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Review

Mycobacterium tuberculosis and myeloid-derived suppressor cells: Insights into caveolin rich lipid rafts

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ABSTRACT

Mycobacterium tuberculosis (*M.tb*) is likely the most successful human pathogen, capable of evading protective host immune responses and driving metabolic changes to support its own survival and growth. Ineffective innate and adaptive immune responses inhibit effective clearance of the bacteria from the human host, resulting in the progression to active TB disease. Many regulatory mechanisms exist to prevent immunopathology, however, chronic infections result in the overproduction of regulatory myeloid cells, like myeloid-derived suppressor cells (MDSC), which actively suppress protective host T lymphocyte responses among other immunosuppressive mechanisms. The mechanisms of *M.tb* internalization by MDSC and the involvement of host-derived lipid acquisition, have not been fully elucidated. Targeted research aimed at investigating MDSC impact on phagocytic control of *M.tb*, would be advantageous to our collective anti-TB arsenal. In this review we propose a mechanism by which *M.tb* may be internalized by MDSC and survive via the manipulation of host-derived lipid sources.

1. Introduction

Mycobacterium tuberculosis (*M.tb*) is capable of evading protective host immune responses using various mechanisms, making it one of the most successful human pathogens [1]. Such mechanisms include prevention of phagolysosome fusion for bacterial degradation, preferential entering of innate immune cells via endocytic pathways to bypass traditional phagocytic pathways, and exploiting other host immune features to promote its own survival and persistence through the action of multiple immune regulatory cell types [2,3]. Regulatory immune cells, such as regulatory T (T_{Reg}) cells, are considered crucial elements for the prevention of immunopathology during chronic infections or inflammatory responses, but are not the only regulatory immune cell playing an important role during Tuberculosis (TB) [4,5]. More recently, a population of myeloid regulatory cells (MRC) known as myeloid-derived suppressor cells (MDSC), have been brought to the fore and act as an important innate immune checkpoint with inhibitory, and often times detrimental, effects on the host immune response [6,7]. This population is composed predominantly of monocytic and granulocytic (i.e. neutrophilic) lineage subsets [6,8] and more recently also cells of eosinophilic lineage [9]. All MDSC subsets are characterized by an immature state particularly in cancer, and more recently in TB. However, reports also exist of MDSC generated from differentiated monocytes [7,10–12]. In this review, we address the role of MDSC in TB, while evaluating the importance of MDSC lipid metabolism in supporting bacilli persistence, including commentary on an alternative internalization pathway which may act as an additional evasion mechanism for *M.tb*.

2. Myeloid cells and the innate immune response to *Mycobacterium tuberculosis*

The immune response to mycobacterial infection is initiated when aerosolized *M.tb* bacilli enter the lungs and encounter the first responders of the innate immune response, namely resident alveolar macrophages (AM), neutrophils, epithelia and conventional dendritic cell subsets (cDCs), which initiate recruitment of various other innate cells, such as blood monocytes [13]. For the purposes of this review, we shall focus on resident alveolar macrophages. During a successful host innate immune response, recruited monocytes differentiate into inflammatory macrophages and monocyte-derived DCs (MoDCs) which recognize bacteria by their pathogen associated molecular patterns (PAMPs), and phagocytosis ensues [14]. Infected macrophages

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Table 1
The cytokines and chemokines involved in the activation, maturation, recruitment and effector functions of myeloid-derived suppressor cells.

	Induction		Recruitment	Effector function	Intracellular Signaling factors
MDSC-ASSOCIATED CYTOKINES AND CHEMOKINES	• LPS • M-CSF • GM-CSF • SCF • IL-6 • VEGF • IL-13 • PGE_2	• IL-10 • S100A8/9 • COX-2 • TNF-α • IL-1β • IFN-γ • IL-6Rα	• CCL2 • CCL5	• NO • ROS • IL-10 • IDO • CO • PGE2 • TGF-β • Arginase 1	• STAT3 • COX-2 • IDO • HO-1 • HIF-1α • iNOS • Arginase 1 • AKT • mTOR • IRF-1

Abbreviations: LPS: Lipopolysaccharide; M-CSF: macrophage-colony stimulating factor; GM-CSF: granulocyte macrophage-colony stimulating factor; SCF: stem cell factor; VEGF: vascular endothelial growth factor; PGE2: prostaglandin E2; COX-2: cyclooxygenase 2; CCL: CC chemokine ligand; NO: nitric oxide; ROS: reactive oxygen species; TGF: transforming growth factor; IDO: indoleamine 2,3-dioxygenase; CO: carbon monoxide; HIF: hypoxia inducible factor; STAT: signal transducer and activator of transcription; iNOS: inducible nitric oxide synthase; HO-1: heme oxygenase 1; AKT: protein kinase B; mTOR: mammalian target of rapamycin; IRF-1: interferon regulatory factor 1.

then migrate to the lung interstitium [13], while cDCs and MoDCs migrate to the draining lymph node and present internalized bacterial peptides on major histocompatibility complex (MHC) class II to antigen-specific T helper 1 (T_H1) lymphocytes, thereby initiating the adaptive immune response. Activated T_H1 $CD4^+$ lymphocytes then migrate to the site of infection in the lungs, via a chemokine gradient, and produce IFN-γ to activate infected alveolar macrophages to carry out their bactericidal activities. These responses are known to clear *M.tb* infection successfully, however many confounding factors influence this process so that the bacteria may survive [14].

Should *M.tb*-specific immune responses fail to contain *M.tb* infection, a chronic inflammatory state is established and results in previously protective responses driving tissue damage. In order to counteract this, a complex repertoire of regulatory mechanisms exist to drive the return to homeostasis. Adaptive regulatory systems are well characterized and understood, however innate regulatory mechanisms are only recently coming to the forefront, with the discovery of MDSC that have strong suppressive characteristics, are present at the site of TB disease [15,16] and potentially even after vaccination with dead *M.tb* in mice [17]. As described by Dorhoi and Du Plessis, MRC constitute a heterogeneous population of myeloid cells consisting of regulatory DCs, tumor-associated macrophages (TAMs), regulatory and alternatively activated macrophages (M2-phenotype) and MDSC, whose expansion have specifically been observed during chronic inflammatory conditions including cancer and chronic infectious diseases [15].

As mentioned above, MDSC are described as a heterogeneous, myeloid cell population with immunosuppressive characteristics and defined subtypes, namely monocytic MDSC [M-MDSC) which resemble conventional monocyte phenotypes, polymorphonuclear MDSC (PMN-MDSC) which resemble conventional neutrophil phenotypes but do not display segmented nuclei (segmented nuclei have been shown to be indicative of mature, non-suppressive neutrophil phenotypes), eosinophilic MDSC (Eo-MDSC) resembling eosinophils, and several stages of progenitors and precursors which can be termed "early" MDSC. These cells are extensively studied in the cancer environment, in both human and mouse models [9,18–21]. While the term "polymorphonuclear" may not be entirely suitable for the description of neutrophil-resembling MDSC owing to it suggesting a segmented nucleus, for the purposes of this review we shall adhere to current nomenclature recommendations as suggested by Bronte et al. [8]. Having not been found to exist in healthy hosts at frequencies detectable through normal techniques, MDSC have been shown to expand during cancer and chronic inflammation and infection [7,18,19,22]. Under steady state conditions immature myeloid cell (IMC) precursors differentiate into mature, non-suppressive cell types (such as monocytes, neutrophils, eosinophils, basophils, dendritic cells, natural killer cells and mast cells) [23]. But, under conditions of chronic inflammation and the presence of certain growth factors, emergency myelopoiesis occurs. IMCs generated under these conditions do not fully mature, and can be activated by certain cytokines and chemokines, to a suppressive phenotype (Table 1) [7,10,23,24]. These MDSC accumulate and contribute to local and systemic immunosuppression.

MDSC function to suppress innate and adaptive immune responses mediated by NK cells, as well as CD4 and CD8 T lymphocytes, in both antigen-specific and non-specific manners, preventing adequate immune responses [7,25–29]. This is problematic when TB disease control is critically dependent on such responses [30]. Mechanisms of MDSC-mediated immune suppression are generally implied from cancer models, since this was the first pathological illness in which these cells were characterized. Most mechanisms may indeed be translated to infectious diseases like TB and include:

1. The production of nitric oxide (NO) via upregulation of inducible nitric oxide synthase (iNOS) which has been demonstrated in both human and murine TB models [22,31–34].
2. The above has been shown to result in the nitrosylation of the T cell receptor (TCR) and ROS production, leading to loss of TCR zeta (ζ) chain, which has been demonstrated in human TB models [7,23], and the killing of DCs in the spleen [17].
3. Inhibition of T cell activation and proliferation via the upregulation of arginase 1 (Arg1) resulting in depletion of arginine, previously demonstrated in human TB disease and murine TB models [7,35,36].
4. Induction of $Foxp3^+$ regulatory T lymphocytes, reducing T_H1 responses critical for *M.tb* infection control, as demonstrated in human TB disease [22,23].
5. Induction of T lymphocyte apoptosis through the upregulation of PD-1 and CTLA-4, as demonstrated in human in vitro TB models [37].
6. Impairment of T cell migration by reducing CD62L expression, as demonstrated in human TB disease [7].

While immune suppression is favoured, in moderation, in order to prevent immunopathology and tissue damage [18], the action of MDSC is excessive and beyond the point of limiting immunopathology. Most importantly, the strong suppression of T_H1 responses by MDSC, and their reduction of innate cytokines, is hugely detrimental towards *M.tb* infection control.

3. The metabolic profiles of mdsc and persistent *M.tb* support *M.tb* growth and survival within the human lung

3.1. MDSC metabolic profile

Myeloid cell subsets have been found to undergo metabolic shifts, under various conditions, from a state of glycolytic energy metabolism to lipid metabolism, allowing for various functional activities, differentiation and increasing hematopoietic activity [38,39]. MDSC have been found to be no exception, with clear metabolic shifts from glycolysis to β-oxidation being observed with subsequent upregulation of lipid-related marker expression (CD36 and Msr1), as well as enzymes linked to fatty acid oxidation, such as lysosomal acid lipase (LAL) and adipose triglyceride lipase [40,41].

In addition to MDSC functioning as immunosuppressive cells, it is hypothesised that these cells may be using their metabolic shift to support the survival and growth of *M.tb*. Studies in the field of cancer have demonstrated that immunosuppressive MDSC favor fatty acid oxidative phosphorylation, and that inhibitory agents are able to successfully block this pathway, thereby inhibiting the immunosuppressive activity of MDSC in vitro [41]. As an example, PMN-MDSC have been found to express lectin-type oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) which acts as a marker of endoplasmic reticulum stress in immunosuppressive PMN-MDSC [42]. In parallel to the metabolic changes and increased lipid accumulation observed, immunosuppressive mechanisms were also induced, including the Arg1 and iNOS pathways and T cell suppressive activities [43]. The central role-player in MDSC lipid metabolism is peroxisome proliferator-activated receptor gamma (PPARγ). PPARs are a family of transcription factors whose role is largely linked to fatty acid metabolism, and demonstrate the link between lipid metabolism and immunity which involves inflammation as the key modulator in the catabolism and anabolism of lipids [44]. PPARγ is primarily controlled by LAL, and, in the context of TB, is upregulated through toll-like receptor (TLR)−2 and TLR-4, which leads to a cascade of events resulting in lipid droplet formation [45–47]. Upregulated expression of CD36, characterised as an LDL receptor, is also a consequence of PPARγ activity which is partly responsible for lipid uptake and intracellular accumulation in *Mtb* [48,49]. In the context of MDSC, PPARγ signaling has been shown to restrict ROS production in the PMN-MDSC subtype, which inhibits cell proliferation [38,50]. Together, this evidence illustrates the ability of MDSC to adapt their metabolic programs to meet their survival and functional demands under varying micro-environmental conditions.

Tumor-associated growth factors such as G-CSF and GM-CSF, signal via STAT3 and STAT5, upregulate lipid transport receptors in MDSC, encouraging lipid accumulation in the tumor environment, well known to be enriched with lipids [43]. Likewise, the TB granuloma environment is also enriched with lipids because of the abundance of lipid-rich foamy macrophages. *M.tb* has been demonstrated to dysregulate host lipid synthesis and metabolism, hijacking the host's cellular metabolic processes to satisfy the nutritional and structural requirements of the *M.tb* bacillus [51–53]. Foamy macrophages are derived from normal macrophage populations and induced as a result of a metabolic shift that leads to an imbalance in LDL influx and efflux [54]. The principal components of these LDL molecules are phospholipids, triacylglycerides (TAG) and cholesterol which normal macrophages are capable of endocytosing via the scavenger receptor, CD36, found on their surface [52,54]. Internalised LDL molecules, therefore, accumulate within macrophages, inducing their differentiation into that of foamy macrophages [55]. The abundance of foamy macrophages present in the TB lung granuloma, has led to the hypothesis that these lipid-laden cells are central to bacillus survival by acting as a nutrient-rich reservoir [56].

3.2. M.tb *metabolic profile*

Lipid droplet-derived cholesterol, cholesteryl esters and TAGs from foamy macrophages have been found within lipid debris of *M.tb* bacilli. Several studies have found, through imaging and metabolic analyses, that *M.tb* favours phagocytosis by foamy macrophages, where the bacterium may undergo metabolic and phenotypic alterations to enhance uptake and use of exogenously derived fatty acids and cholesterol molecules [56–58]. It is thus believed that in TB, foamy macrophages provide an immune-modulatory and bacilli-sheltering niche, which favours disease progression. Murine TB models have illustrated increased PGE_2 and COX-2 production as a result of macrophage-associated lipid bodies, which are known inducers of MDSC expansion and mediators of intracellular signaling pathways associated with MDSC functions [59–61], and contribute towards the suppression of T_H1 responses, TNF production and NO upregulation; all of which are crucial for *M.tb* control and contribute to *M.tb* persistence once suppressed, possibly as a result of MDSC functions [62].

Besides acting as an energy source, fatty acids are also associated with *M.tb* cell wall development, where they act as biosynthetic precursors during resuscitation from a dormant state [57,63,64]. The mycolic acid components of the mycobacterial cell wall are produced via a process of elongation of fatty acids (either produced *de novo,* or via salvaged fatty acids) by fatty acid synthase II complex (FASII) [64]. Fatty acids are also essential for constructing *M.tb* virulence factors, including polyketide lipids phthiocerol-dimycoceroic acid (PDIM), polyacyltrehaloses (PATS) and sulfolipid (SL). In cholesterol metabolism, cholesterol molecules are used as precursors for producing acetyl-CoA, propionyl-CoA and pyruvate. These molecules are used as building materials for PDIM, PATS and SL, as well as acting as intermediates for adenosine triphosphate (ATP) synthesis [65,66].

When carbon sources are limited, *M.tb* upregulates the expression of isocitrate lyase 1 (ICL1), which is involved in the glyoxylate shunt and is required for lipid metabolism and survival [52]. The glyoxylate shunt is described as being an alternative anabolic pathway to the tricarboxylic acid cycle and is essential for acetate and fatty acid metabolism in most bacterial species. This, in turn, assists in regulating oxidative stress, challenge by antibiotics, host infection and overall pathogenesis [67]. In 2000, McKinney and colleagues reported evidence that *M.tb* persistence and virulence in mice is facilitated to a large degree, by ICL1, illustrating the link between infection and expression of ICL1 by bacilli [68].

Another environmental change which induces changes in *M.tb* metabolism is hypoxia. Hypoxia is a major characteristic of the tumor microenvironment and is a strong inducer of MDSC along with its counterpart, Hypoxia-Inducible Factor 1α (HIF-1α). MDSC in particular have been demonstrated to acquire the ability to suppress T cell proliferation and IFN-γ production in response to non-specific stimuli with anti-CD3/CD28 antibodies in a hypoxic culture environment, accompanied by the upregulated expression of *arg1* and *inos* [69]. The biological marker for hypoxia, HIF-1α, is activated under hypoxic conditions and has been demonstrated to promote the immunosuppressive properties of MDSC as well as the recruitment of T_{Reg} cells to tumor sites [70]. Approximately a third of the gene expression repertoire in *M.tb* is altered upon encountering a change in oxygen levels, thus resulting in metabolic changes to accommodate hypoxic microenvironments. Adaptations to hypoxia have been linked to the upregulation of regulators of lipid metabolism, which in turn, affects bacillus physiology through changing redox states and the availability of intermediate metabolism molecules [65,71].

At various stages and sites of infection (be it intracellularly, extracellularly, or at different regions and tissues within the host), the needs of *M.tb* survival are met by variable availabilities of carbon sources. This is supported by the ability of bacilli to catabolize carbohydrates, lipids and amino acids, which is possibly attributed to the heterogeneity of bacilli at various stages and sites of infection [65].

These findings indicate that, not only is *M.tb* directly dependent on host lipid sources, but also that host immune proficiency is affected by lipid availability.

4. MDSC lipid metabolism and host control of *M.tb* infection

Accumulation of MDSC had initially only been described in an *Mycobacterium bovis* Bacille-Calmette-Guerín (BCG) mouse model [33], until Du Plessis et al. confirmed the presence and accumulation of MDSC at high frequencies within pleural effusion fluid, as well as in the peripheral circulation in human TB disease and recent *M.tb* infection [7]. From this study, the inhibition of T cell proliferation and dampened T cell priming was observed, as well as an increased inability to kill mycobacteria, which highlighted the first evidence towards a functional role for MDSC in human TB disease [7]. To build on this work, a study performed by Knaul and colleagues observed that functionally suppressive MDSC internalize *M.tb* and promote a pro-inflammatory milieu that encourages haematopoiesis, MDSC production and recruitment to the lung [35]. Therefore, in a similar fashion to *M.tb* internalization in foamy macrophages, *M.tb* may persist within the lipid-rich environment of MDSC and harness their lipid content as an energy pool for itself [43,72]. It is, therefore, suggested that the high lipid content within MDSC acts as a primary carbon source for *M.tb* survival within the lung and granulomas of TB patients and may be providing a niche for *M.tb* persistence throughout infection and disease [52,56].

5. Caveosomes present a potential secondary avenue for bacterial immune evasion

Although poor antigen presenters, MDSC possess the ability to internalize *M.tb*, and concurrently suppress hostile T_H1 cell responses [18]. The mechanism by which MDSC are capable of achieving this are undefined, but we propose that the mechanism of *M.tb* uptake may be linked to the lipid profile reported for tumor-derived MDSC.

The plasma membrane of human cells is a complex structure characterized by its lipid bilayer which contains conserved, cholesterol-enriched microdomains known as lipid rafts [73]. Lipid rafts are 5–50 nm regions of the membrane with distinct compositions that are more ordered than other regions, making them less fluid. Lipid rafts also appear to act as platforms to colocalize proteins involved in intracellular signaling pathways and are rich in cholesterol, glycosphingolipids, glycosyl phosphatidyl inositol (GPI)-anchored proteins, sphingomyelin, as well as innate immune receptors found in neighbouring regions of the rafts [74]. Known to be involved in crucial cellular functions such as cholesterol transport, chemotaxis, and cell signaling, these structures are also well recognized for their role in extracellular pathogen endocytosis [73,75]. In addition to this, lipid rafts are involved in receptor-dependent and -independent uptake, as pattern recognition receptors (PRRs) have been found located within these lipid-rich regions.

As a mechanism for mycobacterial internalization, bacilli are capable of using lipid rafts to invade and survive within both phagocytic and non-phagocytic cells, such as epithelial cells, mast cells, macrophages, dendritic cells, fibroblasts, type II pneumocytes, and endothelial cells [73,75,76]. *M.tb* is capable of being internalized through multiple mechanisms, other than traditional phagocytosis, through the binding of multiple receptor molecules such as complement receptors (CR), mannose receptors, and Fc receptors, to name a few, which allow the bacilli to bypass lysosomal degradation [72,76,77]. In addition to this, *M.tb* is capable of interacting with plasma membrane steroid cholesterol, whereby the aggregation of lipid rafts is induced [73,78]. An example of one such receptor is CR3, a known receptor for *M.tb* which is implicated in the uptake of bacilli in a lipid raft-dependent manner utilizing the GPI-anchored proteins found at the site of lipid rafts [78,79]. Importantly, the CR3 receptor, also known as CD11b, is one of the few key surface receptors identified as a phenotypic marker for MDSC, albeit not unique to this cell subset [8,80]. Cholesterol disruption has been shown to redistribute the accumulation of CR3 receptors, normally found surrounding lipid rafts, to non-lipid raft regions so that the formation of the CR3/GPI-anchored proteins/cholesterol complex is inhibited, thereby inhibiting *M.tb* uptake into the cell [79], supporting our hypothesized involvement of CR3. The activation of lipid raft aggregation results in the increase of the local cholesterol concentration and the spontaneous curvature of the membrane to the point where surface bound bacteria is internalized [78]. It has been shown that mycobacterial infection induces cholesterol accumulation at the site of bacterial internalization on the plasma membrane, as well as around the internalized, intracellular mycobacteria [73], and evidence supporting the role of cholesterol in persistence is compelling, as various studies have reported its depletion severely disrupting lipid raft formation and aggregation via lipid raft disruption agents [75].

In conjunction with the cholesterol enriched domains, scaffolding domains known as caveolin proteins bind cholesterol in the lipid rafts, and provide a structural backbone for the formation of structures known as caveolae [75,81,82]. These structures are cup-shaped invaginations of the plasma membrane, well known for their highly cholesterol- and sphingolipid-enriched centres, that perform various homeostatic functions within a cell including, endothelial transcytosis, maintenance of lipid and glucose homeostasis, cellular signaling, endocytosis, viral and bacterial cell entry, as well as tumor development [81–84]. The composition of caveolae strongly mimic that of lipid rafts and their formation is dependent on the phosphorylation of caveolin-1 (cav-1) proteins – hypothesized to be dependent on the activation of lipid raft aggregation following the binding of *M.tb* to lipid raft cholesterol molecules during TB [79].

Well characterized in the cancer tumor microenvironment as well as liver disease, caveosomes have been suggested to harbor tumor-suppressor or tumor-promoter properties depending on the location of the cancer, as well as the cancer type and tissue of interest [81,85]. In infectious diseases, caveosomes have been found to harbor pathogens like the SV40 virus [86], cholera toxin [87], *E. coli* [88], HIV-1 [89,90] and *Plasmodium falciparum* [91], to name a few, with many displaying evidence of the successful prevention of lysosomal compartment fusion. Of particular interest, mycobacteria have also been found to be internalized and reside within these caveosome structures in an attempt to avoid the lytic activity of lysosomal enzymes and the activity of oxygen and nitrogen reactive species, as observed by Muñoz et al. in mast cells [73,92,93]. The above-mentioned findings suggest that these structures may play a role in serving as a reservoir for *M.tb* and, most importantly, masking internalized *M.tb* from the host innate immune response, providing a successful mechanism for immune evasion (Fig. 1).

6. Lipid metabolism in *M.tb* facilitates its internalization, survival and persistence in caveosomes

Caveosomes are capable of internalizing pathogens which are 500 nm in size, and are themselves only 60–80 nm in diameter as observed by scanning electron microscopy [94,95]. Since an *M.tb* bacillus can range from 200–500 nm in diameter, these may be feasible structures for the internalization of *M.tb* as they would be large enough to internalize a bacillus or encapsulate parts thereof. The sequence of events leading to successful internalization of bacteria, currently agree on the hypothesis that (1) receptors housed within regions of lipid rafts bind to bacterial surface receptors, (2) caveolin, and the accessory proteins known as cavins, then bind to the intracellular face of the lipid raft via cholesterol binding, (3) spontaneous curvature of the plasma membrane occurs [78], (4) dynamin activation at the neck of the invagination causes budding off into the cytosol [82,83], (5) free caveolar structures then fuse to the early

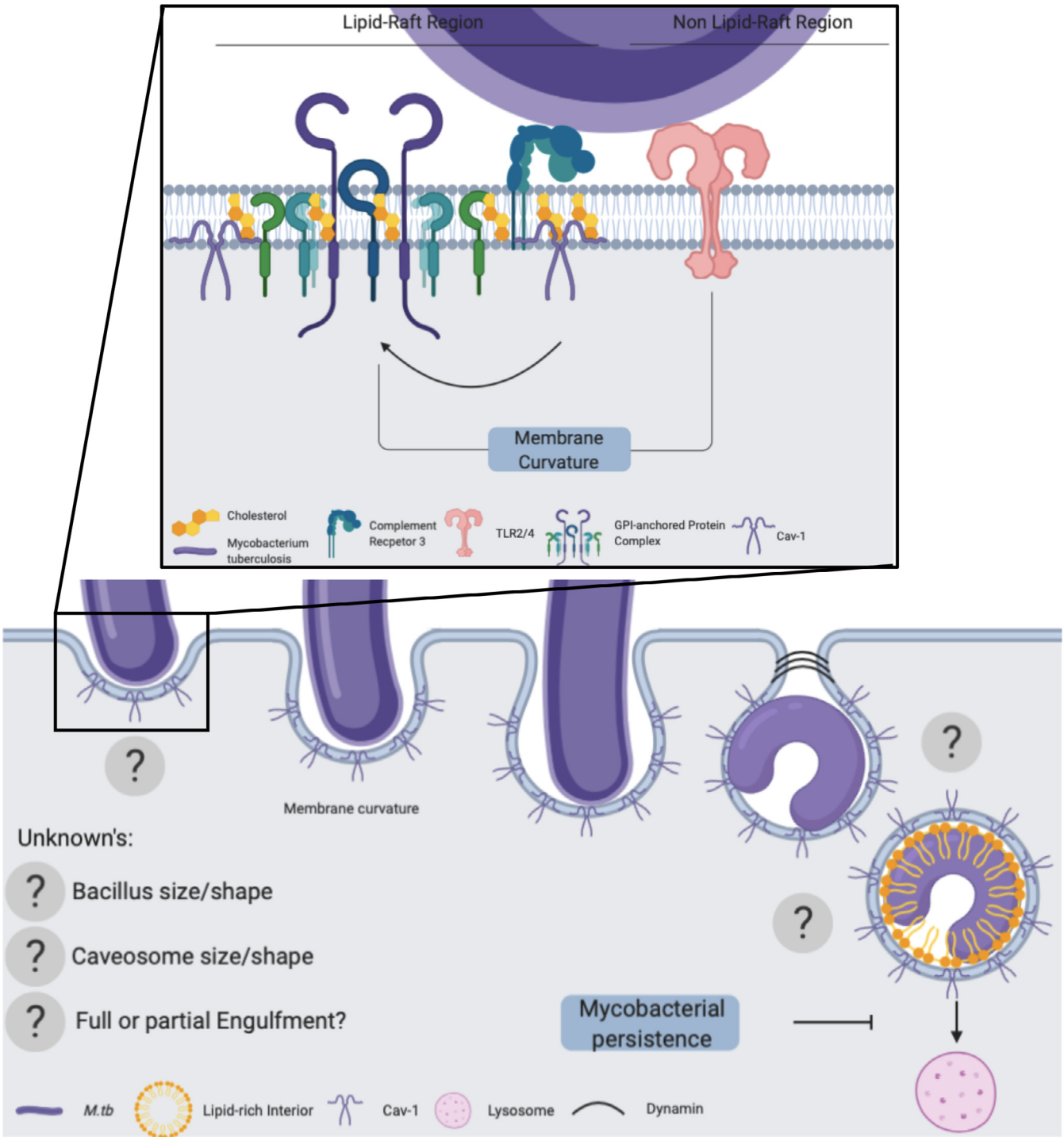

Fig. 1. Conceptual demonstration of the proposed internalization mechanism employed by *Mycobacterium tuberculosis* (*M.tb*) to enter myeloid-derived suppressor cells (MDSC) in order to evade traditional entry mechanisms whereby lysosomal fusion and subsequent mycobacterial degradation is averted. Lipid receptors found on the surface of *M.tb* bacilli bind to various receptors found within and neighbouring lipid-raft domains on the surace of MDSC plasma membranes which are known to contain GPI-anchored proteins, cav-1 proteins and receptors like complement receptor 3 (CD3/CD11b). These receptors bind pathogenic molecules along with pattern recognition receptors, like those from the toll-like-receptor family, which are located close to these lipid-raft regions (insert). Binding of the receptors within the lipid-rafts activates cholesterol accumulation and subsequent spontaneous curvature of the membrane, facilitated mainly by the cav-1 proteins. We propose that this process may result in the complete or partial engulfment of individual bacilli into lipid-rich cells like MDSC, and support pathogen growth and survival through the prevention of lysosomal fusion to these endocytic vesicles (caveosomes). It is not yet known biologically how this is achieved; as such outstanding questions remain as to whether these structures partially or fully surround bound bacteria, how they achieve this, or if they recruit multiple lipid-rafts to a single bacillus for complete encapsulation.

endosome, and (6) multiple caveosomes are capable of binding to the early endosome and redirecting what would normally be its natural path to the late endosome, where the contents would surely be degraded by the lysosome [82]. Via this mechanism, bacteria internalized in caveosomes are capable of hijacking the host defensive mechanisms and preventing fusion of the lysosome to the endosome and thus evade eradication. It should be noted, however, that caveolar internalization is not constitutive, but requires bacterial/viral binding for activation [82].

To date, it is still not certain which other surface receptors are utilized by *M.tb* to enhance this endocytic form of cellular entry, if any. A pivotal study conducted by Shin et al. demonstrated the translocation of TLR2 to lipid rafts in macrophages treated within *M.tb* lipoproteins [96]. However, Fine-Coulson and team deemed this observation incorrect as further studies produced no such translocation of either TLR2 or TLR4 in response to *M.tb* infection in epithelial cells [75]. Owing to the differences in cell types used to assess this mechanism, it is plausible that the observed differences may be related to this. Our most recent results on the role of Cav-1 in MDSC infected with *M.bovis* BCG, demonstrated that MDSC generated from the bone marrow of wild-type (WT) or Cav1-/- mice, upregulated surface expression of Cav-1, TLR4 and TLR2 expression after BCG infection. Cav-1 deficiency resulted in a selective defect of intracellular TLR2 levels, predominantly in the M-MDSC subset. Although our current experimental setup failed to show a difference in the phagocytosis of BCG by M-MDSC from WT and Cav1-/- mice or caveosome formation, we did measure a reduced capacity of MDSC to 1) up-regulate surface markers, 2) secrete various cytokines, and 3) induce iNOS and NO production. The effect of these on mycobacterial control, specifically killing of chronic Mtb infection at various time points and infectious doses, remains to be examined. Among the signaling pathways affected by Cav-1 deficiency, we found lower phosphorylation of the p38 mitogen-activated protein kinase (MAPK). Our findings reveal major differences to macrophages and dendritic cells (DCs) and implicate that Cav-1 is dispensable for the internalization of BCG, but that vesicular TLR2 signaling in M-MDSC is a major signaling pathway induced by BCG, controlled by Cav-1, and lastly, that vesicular TLR2/Cav-1 signaling is required for T cell suppressor functions [97].

For this reason, the hypothesized TLR involvement should be further studied in specific host-*M.tb* models using standardized cell types and investigative techniques where possible.

In addition to the above-mentioned findings, research in pancreatic cancer observed that the overexpression of cav-1 is associated with worsened tumor infiltrations and clinical outcomes [85,98]. From these studies it was identified that cav-1 is essential for the growth and metastases of tumours and is responsible for promoting tumor resistance to therapies such as chemotherapy and radiation. Upon the loss of cav-1 expression through blockage models, tumor cells became significantly less invasive, had reduced proliferation and migration, and became highly sensitized to chemotherapy. This was in conjunction with an increased activation of apoptotic response pathways [85,98]. Based on these findings, the loss of cav-1 expression on innate immune cell types could well increase treatment efficacy by reducing the number of bacilli able to harbor within MDSC, and by that manner, evade the presence of anti-TB drugs, thereby supporting our hypothesis.

7. Concluding remarks

The immunosuppressive mechanisms of myeloid-derived suppressor cells are clearly defined in the field of cancer research, however these have not clearly been demonstrated in most infectious diseases characterized by chronic inflammatory states, such as active TB disease. We speculate that similar mechanisms observed in cancer exist in TB due to the chronic inflammatory state observed in both. The opinion suggested from this review is that *M.tb* bacilli could successfully survive and persist in caveosomes, with no lack for carbon resources, owing to the lipid metabolism characteristics of *M.tb*, as well as the proposed persistence-mediator: MDSC. The observation that CR3/CD11b is a dominant phenotypic marker for MDSC is important for this opinion. Additionally, with the observation that cav-1 proteins are present in great numbers in the plasma membrane of MDSC, suggests a relationship between lipid raft-mediated endocytosis and caveolin expression, and further supports this opinion. This also demonstrates a cause to believe that there is a link between immune evasion strategies and immune suppression activities through the action of caveolin-mediated internalization into cells such as MDSC, to contribute to the protection of *M.tb* from host responses.

It is tempting to speculate that the suppressive action of MDSC may promote the initiation of the initial collapse of early protective immunity against *M.tb* infection, with the process being augmented through additional assistance from caveolin-mediated *M.tb* endocytosis. The repercussions of such findings may accelerate targeted drug therapy development, enrich our understanding of *M.tb* evasion strategies, as well as possibly contribute towards advances in measuring treatment efficacy in individual patients at early time points during treatment. This proposed mechanism, however, will require extensive research, the pilot study for which is indeed underway.

8. Outstanding questions

Future research should consider the following important questions highlighted throughout this review: (1) Does the proposed caveolin-mediated endocytosis indeed occur in MDSC during *M.tb* infection? (2) If so, could this process be targeted as a host-directed therapy approach for active TB disease interventions? (3) Additionally, are the caveosomes partially or fully engulfing bacteria? (4) If fully, are the caveosomes altering the shape of the internalized bacilli? (5) Do *M.tb* bacilli prefer to activate the MDSC route of uptake as a mechanism of immune evasion, or rather other molecular immune evasion pathways known to be targeted in alveolar macrophages? (6) In terms of the pathogen itself, do *M.tb* bacilli display altered metabolic activity following internalization via different pathways (caveolin-mediated vs phagocytosis) and within different cell types (MDSC vs alveolar macrophages)?

9. Search strategy and selection criteria

Data for this review were identified by searches of PubMed, and references from relevant articles using the search terms “Caveolin”, “lipid-rafts”, “myeloid-derived suppressor cells”, “TB”, “*Mycobacterium tuberculosis* infection”, “lipid metabolism”, “cav-1″, “MDSC metabolism”, and “*M.tb* metabolism”. Only articles published in English between 1997 and 2019 were included, however only 16% of the references were published before 2008.

Declaration of Competing Interest

All authors disclose that there are no conflicts of interest.

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