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Interaction of *Mycobacterium tuberculosis* H₃₇Rv with Microfold Cell leads to a New Era of Infection in Host

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Abstract

Tuberculosis (TB) is widely distributed dangerous disease that spreads at faster rate and caused by *Mycobacterium tuberculosis* H₃₇Rv (*M. tuberculosis* H₃₇Rv) which is highly successful lipid and GC rich bacteria. The bacterium gains its success as it utilizes host macrophages for its survival and replication. *M. tuberculosis* H₃₇Rv uses at least two separate pathways to recruit macrophages. It uses its PGL surface lipid to recruit macrophages through host CCL2 and Micro fold cells to enter deep in tissues. M-cells express many different carbohydrate markers on their surface which helps in cell and pathogen or antigen interaction. These cells transfer substances from gut across epithelium and to immune cells. M-cells have the potential to intricate the life cycle of this pathogen by internalizing pathogen. M-cells are targeted for vaccine to induce immunity and it has experimented on mice, humans and primates. Nanoparticles and microspheres can also be used successfully for drug or vaccine delivery through micro fold cells because microspheres used for vaccine delivery system to increase mucosal antibody responses that provide prolonged therapeutic effects and nanoparticles are easily accepted by cells so these particles also useful in drug delivery. In this manuscript we have describe the significance of M- cells that could be helpful in TB treatment.

Keywords: Alveolar macrophages; Cytokines; Microfold cell; *Mycobacterium tuberculosis*; Phagosome

Abbreviations: Micro fold cells (M-Cells); Tuberculosis (TB); Human Immunodeficiency Virus (HIV); *Mycobacterium tuberculosis* (*M. tuberculosis* H₃₇Rv); Phenolic Glycolipids (PGL); World Health Organization (WHO); Fatty Acid Synthase (FAS); Twin-Arginine Translocation (TAT); Receptor activator of nuclear factor- κ B ligand (RANKL); Receptor Activator of Nuclear Factor Kappa-B (RANK); Nuclear Factor- Kappa B (NF- κ b); Multiple Drug Resistance-Tuberculosis (MDR-TB); Extensively drug-resistant tuberculosis (XDR-TB); Follicle-Associated Epithelia (FAE); Peyer's Patches (PP); Mucosa-Associated Lymphoid Tissue (MALT); Nasal Associated Lymphoid Tissue (NALT); Bronchial Associated Lymphoid

Tissue (BALT); Fatty Acid Synthase (FAS); Phospholipids (PL); Phenolic Glycolipids (PGL); Tumor Necrosis Factor Receptor Superfamily 11a (TNFRSF11a); Receptor Activator of Nuclear Factor- κ B Ligand (RANKL); Receptor Activator of Nuclear Factor- κ B (RANK); Type 1 fimbriae D-mannose specific adhesin (FimH).

Introduction

Tuberculosis is the global threat which is widely distributed and communicative [1]. It is caused by *Mycobacterium tuberculosis* H₃₇Rv (*M. tuberculosis* H₃₇Rv) strain which is one of the pathogenic strains among Mycobacterium species. Primarily tuberculosis is a respiratory disease, but it also infects other parts of the body including bone, brain, urinary tracts etc. which is known as extra-pulmonary tuberculosis [2]. Besides through respiration, *M. tuberculosis* H₃₇Rv can also enter in host circulation by Micro fold cells (M-cells). These cells originate within the gut-associated lymphoid tissue (GALT) in Peyer's patches of the small intestine, and in the mucosa-associated lymphoid tissue (MALT) of additional division of the human gastrointestinal tract [3]. M-cells are famous for instigating mucosal immunity response on the apical membrane of these cells then allocate for conveying of microorganisms and particles throughout the epithelial cell layer through the gut lumen to the lamina propria where communications with immune cells occurs [4]. M-cells are very specialized cells for phagocytosis of intestinal macromolecules, particulate antigens and microorganisms across epithelium. These cells can directly mediate leading infection by *M. tuberculosis* H₃₇Rv bacterium and ease distribution beyond mucosa [5]. These cells are found in the follicle-associated epithelia (FAE) of Peyer's patches (PP), isolated lymphoid follicles, NALT (nasal associated lymphoid tissue), BALT (bronchial associated lymphoid tissue), appendix and in MALT outside the gastrointestinal tract. These cells might be deficient in cilia and enzymatic movement, so they are not engaged with processing or ingestion but rather their essential capacity is to translocate particles from gut lumen to host immune cells [6]. Various earlier studies proved that M-cells receptor work very cleverly with host immune cells and inhibit the entry of the pathogen therefore play crucial role in vaccine development or drug delivery [7-9]. In our study, we focused

on various characteristics that M-cells shows and its essentiality in manufacturing vaccine against TB.

Literature Review

Tuberculosis epidemics

According to WHO report of 2016, 61 lacs novel cases of tuberculosis were reported by national power system and informed to WHO. The respective data is an increment from the observed data of 2013-2015. 2016-year data shows 6 lac new TB cases which shows resistance to rifampicin (RRTB), which is the major efficient first-line anti tuberculosis medicine, of these 49 lac rifampicin resistance people had been generated multidrug-resistance TB (MDR-TB) and along with this, roughly partially (almost 47%) of these cases were only present in India, China and the Russian countries [10]. According to WHO report of 2017, TB mortality rate has fallen about 3% per year. Occurrence of TB has dropped 2% per year and death due to TB reduced up to 16%; but still there is an immense gap in TB recognition and cure. In 2017 WHO has mentioned TB- Sustainable Development Goals (SDG) monitoring framework of 14 indicators that are associated with TB incidence. In the end TB strategy specific goals are set that include 90% reduction in TB death and 80% reduction in new TB cases per year by 2030, compared with 2015 [11]. TB is a challenge to fitness and analytical tests such as polymerase chain reaction (PCR) and smearing test [12] as these are costly and time-consuming, so we still need a key to control TB. There are some assays that are cheap like TB biomarker assay which is accurate and easily available for TB identification of patient [13]. Now days, Human Immunodeficiency Virus (HIV) and TB drug resistance is a global risk [14,15]. *M. tuberculosis* H₃₇Rv and Human Immune deficiency Virus (HIV) together weaken the immune system by decreasing CD4+ T lymphocytes and decrease the host survival [16]. People with this co-infection have unfavourable treatment outcome due to executive complexity of these pathogens [17]. Antiretroviral Therapy (ART) is recommended for HIV-TB co-infected patient in early stage of infection because it shows increased HIV-TB co-infected host survival [18]. TB has complicated and long-lasting effects on human body that are not common but may be danger to life. In 1.5% to 3.5% cases, Deep Vein Thrombosis (DVT) is associated with TB and critical to isolate TB patients who are at high risk. Anti -Tubercular Treatment (AAT) and anticoagulant therapy can prevent the fatal complication of this disease [19].

M. tuberculosis pathogenesis resistance inside the phagosome

This bacterium is gram positive, aerobic and classified as intracellular parasite of alveolar macrophages of humans. Although this bacterium is classified as gram positive, but it is difficult to stain the bacterium by gram staining procedure due to its complex cell wall nature. Strains of *M. tuberculosis* H₃₇Rv enters in host through inhalation as these pathogens released by sneezing or coughing of *M. tuberculosis* H₃₇Rv infected

person and ultimately spread to the lungs and utilizes macrophages for its replication, causing primary infection and secondary infection in lymphoid organs. In the alveolar macrophages, this bacterium persists for longer period; enhance its progenies by replication that results in formation of granuloma which is characterized by *M. tuberculosis* H₃₇Rv cells surrounded by various immune cells like B cells, T cells, NK cells and dendritic cells etc. When *M. tuberculosis* H₃₇Rv outbreaks in host, it uses its Phenolic Glycolipids (PGL) surface lipid to recruit macrophages through recipient chemokine (C-C motif) ligand 2 (CCL2), which benefit *M. tuberculosis* H₃₇Rv in inflowing deep in host tissue [20]. *M. tuberculosis* H₃₇Rv synthesizes Lipomannans (LM) and Lipoarabinomannans (LAM), the two essential cell wall components and necessary for its virulence. *M. tuberculosis* H₃₇Rv survives by regulating membrane lipid composition in favour to diverse environmental conditions. Many components of lipid rich cell wall are important for causing infection [21,22]. For lipid adjustment Fatty Acid Synthase 1 (FAS 1) and FAS 1 products depending metabolic pathways with membrane Phospholipid (PL) and cargo space compound Triacyl Glycerol (TAG) are essential [23]. In *M. tuberculosis* H₃₇Rv, biogenesis of FAs is started by the enzyme FAS 1 which is a multifunctional enzyme catalyses de novo pathway and synthesizes long chain acyl CoAs from acetyl CoA via malonyl CoA as extender unit. These acyl-CoAs are consequently used for the production of membrane phospholipids (PL) or they are shambled into the FAS II organization for the production of mycolic acids. In *M. tuberculosis* H₃₇Rv, the type II FAS organization is dependable for the strengthening of these acyl-CoAs during repetitive compression effect with malonyl-ACP, leading to the formation of long-chain meromycolyl-ACPs [24-26]. *M. tuberculosis* H₃₇Rv cell wall core is made of peptidoglycan and linear galactofuran which are connected by a link unit L-Rha-D-GlcNAc-P. Peptidoglycan also attached to highly branched arabinofuran and mycolic acid. Mycolic acid is arranged perpendicular to the membrane and responsible for virulent character of *M. tuberculosis* H₃₇Rv [27]. *M. tuberculosis* H₃₇Rv hard cell wall could work as permeability barrier which provide resistance against antibiotics and help in diffusion of hydrophilic molecules through liposome [28]. *M. tuberculosis* H₃₇Rv cell wall alone is unable to show resistance against drug so it requires enzymatic activity, when cell wall is treated with lysozyme, oligomers of weight 20,000 and amino acids, muramic acid, glucosamine are separated [29,30]. *M. tuberculosis* H₃₇Rv obstructs the biogenesis of the phagolysosome [31]. The inclination of *M. tuberculosis* H₃₇Rv to enter the host macrophages, where it is securely stowed in a pathogen-accommodating phagosome which does not develop into the phagolysosome [32] is essential to tuberculosis infection, inertness, spread, and concealment of immunological identification by the host.

M. tuberculosis H₃₇Rv dependence on M-cell for infection

Different systems have been proposed to clarify enteral take-up of microorganisms, including interruptions of the epithelial obstruction, transcytosis transversely over

enterocytes, infection of dendritic cells or potentially lymphocytes, and via M-cells situated inside the follicle-related epithelium (FAE) which covers Peyer's patches [33], and speckled along the villus-autonomous of Peyer's patches [34]. As mentioned in the introduction part of this manuscript that these cells also help in instigating immune response, also convey a noteworthy position for the testing of gut luminal antigens, as well as are vital for take-up of different commensally residence bacterial and viral pathogens together with *Salmonella enteric* (*S. enteric*), *Vibrio cholerae* (*V. cholerae*), HIV, Reovirus, Poliovirus etc. [35-38]. M-cells are developed by Receptor activator of nuclear factor- κ B ligand and receptor activator of nuclear factor kappa-B (RANKL-RANK) pathways [39,40]. If RANKL is absent, then M-cell number has been drastically reduced. The stromal cells below epithelial layer may be source of RANKL [41].

RANKL binds to its receptor, RANK (Tumor Necrosis Factor Receptor Superfamily 11a TNFRSF11a) that activate non-canonical transcription factor RelB and NF- κ B related signaling

pathway and induces expression of SpiB, an electron transport chain transcription factor which is important for M-Cells providence, determination and maturation [42-44]. M-Cells morphology is different in different animal species. These cells express many different carbohydrate markers on their surface which helps in cells to pathogen or antigen interaction [45]. M-cells allocate substances from gut across epithelium to immune cells and known to transport latex beads, carbon particles, liposome and macromolecules including ferritin, horseradish peroxidase, cholera toxin-binding subunit, lectins and antiviruses antibodies [46]. One of the studies shows that these cells are also known for transporting microorganisms from outside the body like *V. cholerae* and *S. typhimurium* [47,48]. Distribution through M-cell occurs via transmembrane endocytosis that might be inhibited at 25°C or below and is a fast process in comparison to complete endocytosis and exocytosis that takes at least ten minutes to complete [49,50].

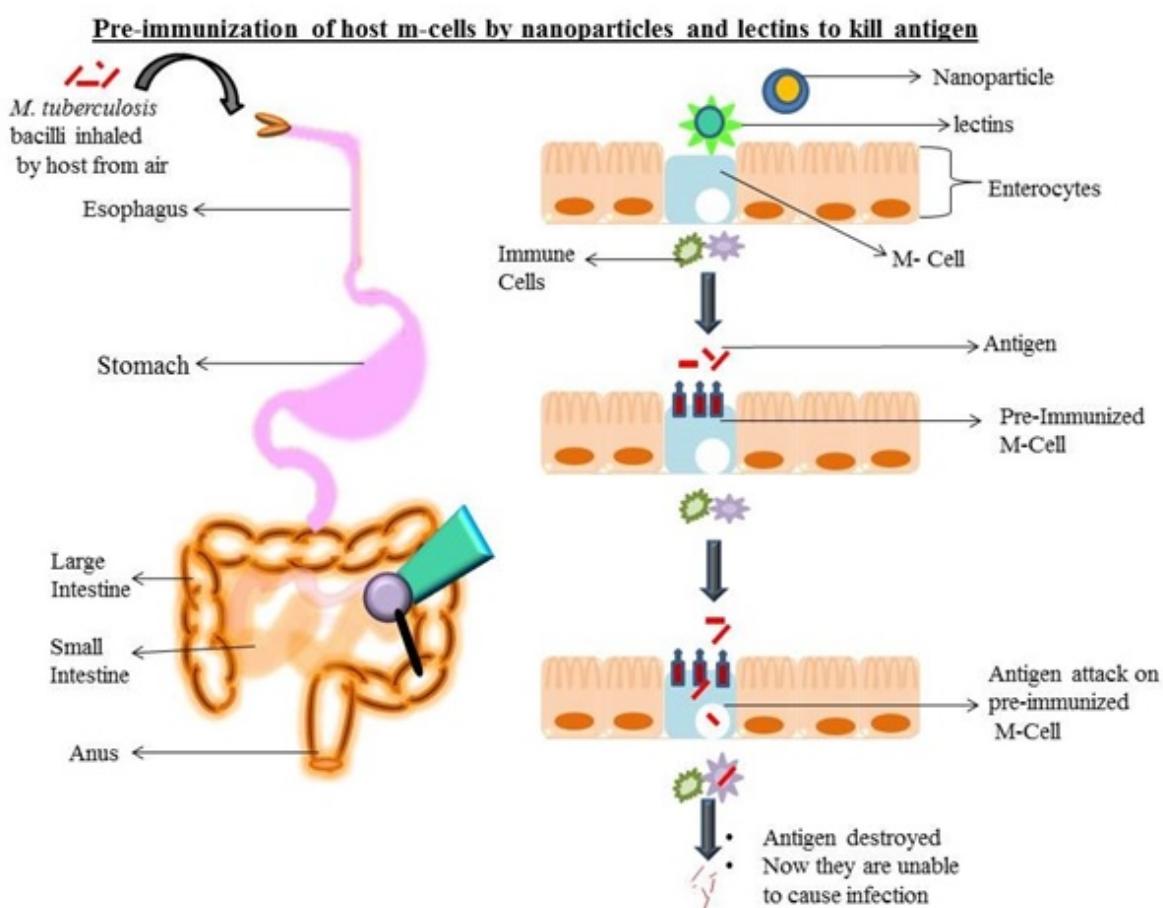


Figure 1 Pre-immunization of host m-cells by nanoparticles and lectins to kill antigen. The figure shows that *M. tuberculosis* H₃₇Rv inhaled by host from air and reaches deep in tissues through M-Cells and microscopic view shows the drug delivery through nanoparticles and microspheres by which pre-immunization of M-Cells can be done to kill *M. tuberculosis* bacterium.

M-cells do not have compactly packed elongated microvilli which are normal characteristics for enterocytes, rather than they have disrupted squat microvilli alongside their apical fringe. Furthermore, these cells contain profound basolateral

invaginations that are frequently establish to port different immune cells. This morphology takes into consideration for productive antigen and microbial interaction along fast transcytosis [51-53]. Current revisions in mice model had been

confirmed the phenomenon of generation of M-Cells which showed that the origin of these cells is Lgr5+ intestinal stem cells (ISCs) [54]. The separated epithelial lineages of the intestinal system incorporate Paneth cells, cup cells, tuft cells, enterocytes, enteroendocrine cells, and M-cells which specific parts have been requisite for appropriate nourishment inside the setting of a composite advantageous luminal microbiota.

By means of temperament, these ISCs are especially delicate toward damage underneath unpleasant situation such as in infection. In case of such unpleasant environment, stagnant cell populaces of secretory ancestor cells (+4 cells) can de-differentiate into authentic LGR5+ quickly multiplying ISCs

[55]. In both cases homeostatic as well as in adverse conditions, the capacity of stem cells is accepted as affected through different cells inside the position containing subepithelial myofibroblasts, lymphocytes, macrophages, and dendritic cells. Several precise interactive molecules of these cells had been identified such as GP2, Marcks I1, M-Sec, Sgne-1, annexin V and CCL9, of which GP2 mediates bacterial uptake that express Type 1 fimbrial D-mannose specific adhesin (FimH), which is a constituent of type I Pili on the outer membrane of bacteria and commence consequent definite immune response against bacteria [56-61].

Drug delivery to m-cells by nanoparticles and lectins to block oxygen uptake system of antigen

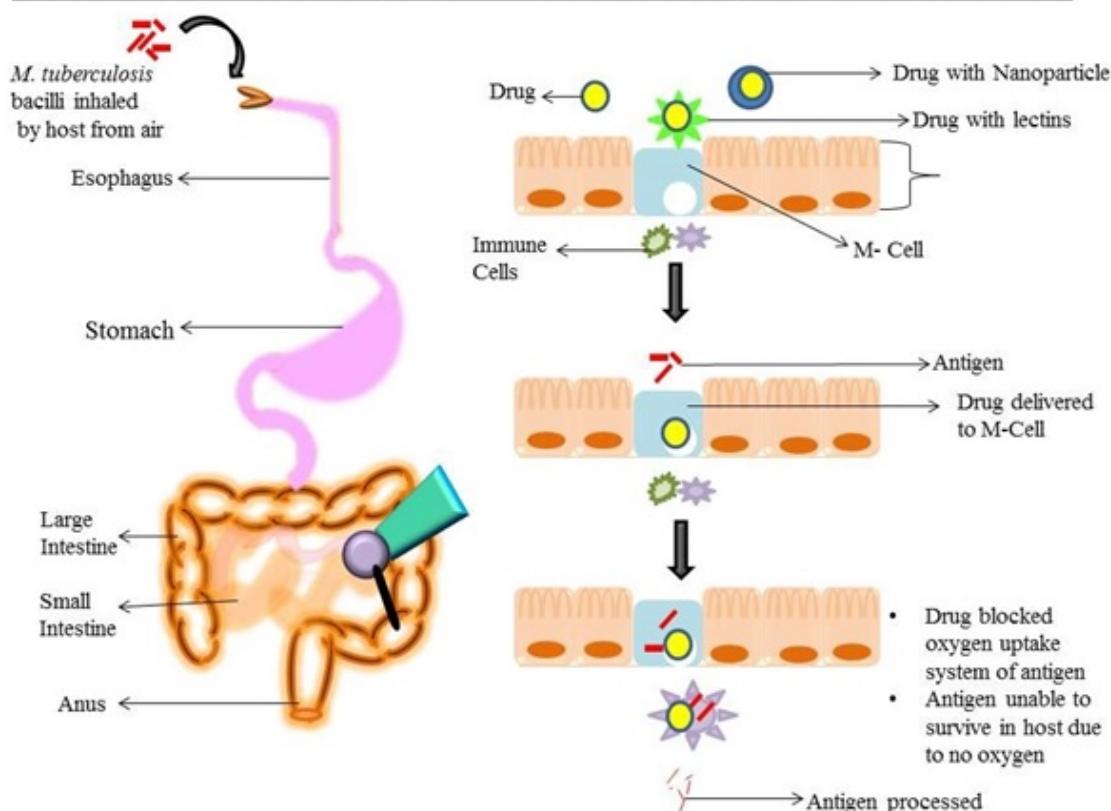


Figure 2 Drug deliveries to m-cells by nanoparticles and lectins to block oxygen uptake system of antigen. The figure shows that *M. tuberculosis* causes infection in host. Microscopic view of M-Cells shows the delivery of drug to M-cells by using nanoparticles and lectins that targets oxygen uptake system of antigen to block it. When antigen will enter in host, it would not be able to get oxygen and survive in cells.

Treatment with Anti - RANKL antibody leads to depletion of M-Cells [62] which efficiently blocks uptake of antigens and prevent development of disease [63]. M-cell specific monoclonal antibodies are established for M-cell targeted mucosal vaccine [64]. It had been demonstrated in many studies that lectin compounds which possess specificities for different carbohydrate molecules can boost uptake of drug in intestine [65-71]. The explicitly of M-Cell for lectins had further recognized through position of horseradish peroxidase (HRP)-conjugated lectins in the transmission electron microscope (TEM). In the glycocalyx of M-cells, carbohydrates are first

demonstrated which are not expressed elsewhere on the FAE surface [72]. The phenomenon of targeting the antigen specific M-Cells at immune stimulation site grant a prospective influential approach for civilizing the efficacy of mucosal immunization whereas decreasing probably detrimental side-effects. Advancement has been made during emergence of such targeting approaches by means of exclusive cell surface assets and the carbohydrate specificity of lectins and these approaches as well confirm valuable effect in treatment of tuberculosis [73].

Microspheres efficiently used for controlled drug delivery and as carrier. Drug delivery through microspheres has several advantages over conventional method. Biodegradable microspheres provide sustain release and eliminate the need of several doses with controlled drug delivery they provide prolonged therapeutic effects; these are reliable and reduce dosing frequency [74,75]. Nanoparticle based drug delivery is advance system and correspond as a marketable, convenient moreover main capable alternate meant for probable TB treatment. Cells intake nanoparticles more efficiently than bigger molecules delivery of drug and are adapted to enable controlled, slow and persistent discharge of drug through the matrix. Nanoparticles comprises of mainly polymers which are biocompatible and biodegradable material, they may be either natural (e.g., gelatin and albumin), synthetic (e.g., polylactides and polyalkylcyanoacrylates), or solid lipids [76].

As we know from above lines that microspheres and nanoparticles are successfully used for drug or vaccine delivery so that we can pre-immunize our system by delivering effective antibody or drug in microfold cells which can work against *M. tuberculosis* H₃₇Rv pathogen (**Figure 1**). We can also deliver drugs through microfold cell by using lectins or microspheres which can target *M. tuberculosis* H₃₇Rv system to exterminate it (**Figure 2**) such as artemisinin which target *M. tuberculosis* H₃₇Rv system and hinder in oxygen uptake [77].

Discussion and Conclusion

The above content is summarized as, we know that, as TB is a major threat and co-infection of TB with HIV is riskier, therefore we need to discover a novel way to treat this lethal disease. In the above content, we find that M-cells have higher rate of transferring various particles beyond mucosa that provide a different and efficient way of *M. tuberculosis* H₃₇Rv entry in host and this entrance in most of the people is the primary cause of TB. As M-Cells provide favorable condition like acidic pH for *M. tuberculosis* H₃₇Rv to survive therefore we can predict that this inhibiting way of transferring of this pathogen from M-Cells can inhibit further infection of *M. tuberculosis*. M-Cells can be targeted to produce immunity and by using receptors of these cells, pathogen entry can be inhibited either by generating immune response, vaccine or drug delivery. In this way we can predict that such nanoparticle should be synthesized that can recognize the selected M-Cells, carrying specific markers after infection by *M. tuberculosis* H₃₇Rv. M-Cells represent different carbohydrate receptors that help antigen to get entry so by raising antibodies against these carbohydrate markers may successfully eliminate infected M-Cell. We can use lectins as they increase drug uptake, nanoparticles as they are efficiently accepted by cells and microspheres as they reduce dosing frequency, to convey drug and vaccine which can pre-immunize our system or can target *M. tuberculosis* H₃₇Rv system to eliminate it.

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Conflict of Interest

There is no conflict of interest.

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