composed of mycolic acids linked to terminals of arabinan chains. The bacterium outer leaflet consists of a variety of intercalating mycolic acids, namely i) trehalose monomycolates (TMM), ii) mycoseroic acids of phthiocerol dimycocerosate (PDIMs), and iii) phenolic glycolipids (PGLs), which consists of three methylated glucose molecules linked via phenol molecules to fat (phthiocerol) forming the electron-transparent zone. The bacterium is encased in a capsule comprised mainly of PGLs and other compounds, including PDIMs, phosphatidylinositol mannosides, and phospholipids. The

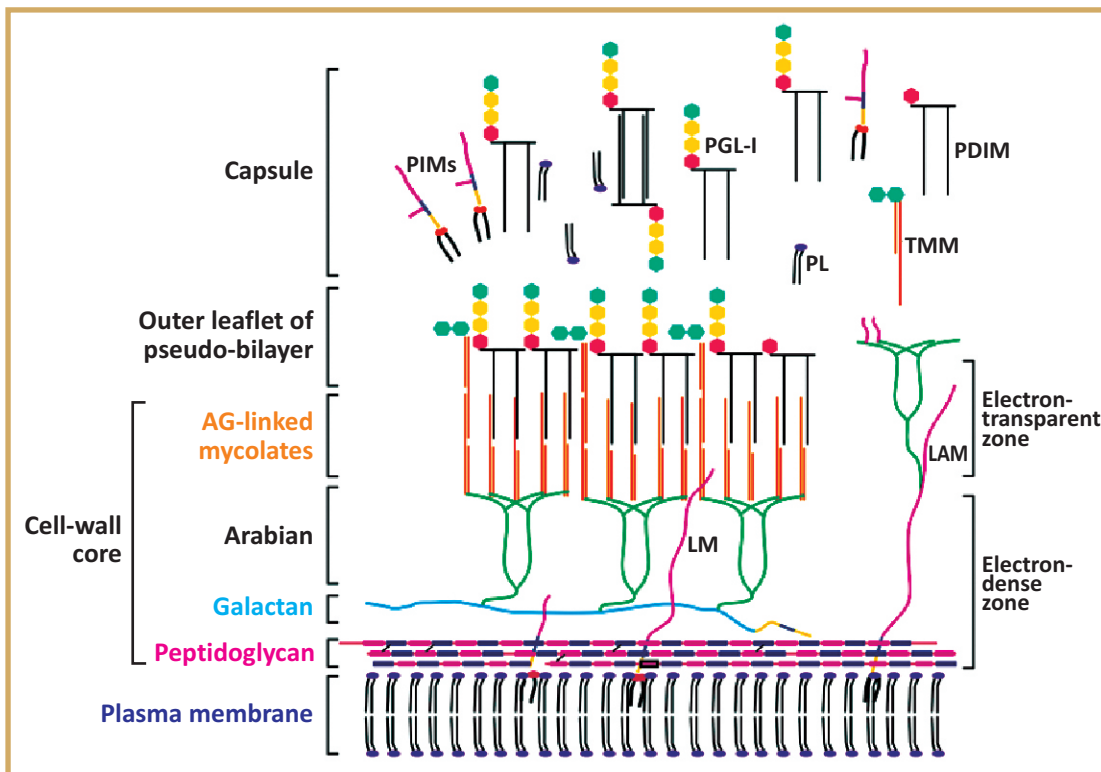


Fig. 1 : Schematic model of the cell envelope of *M. leprae*. PIMs: phosphatidylinositol mannosides; PGL-1: phenolic glycolipid-1; PDIM: phthiocerol dimycoerolates; PL: phospholipids; TMM: trehalose mono-mucolates; LAM: lipoarabinomannan; LM: lipomannan (Sekar 2017)

bacterial membrane of *M. leprae* comprises of lipids and proteins and facilitates the transport of molecules in and out of the bacteria. Biochemical fractionation studies have identified two major polypeptides – major membrane protein-I (MMP-I) and major membrane protein-II (MMP-II) associated with the cell membrane of *M. leprae* (Fig. 1) (Sekar 2017).

Innate Immunity in Leprosy

The term 'immunity' comes from the Latin word *immunitas*, which means protection from prosecution during the Roman senator's tenure. Historically, it refers to protection against disease, more specifically, protection against infectious

disease. Cells and molecules that have a role in immunity form the immune system, and the response that occurs from the recognition of foreign bodies is called the immune response (Silva & De Castro 2008). The term innate immunity refers to a residential pre-existing mechanism or immediate defence to prevent infection from pathogens (Wood 2006, Abbas et al 2012).

Innate immunity serves as a first-line response to prevent, control, or eliminate the microbial infection in the host. Another function of this immune system is recognizing and eliminating damaged or dead cell products and triggering an

adaptive immune response (Silva & De Castro 2008, Abbas et al 2020). In 1884, Metchnikoff explained that the innate immune system works in rapid detection of germs, phagocytosis, and antimicrobial activity (Modlin et al 2012, Modlin 2010).

Components of the Innate Immune System

The innate immune system comprises of cellular and biochemical mechanisms that act prior to the infection, rapidly respond to infection, and react to microbes and their products. The essential components of the innate immune system are i) physical and chemical barriers, such as the epithelium and antimicrobial chemicals, which are produced on the surface of the epithelium, ii) phagocytes (neutrophils and monocytes/macrophages), DCs, and NK cells, iii) blood proteins (i.e., member of the complement system and other inflammatory mediators, and iv) cytokines that regulate and control the innate immune cells. Several components of the innate immune system recognize foreign bodies by detecting certain carbohydrates or lipids on the microorganism's surface other than that of human cells. In addition, the innate immune system component has unique receptors called pattern recognition receptors or PRRs, which recognize the molecular structure on the surface of microbes. This strategy is utilized to differentiate self from non-self (Abbas et al 2020).

When microbes successfully pass through the physical barrier or the epithelium, the microbes face cells from the innate immune system. Cellular innate immune response to microbes occurs in a process called inflammation which leads to the recruitment of WBCs and plasma proteins from the blood leading to the accumulation and, subsequently, their activation to eliminate the microbes. These reactions involve cytokines produced by DCs, macrophages, and other cell types during the innate immune reactions.

The most commonly recruited WBCs during inflammation are phagocytes, neutrophils, and monocytes.

The phagocytes express receptors on their cell surface, which recognize, bind, and engulf different microbes and activate the cells. Phagocytes produce reactive oxygen species (ROS) and lysosomal enzymes that destroy the microbes during receptor binding. Microbes that can escape the tissues' defence mechanism enter the blood, further recognizing the innate immune circulating proteins. The alternative pathway component, namely the complement system, is considered a critical plasma protein in innate immunity. When the microbial surface activates this pathway, the proteolytic cleavage products are generated, which mediate the inflammatory response, encapsulating the microbes for subsequent phagocytosis and directly lysing the microbes (Abbas et al 2012, Abbas et al 2020).

Recognition of *Mycobacterium leprae*

Mycobacterium leprae can be recognized and eliminated by the innate immune system through receptors on the phagocytes (Modlin et al 2012). In leprosy, receptors to complement fragments of CR1, CR3, and CR4 aid in phagocytosis (Sekar 2017). Mycobacterial cell walls contain several antigens that are involved in the immune response. One of the potent immunoglobulins (Ig) triggers specific to *M. leprae* is phenolic glycolipid-1 (PGL-1), yielding potent IgM response and is recognized by complement 3 (Bhat & Prakash 2012, Sekar 2017, Salgado et al 2019).

PGL-1 has a selective affinity towards the $\alpha 2$ LG G-domain module of the $\alpha 2$ laminin 2 chain, a basal lamina component in Schwann cells. It is highly specific due to the trisaccharide units (Gautam et al 2021). Laminin $\alpha 2$, which is present only in the peripheral nerves, explains this specific neurotropism of *M. leprae*. The uptake

of *M. leprae* by Schwann cells depends on α -dystroglycan (DG), namely the laminin receptor present on the cell membrane and other intracellular components that has a role in the process of early nerve degeneration and interiorization (Bhat & Prakash 2012, Pinheiro et al 2011, Schenk et al 2012, Nath & Chaduvula 2017).

Lipoarabinomannan (LAM) is another main component present on the cell wall of *M. leprae* that cross-reacts with other mycobacteria. Together with its non-arabinosylated precursor (lipomannan [LM]), these lipoglycans serve as essential constituents of all mycobacteria, modulating innate and adaptive immune responses (Angala et al 2020). This antigen induces IgG antibodies and can inhibit the activation of interferon (IFN)- γ in macrophages. In addition, some of the proteins involved in cell wall synthesis, identified as *M. leprae* antigens, can also trigger cellular immunity due to the potency against T-helper (Th)1 (Byceson et al 1990, Bhat & Prakash 2012).

The innate immune system is equipped with germline encoding the PRRs to recognize pathogen-associated molecular patterns (PAMPs) (Abbas et al 2020). This idea was brought in 1989 by the late Charles Janeway, who proposed the presence of cellular receptors that may sense pathogens and deliver “danger” signals to cells (Vogel 2012). PAMPs are specific to pathogens and are not expressed by the host. Due to this nature, the innate immune system can differentiate between self and non-self and send the required signal to the adaptive immune system (Modlin et al 2012). The PRRs are expressed on phagocytes, DCs, and many other cell types and are in diverse cellular compartments based on where microbes or their products may be found (Abbas et al 2020).

Toll-like receptors

Toll-like receptors (TLRs) are a family of receptors

that can recognize various types of PAMPs. This family of receptors has a structural relationship with *Toll*, a protein product that plays a role in innate immunity and the dorsoventral development of fruit flies, *Drosophila* (Gandhi & Ravindra 2021, Modlin et al 2012, Abbas et al 2012). In 1997, Medzhitov et al (1997) described the first human homolog of the *Drosophila* toll receptor, which is now known as TLR4. Further advancement by Bruce Beutler led to the finding of TLR4 as a critical component of the mammalian lipopolysaccharide (LPS) receptor complex. At least a range of eleven TLRs has been identified in humans and other species (Steensma et al 2014, Abbas et al 2020). It can be inferred that TLR1, TLR2, and TLR6 are involved in interaction with peptidoglycan, a lipopeptide found in *M. leprae*, recognition by the innate immune system (Wood 2006, Abbas et al 2020).

Toll-like receptors are transmembrane proteins characterized by an extracellular leucine-rich repeat domain and a cytoplasmic domain containing a conserved region called the Toll/IL1 receptor (TIR) domain and the transmembrane domain (Vu et al 2017). Plasma membrane TLRs are specific for bacterial cell wall components, and endosomal TLRs recognize nucleic acids. When activated by ligand exposure, the intracellular domain of the TLR may trigger a MyD88-dependent pathway that ultimately leads to the nuclear translocation of the transcription factor NF- κ B. NF- κ B modulates the expression of many immune response genes, including the genes for various cytokines and chemokines. Upon activation, TLRs are also involved in the phagocytosis of pathogens by host cells, maturation of host phagosomes, and direct antimicrobial pathways that promote the release of non-specific antibacterial molecules, such as antimicrobial peptides (AMPs) (Gandhi & Ravindra 2021, McInturff et al 2005).

Toll-like receptor 2/1 (TLR2/1)

Toll-like receptor 2/1 is a heterodimer cell surface receptor that detects mycobacterial lipoproteins and requires a triacyl group to be active (Modlin et al 2012, Silva & De Castro 2008). The distribution of TLR2/1 in leprosy lesions is associated with pathogen resistance (Krutzik & Modlin 2004). The activation of TLR2/1 heterodimer will induce the differentiation of monocytes to macrophages and DCs (Lockwood 2008). It also triggers cytokine production, such as TNF- α and IL-12 (Silva & De Castro 2008, Montoya & Modlin 2010). Cytokines are classified into two groups, i) type 1 cytokines, including IFN- γ , IL-12, and IL-18, which increase the response of TLR 2/1 heterodimer, whereas IFN- γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) can increase the TLR1 expression in monocytes, and ii) type 2 cytokines, including IL-4 and IL-10, exerting an inhibitory effect on TLR2/1 activation. IL-10 does not decrease TLR2 or TLR1 expression, contrary to IL-4, which inhibits TLR2/1 activation and TLR2 expression (Montoya & Modlin 2010). Increased expression of TLR2 and TLR1 are found in the lesions of tuberculoid leprosy as compared to the disseminated lepromatous form (Silva & De Castro 2008, Montoya & Modlin 2010).

Other findings in the field of gene polymorphism of TLR 2/1 have shown a role in the pathogenesis of leprosy. TLR2 single nucleotide polymorphisms (SNPs) have been associated with the susceptibility to lepromatous leprosy. Several lines of data have shown that TLR1 genetic polymorphism might play a role in the decreased TLR2/1 response towards lipopeptides and leprosy pathogenesis. Johnson et al (2007) have suggested that TLR response is vital during acute infection; however, moderation of the innate response may benefit chronic infection, such as leprosy. Consistent with these other TLR 2/1 activation findings, it can also lead to tissue damage,

including nerve damage in leprosy (Modlin 2010, Montoya & Modlin 2010).

The ability of the innate immune response to bridge over to the adaptive immune response is initiated and modulated by DCs expressing the leukocyte Ig-like receptor A2 (LILRA2). Activation of LILRA by DCs inhibition will switch the surface phenotype of monocytes and its cytokines secretion profile and prevent the immune system's ability to trigger adaptive T cell response (Lee et al 2007). The LILRA2-expressing cells identified in lepromatous leprosy lesions belong to a monocyte/macrophage lineage and co-express CD209, which is pivotal in mediating the uptake of mycobacteria by macrophages (de Lima Fonseca et al 2017). Activated LILRA2, which is more common in lepromatous leprosy form, inhibits TLR 2/1 induced IL-12 yet releases IL-10 as well as suppression of TLR 2/1-induced antimicrobial activity (Modlin 2010, Röltgen et al 2020).

Other TLRs and PRRs

Mycobacterium leprae has also been observed to be recognized by the TLR2/6 heterodimers and it induced the formation of foamy macrophages or lipid droplets (LD). LD originates from the endoplasmic-Golgi reticulum complex, and the ability of LD formation depends on motor and microtubule proteins (Mattos et al 2011). It is considered an essential source of nutrition for pathogens and plays a pivotal role in the infection and resistance against antibiotics (Mattos et al 2011, de Macedo et al 2020). The TLR2 and TLR6 pathways become active during LD biogenesis after being triggered by *M. leprae* infection in macrophages (de Macedo et al 2020). A study by Mattos and coworkers has shown that the accumulation of oxidized lipids could suppress innate immune responses beneficial for mycobacteria growth in the host (Mattos et al 2011, White 2008). Vital markers for lipid accumulation in adipocytes or macrophages are adipose diff-

erentiation-related protein (ADRP) and perilipin (de Macedo et al 2020). ADRP is one of the main proteins on the LD surface that influences LD formation. After phagocytosis of live *M. leprae*, ADRP expression increases in human monocytes (Mattos et al 2011, de Macedo et al 2020).

A study by Mattos et al have reported a new cellular heterodimer receptor, identified as the TLR4/6 (Mattos et al 2011). Recent investigations have revealed that TLR4 polymorphism is associated with susceptibility to leprosy and demonstrated that *M. leprae* decreased TLR4-mediated IL-1 α and IL-6 production in monocytes (de Macedo et al 2020). TLR9 has a role in recognizing bacterial CpG DNA, contributing to a role in response to mycobacteria. Polymorphisms in Toll-IL1 receptor domain-containing adaptor protein 1 (TIRAP), the downstream signaling molecules of TLR, TIRAP S180L are associated with protection against leprosy infection (Modlin 2010).

Nucleotide-binding oligomerization domain (NOD) - like receptor (NLR) also plays a role in the innate immune response to mycobacterial infections (Schenk et al 2012). NLR functioned to identify the pathogen in the cytoplasm, for example, NOD-2 recognizes muramyl dipeptide (MDP), a part of the peptidoglycan mycobacterial cell wall. NLR is a type of PRRs, together with an adapter protein and an effector enzyme (caspase), which formed the inflammasomes (Silva et al 2018). Inflammasomes are multi-protein complexes that assemble in the cytosol of cells in response to microbes or changes associated with DAMPs (Abbas et al 2020). Depending on the type of the caspase involved, the inflammasomes can be categorized into two types of signaling pathways: classical (or canonical), which activates caspase-1, and noncanonical, which involves other caspases to provoke inflammation. Caspase-1, a critical inflammatory caspase, plays a role in innate immunity through two mech-

anisms, a) activation of pro-inflammatory cytokines (pro-IL-1 β to active IL-1 β and pro-IL-18 to active IL-18) and b) induce a type of cell death called pyroptosis (Silva et al 2018). A study conducted by Mendes et al (2020) has reported that low expression of caspase-1, IL-1 β , and IL-18 is found both in tuberculoid as well as lepromatous leprosy.

Monocytes/macrophages

The two primary types of phagocytes are monocytes/macrophages and neutrophils. The monocytes/macrophages are further categorized in leprosy skin lesions into M1 type and M2 type. In tuberculoid leprosy granulomas, epithelioid macrophages with the M1 phenotype (CD68+CD163-) predominate, whereas macrophages in lepromatous leprosy granulomas are foamy and mostly have the M2 phenotype (CD68+CD163+) (Mi et al 2020). The monocytes/macrophages are an integral part of the innate immune response, i.e., phagocytosis, and the major host of leprosy pathogens. Phagocytosis is the process of ingestion and destruction of the pathogen by phagocytes (Abbas et al 2012).

Phagocytosis of *M. leprae* by macrophages originated from monocytes and can be mediated by complement receptor CR1 (CD35), CR3 (CD11b/CD18), CR4 (CD11c/CD18), and regulated by protein kinase (Bhat & Prakash 2012, Pinheiro et al 2011). After the ingestion of pathogens via phagocytosis, the TLR-mediated internalization by macrophages plays an important role in resolving intracellular bacterial infection. The TLR stimulation subsequently causes NF- κ B activation, which leads to an increase in inducible nitric oxide synthase (iNOS) and NO production and secretion of pro-inflammatory cytokines (Mak & Saunders 2006).

Cytokines of the innate immune response are known to regulate the action of macrophages, i.e., tuberculoid leprosy lesion expressed IL-15

whereas lepromatous leprosy lesions expressed IL-10 (Modlin 2010, Pinheiro et al 2011). Both IL-10 and IL-15 increase the expression of CD209 in monocytes. IL-10 induces a phagocytic pathway in the form of a programmed scavenger receptor resulting in mycobacterium phagocytosis and oxidized lipids. On the contrary, IL-15 reduces phagocytosis but induces an antimicrobial vitamin D dependent pathway (Montoya et al 2009).

A study by Sinsimer et al (2010) has shown that *M. leprae* plays an active role in controlling the release of cytokines from monocytes by providing positive and negative regulatory signals through multiple signaling pathways, including NF- κ B, phosphatidylinositol-3-kinase (PI3K), and IL-1 β -converting enzyme, ICE (caspase-1). The production of pro-inflammatory cytokines on monocytes, namely IL-1 β , IL-18, IL-6, IL-10, IL-12, and TNF- α in response to stimulation of *M. leprae* was identified to be very low or absent. These investigators also reported that *M. leprae* induced high levels of negative regulatory molecules IL-1 receptor antagonist (IL-1Ra) and monocyte chemoattractant protein-1 (MCP-1) and that this induction involved the PI3K signaling pathway. In addition, the production of IL-6 was suppressed by *M. leprae* via a PI3K-dependent mechanism, while the delay in the activation of caspase-1 together with the decreased activation of NF- κ B appeared to contribute to the low levels of IL-1 β and IL-18 produced by *M. leprae* (Sinsimer et al 2010, Mattos et al 2011).

Neutrophils

Neutrophils are the most abundant polymorphonuclear (PMN) cells in circulation. These cells were traditionally characterized by their phagocytic ability, the release of lytic enzymes from their granules, and the production of reactive oxygen intermediates with an anti-microbial propensity (Schmitz et al 2019). Gomes et al

(2020) reported that a high number of circulating neutrophils were found in patients with leprosy reaction, either type 1 or type 2. This was further supported in study findings of erythema nodosum leprosum (ENL) pathogenesis in which patients' neutrophils are thought to contribute an important role in the occurrence of ENL (Darmaputra et al 2018). In addition, a notable cellular dysfunction and elevated antigen-antibody immune complexes levels were evident and these were associated with increased levels of pro-inflammatory cytokines, specifically predominant neutrophilic inflammatory infiltrate. Nonetheless, pathogenetic mechanisms to explain this phenomenon requires further studies (Gomes et al 2020).

Immune evasion mechanism by Mycobacteria

Mycobacteria are able to evade the host immune response through a variety of strategies. First, phagocytes engulf *M. leprae* via the aid receptors to complement fragments of CR1, CR3, and CR4; this is beneficial for the mycobacteria because they can evade triggering the oxidative burst and further protect themselves from exposure to damaging oxygen radicals. Second, after the ingestion, the mycobacteria inhibit macrophage activation by LAM, which will further inhibit the release of IFN- γ and TNF- α . Other defense strategies undertaken by mycobacteria include the inhibition of phagolysosome formation, invasion of the cytoplasm of macrophages, and hiding in Schwann cells (Chapel et al 1999).

Numerous studies have shown that macrophage surface receptors, type C lectin receptors, and mannose receptors are involved in the uptake of *M. leprae*. The type C lectin receptors recognize the specific structure of carbohydrates found in the mycobacterial cell wall components. Dendritic cell signaling ICAM3 grabbing non-integrin (DC-SIGN)/CD209 and mannose receptors bind to mannose-capped lipoarabino-

mannan (ManLAM) on mycobacteria cell walls. CR3 can facilitate phagocytosis of mycobacteria through complement opsonins or phagocytosis through lectins that require cholesterol (Montoya & Modlin 2010). Cholesterol in *M. leprae* mediates the recruitment of tryptophan aspartate-containing coat protein (TACO) from the plasma membrane to the phagosomes. TACO or coronin-1A (CORO1A), is a sheath protein that prevents phagosome-lysosome fusion and subsequently causes the degradation of mycobacteria on lysosomes (Montoya & Modlin 2010, Elamin et al 2012). The ability of *M. leprae* to increase TACO, which is expressed in macrophages containing *M. leprae in vitro*, reduces TLR2 mediated signaling (Modlin 2010).

Antimicrobial activity

The antimicrobial mechanism that occurs after phagocytosis plays an important role in killing pathogens and induction of vitamin D-dependent AMPs in the form of cathelicidin. Mycobacterial infection of macrophages also causes the induction and accumulation of oxidized lipids, as seen in lepromatous leprosy. The ability of mycobacteria to form LD depends on TLR2 signaling and PPAR activation γ (Pinheiro et al 2011).

Innate immunity and vitamin D

Vitamin D, a dietary supplement, has shown its share in the effects on Bacillus Calmette-Guérin vaccination, prostaglandins, vascular endothelial growth factor, reactive oxygen species, reactive nitrogen intermediates, matrix metalloproteinases, antiphospholipid syndrome, nerve growth factor, and a potent AMPs inducer (Luong & Nguyễn 2012, Bergman et al 2020). This critical antimicrobial mechanism in TLR-activated human monocytes is the role of vitamin D and its receptor, specifically the induction of 25-hydroxyvitamin D3-1 α -hydroxylase (CYP27B1), which converts 25-dihydroxy vitamin-D3 into the active

form of 1, 25-dihydroxy vitamin-D3 (1, 25-D3). The latter interacts with vitamin D receptors (VDRs) to influence macrophage capability in killing the pathogen by increasing expression of AMP cathelicidin (Modlin 2010, Sinsimer et al 2010, Rusyati et al 2019). However, in leprosy, *M. leprae* inhibits VDR activity through down-regulation of CYP27B1 (Darus et al 2019). Additionally, a previous study has demonstrated that vitamin D deficiency has a strong negative correlation with the rise in pro-inflammatory cytokines, such as TNF- α . The high levels of TNF- α have been implicated in the direct damage of myelin sheath, stimulation of bone reabsorption, and inhibition of bone collagen synthesis (Mandal et al 2015).

In leprosy, the vitamin D AMP may play an important role in the disease outcome based on several factors: i) the tendency for antimicrobial pathway gene expression in tuberculoid versus lepromatous leprosy, ii) correlation of SNP VDR in lepromatous leprosy subjects, and iii) reports of successful use of vitamin D as adjuvant therapy for leprosy (Sinsimer et al 2010, Modlin 2010). In addition, several studies have recommended the use of supplemental vitamin D for mediating the immune system (Salgado et al 2019, Rusyati et al 2019, Darus et al 2019).

Dendritic cells in leprosy

Dendritic cells are myeloid derivatives of hematopoietic cells and are derived from precursors, which differentiate into monocytes but not granulocytes. Similar to these cells, the DCs express receptors that recognize pathogenic molecules and respond to pathogens by cytokine secretion. The majority of DCs are referred to as conventional DCs. In response to pathogen activation, conventional DCs on the skin, mucosa, and parenchyma of organs becomes mobile, migrate to lymph nodes, and present pathogenic antigens to T cells. Therefore, these cells act in an

innate and adaptive immune response (Abbas et al 2020).

As professional antigen-presenting cells, DCs, mainly mediate this instructive role in the innate immune system, which are highly efficient in activating the T cell response against pathogens (Modlin et al 2012, Modlin 2010). Activation of TLRs in monocytes induces GM-CSF and GM-CSFR that trigger differentiation into immature DCs, releasing cytokines and presenting antigens to T cells (Modlin et al 2012). Langerhans cells (LCs), resident DCs, are in the epidermis expressing CD1a and CD207 (Langerin). Various studies have indicated that the number of LCs in the epidermis of tuberculoid leprosy patients is significantly larger than those observed in patients with lepromatous leprosy (de Lima Fonseca et al 2017, Azadeh & Dabiri 2004). These findings are consistent with the concept of cell-mediated immune response in the lesions of tuberculoid leprosy, while the humoral immune response in lepromatous leprosy (Salgado et al 2019).

Further, lepromatous leprosy lesions are characterized by a significant deficit of DCs both in the epidermis as well as dermis layers (plasmacytoid DCs [pDCs] and dermal dendrocytes [DDs]). This is a potential mechanism for the observed reduced cell-mediated immune response in these lesions. DCs differentiation from myeloid precursors can be inhibited through inhibitory receptors via CD209 (Bokhary & Phung 2016). Peripheral monocytes from lepromatous leprosy patients do not differentiate to CD1+ DCs after TLR activation. This study shows that *M. leprae* has a role in decreased cell-mediated immune responses by DCs differentiation and antigen presentation ability impairment (Modlin 2010).

An individual's susceptibility towards a clinical disease outcome can be traced to different variables, such as the environmental factors, divergence in virulence of a pathogen, and the

complex interplay between host and pathogens. A recent study by Prakoeswa et al (2020) reported a significant correlation between environmental factors, such as physical environment of the house, clean water facilities, availability of latrines, waste disposal facilities and personal hygiene and female in Gresik Regency (Prakoeswa et al 2020). The competence of an immune system affects a person's susceptibility, in this case, towards *M. leprae*. In specific, the immune system is influenced by genetics and nutritional status. Genetic variants in the class-II HLA-DR-DQ locus have been consistently associated with protection against leprosy have been identified (Cambri & Mira 2018). In Vietnamese and Brazilian cohorts, however, alleles in the PARK2 and PACRG regions of chromosome 6 were linked to susceptibility towards leprosy (Mira et al 2004, Jin et al 2018). Notably, decrease in serum levels of substances with antioxidant potential (such as retinol [vitamin A], tocopherol [vitamin E], ascorbic acid [vitamin C], zinc, magnesium, and selenium) have been observed in different forms of leprosy, mainly in lepromatous leprosy (Jyothi et al 2008). These findings suggest the need to further deepen the knowledge of the immune system on a genetic basis through the advances in genome sequencing technology for further advancement in infectious diseases, specifically leprosy.

Conclusions

The innate immune system is the first line of defence mechanism to pathogens like *M. leprae*. This immune system consists of physical barrier components, solvent factors, and cells (e.g., macrophages, monocytes), and AMPs. The innate immune system in *M. leprae* recognizes *M. leprae* antigens, such as PGL1 or LAM by TLR1, TLR2, and TLR6, either homodimers or heterodimers, followed by phagocytosis involving macrophages and monocytes as well as antimicrobial activity.

Antimicrobial activity in leprosy is associated with LD formation and vitamin D, both contributing to disease outcomes. DCs play a role in providing instructions. Adaptive immunity is activated subsequently after DCs on innate immune response signal the host body defences' adaptive immune response. Evasion of immune response by the leprosy bacillus is a complex synergy between environmental factors, divergence in pathogen virulence, and complex interplay between the host and pathogens, particularly the immune system dictated by genetic and nutritional factors. Further genomic studies, specifically focused on the immune system, will further enhance the understanding of leprosy and aid in the eradication of leprosy.

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