

# Exploitation of the Macrophage Mannose Receptor (CD206) in Infectious Disease Diagnostics and Therapeutics

## Abstract

The macrophage mannose receptor (MR, CD206) is a C-type lectin expressed predominantly by most tissue macrophages, dendritic cells and specific lymphatic or endothelial cells. It functions in endocytosis and phagocytosis, and plays an important role in immune homeostasis by scavenging unwanted mannoglycoproteins. More attention is being paid to its particularly high expression in tissue pathology sites during disease such the tumor microenvironment. The MR recognizes a variety of microorganisms by their mannan-coated cell wall, which is exploited by adapted intracellular pathogens such as *Mycobacterium tuberculosis*, for their own survival. Despite the continued development of drug delivery technologies, the targeting of agents to immune cells, especially macrophages, for effective diagnosis and treatment of chronic infectious diseases has not been addressed adequately. In this regard, strategies that optimize MR-mediated uptake by macrophages in target tissues during infection are becoming an attractive approach. We review important progress in this area.

## Introduction

Infectious diseases account for more than 25% of human deaths in the world annually and are currently the third leading cause of death in the United States [1,2]. The optimism in the early 1970s of eradicating infectious diseases with the discovery of powerful antibiotics, vaccines, and antiviral drugs has been lost with the emergence of multiple anti-infective resistant microbes. As infectious diseases remain very significant health problems at home and around the world, a central component of the world-wide effort to eliminate or thwart these diseases will rest on having markedly improved imaging/diagnostic capabilities as well as more effective therapies and vaccines. The lack of accessibility of many diagnostic and chemotherapeutic agents to infected or diseased tissue sites in patients with a variety of infectious diseases remains a formidable obstacle to effective cures, especially for chronic and recurring infections due to intracellular pathogens of macrophages. Despite the continued development of drug delivery technologies, the targeting of agents to immune cells, especially macrophages, for effective diagnosis and treatment of the underlying diseases has not been addressed adequately. Through ongoing efforts in the Center for Microbial Interface Biology at The Ohio State University, attention is being paid to the mannosylation of clinical agents for advanced imaging, diagnostics and therapeutic strategies that optimize MR-mediated uptake by macrophages in target tissues during infection. It is anticipated that such strategies will also enable the use of smaller amounts of agents for optimal clinical effects, thereby increasing efficacy and reducing toxicity.

## Macrophage Mannose Receptor

The mannose receptor (MR, CD206 or MRC1) is a transmembrane



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**Submission:** 28 November 2013

**Accepted:** 06 January 2014

**Published:** 10 January 2014

**Reviewed & Approved by:** Dr. Sankar N. Maity

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glycoprotein that belongs to the C-type lectin family and is expressed predominantly by most tissue macrophages, dendritic cells (DCs) and selected lymphatic or liver endothelial cells [3]. Its extracellular regions include an N-terminal cysteine-rich (CR) domain that binds glycoproteins bearing sulfated sugars that terminate in 4-SO<sub>4</sub>GalNAc, a fibronectin II (FNII) domain, and eight carbohydrate recognition domains (CRDs) that bind sugars like mannose and fucose with high affinity. The MR serves as a homeostatic receptor by binding and scavenging unwanted high mannose N-linked glycoproteins as well as pituitary hormones from the circulation [4-6]. Since many pathogenic microbes are coated with mannose-containing structures, the macrophage MR interacts with those pathogens in a form of the host molecular mimicry [7].

## MR and Pathogen Recognition

The MR recognizes a variety of microorganisms, including bacteria, fungi, virus, and parasites. Notably, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Yersinia pestis*, *Candida albicans*, *Pneumocystis carinii*, *Cryptococcus neoformans*, HIV, influenza virus, dengue virus, and *Leishmania* species bind to the MR and are endocytosed by macrophages [5,8]. Many MR-binding organisms are intracellular pathogens. Since the MR is an abundantly expressed endocytic, recycling receptor [9], targeting this receptor is a viable and attractive strategy for the delivery of carbohydrate-containing imaging/diagnostic agents as well as the intracellular delivery of therapeutics for many infectious diseases.

## Rationale for MR Targeting

The lack of accessibility of many diagnostic and chemotherapeutic agents in infected or diseased sites of patients with diseases like cancer and infectious diseases has remained a clinical challenge. Despite the continued development of drug delivery technologies, the effective targeting of drugs to macrophages for the diagnosis and treatment of the underlying diseases remains to be proven. Based on a growing literature, the feasibility that mannosylation of imaging agents,

diagnostics and/or therapeutics will lead to clinically relevant MR-mediated uptake by macrophages in target tissues or organs is much increased. Furthermore, enhanced uptake is predicted to require smaller doses of the agents sufficient for optimal clinical effects, thereby reducing the toxicity of administered substances.

### Strategies for Small Molecule Delivery to Macrophages

For effective and targeted delivery, small molecule (e.g., drug) carriers such as liposomes, microparticles, nanoparticles, dendrimers, etc. are used. Most of the carrier molecules are inert and exhibit multiple advantages of biocompatibility, biodegradability, high charge density and non-toxicity. In most cases, these carriers are functionalized with mannose (mannosylated) or with anti-MR nanobody to use as substrates for targeting the MR. The small molecule carriers are able to traverse the different membrane barriers of macrophages and release their cargo inside the cells. The same principle has been exploited recently by using mannosylated nanoparticles (polymeric micelles) for high efficiency delivery of MR-targeted siRNA into macrophages [10]. The MR has recently been shown to mediate the uptake and endosomal delivery of the intracellular TLR9 ligand CpG motif-containing oligodeoxynucleotides in murine peritoneal macrophages without using any mannosylated carriers [11]. More advanced delivery systems targeting the macrophage MR have also been reported in recent years in various disease models [12-14]. Singodia et al. used an alternate approach to target the macrophage MR by coating antibiotic-loaded liposomes with a sulfated sugar ligand of the receptor (4-SO<sub>4</sub>GalNAc) rather than mannoses, which led to enhanced antibiotic uptake by the CR domain of the MR and thus opens up a new drug delivery strategy via this receptor [12]. Two novel polyethylene glycol-treated (PEGylated) nanocarriers have been developed for high efficiency MR-mediated macrophage target-

ing: one by using PEG-sheddable, mannose-functionalized nanoparticles in the acidic tumor microenvironment [13] and the other by incorporating PEG and peptide in a dendritic structure device known as “PEGtide dendrons” functionalized with mannose residues [14]. Because of their modular design, PEGtide dendrons were suggested to be advanced carriers in imaging and drug delivery applications.

### Targeting the MR to Tumor-Associated Macrophages (TAMs) in Cancer

Tissue macrophages, the major patrolling immune cells of the innate immune system as well as the communication cells for the development and maintenance of the adaptive immune system, represent a highly diverse population in terms of phenotype and function depending on their tissue sites and state of activation [15]. They have variably been termed M1, M2, immunoregulatory, etc. highlighting their spectrum of activities. Alveolar macrophages are prototypic alternatively activated macrophages and express abundant MR which mediates interaction with various air-borne pathogens [5]. In the cancer tissue microenvironment, tumor-associated macrophages or TAMs have been described which play important roles in tumor invasion, proliferation and metastasis [16]. These are M2-type macrophages and express high levels of the MR [17]. Studies in cancer research are targeting the MR expressed on TAMs. These studies are providing proof of principle that the MR is an appealing bio-target for cancer diagnosis and therapy. Mannose-coated liposomes have been used for imaging of TAMs by positron emission tomography (PET) [18], and to mediate MR-directed anti-cancer drug delivery in a mouse model of peritoneal metastasis and in human gastric cancer [19]. Using single-photon emission computed tomography/micro-computed tomography (SPECT/micro-CT) has enabled *in vivo* imaging of TAMs by nanobody-based targeting of

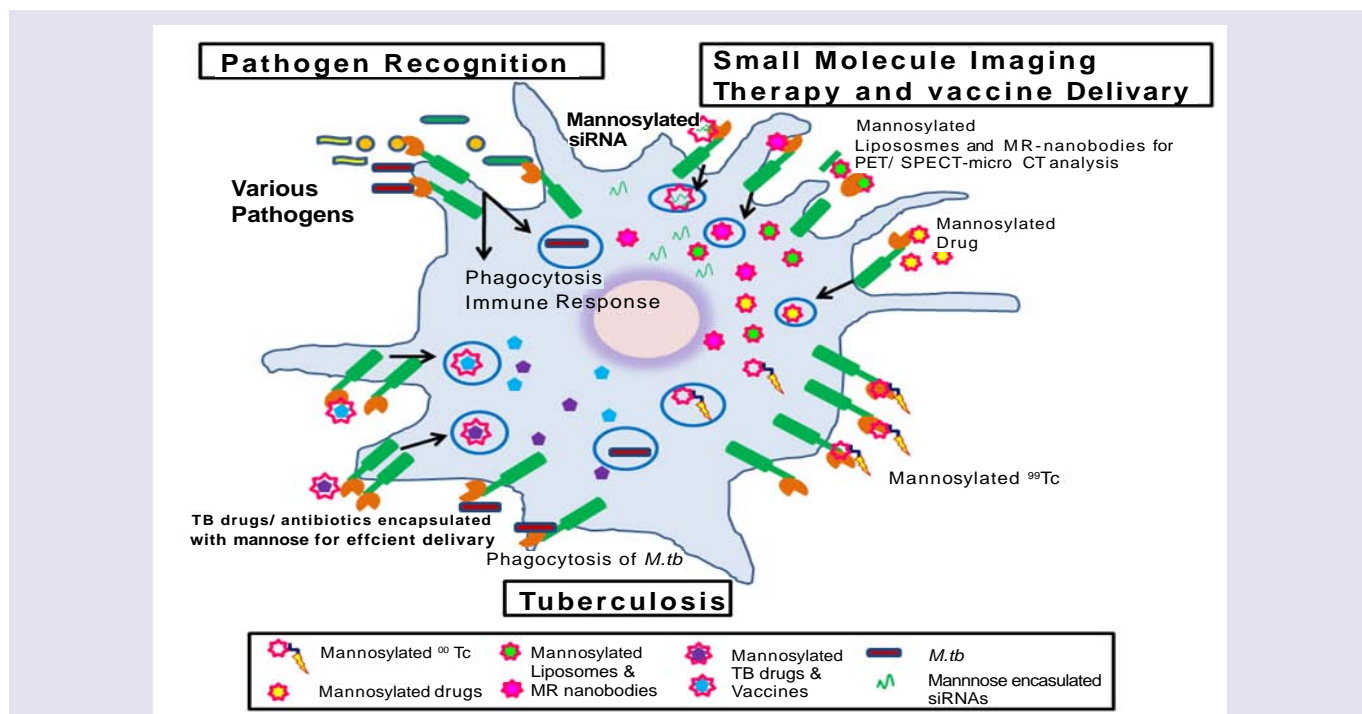
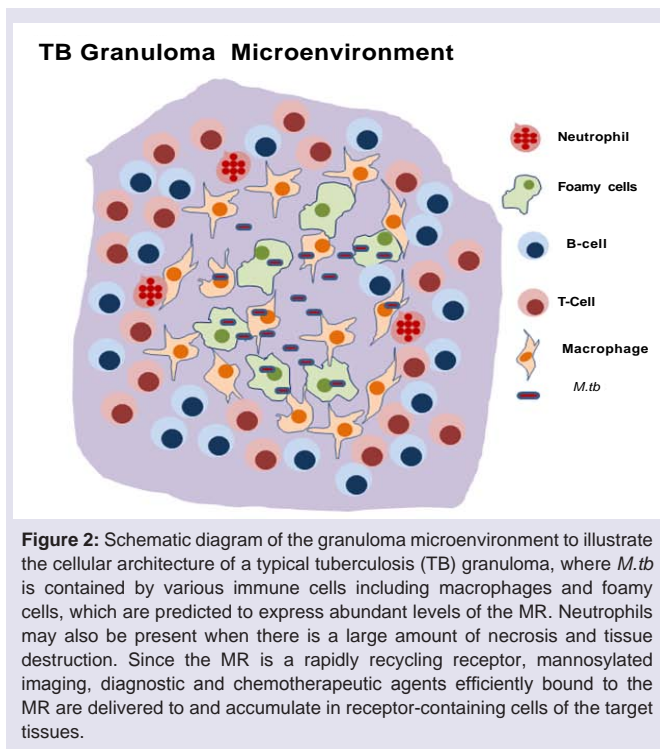


Figure 1: Schematic diagram for the roles of the macrophage mannose receptor (MR, CD206) in pathogen recognition [e.g., *Mycobacterium tuberculosis* (*M.tb*)]; and in specific binding of mannosylated liposomes, siRNAs, various drug molecules, and radioisotopes like <sup>99</sup>Tc as well as MR-nanobodies.



the MR in mouse models of lung and breast cancer [20]. A similar approach has been recently applied to autoimmune disease where a SPECT/micro-CT imaging of joint inflammation was performed by targeting nanobody-based macrophage MR in a mouse model of rheumatoid arthritis [21]. Thus, TAMs represent an appealing bio-target for cancer imaging, diagnosis and therapy. The successful optimization of techniques for efficient MR-mediated delivery of imaging and therapeutic agents in these types of studies set the stage for targeting MR-expressing macrophages for similar types of clinical applications in infectious diseases including tuberculosis (TB).

## Targeting the MR in Infectious Diseases

### TB as a model

As noted above, many pathogens are surface-coated with specific carbohydrates that are recognized by the macrophage MR and other C-type lectins. Research is beginning to exploit the strategy of receptor and ligand interaction, and make use of mannoseylated “pathogen-like” polyanhydride nanoparticles for delivery of antigens into macrophages and DCs, the latter being another critical immune cell in innate and adaptive immunity [22,23]. Liposomes are often used as a carrier system for antimicrobial agents and, in general, to improve their bio-distribution, reduce cell toxicity, and increase the therapeutic index at the infection site [24]. In this regard, the MR represents a potential target for efficient delivery of mannoseylated liposomes.

TB is a respiratory infection caused by the bacterial pathogen *Mycobacterium tuberculosis*. It currently infects approximately one third of the world’s population and is the second leading cause of death globally due to a single infectious agent with ongoing worldwide efforts to improve therapies and vaccines [25]. *M.tuberculosis* is a prototypic intracellular pathogen of macrophages which play

a major role in both latent and active TB. Macrophages are an essential component for granuloma formation and maintenance. The granuloma is where *M.tuberculosis* is controlled and persists, yet this unique environment remains one of the least understood aspects of the host-pathogen relationship [26]. What is widely recognized, however, is that the granuloma microenvironment represents a formidable barrier to the delivery of diagnostic agents and therapies, akin to the tumor microenvironment, and some parallels can be drawn, including physiological barriers such as reduced oxygen tension and altered phenotype and function of macrophages [27]. We currently lack the ability to accurately image granulomas in patients with latency, a condition in which treatment can reduce the risk of developing active TB.

Targeting the macrophage MR is a potential and an attractive strategy for the imaging, diagnosis and therapy of TB (Figure 1). Our group discovered the role of the MR in the phagocytosis of *M. tuberculosis* by human macrophages 20 years ago [28], and, more recently, MR’s role in regulating macrophage responses to this pathogen [29,30]. To date, there has been no report on the systematic evaluation of the MR on macrophages within TB granulomas akin to TAMs. However, it is likely that such macrophages express the MR (CD206) in addition to CD163 [27]. The MR has been implicated in macrophage adhesion and fusion during granuloma formation [31]. In addition, PPAR $\gamma$  mediates induction of the MR and foamy cells, the latter found in granulomas [32]. We have found that PPAR $\gamma$  is up-regulated by *M. tuberculosis* engagement of the MR [30] which could potentially help sustain the regulated inflammatory environment within granulomas [27]. Since the TB granuloma provides a tangible barrier to antibiotic penetration [33] and the MR is predicted to be abundantly expressed on macrophages, foamy cells and DCs, all cells being present in the granulomas, the MR could be an attractive target for imaging agents and drug delivery systems in this microenvironment akin to strategies being developed in the cancer field (Figure 2). In a recent study, D’Addio et al. optimized macrophage MR-specific targeting for anti-TB drugs by using nanocarriers functionalized with polymannoses and found that maximum macrophage uptake of the drugs occurred with increased mannose density on nanocarrier surfaces [34].

### Targeting the MR for Vaccine Delivery

The macrophage MR can mediate the presentation of mycobacterial antigens to T cells in the development of an adaptive immune response [35]. This property raises the potential for targeting the MR and other C-type lectins in the development of effective vaccines [36]. In this context, several studies provide evidence that the MR pathway can be targeted for vaccine delivery [37-39]. For example, a novel DNA vaccine formulation enhances cytotoxic T-lymphocyte activity through efficient gene delivery to DCs by MR-mediated endocytosis [40]. The MR endocytic pathway can also be used to deliver DNA-based vaccines into antigen-presenting cells using mannoseylated liposomes [41]. The use of cationic mannoseylated liposomes complexed with plasmid DNA has shown high transfection efficiency due to recognition by the MR both *in vitro* and *in vivo* [42]. The concept of T-cell activation by this approach is supported by a study where CD8<sup>+</sup> T-cell activation was successfully induced via the MR-mediated endocytic pathway providing further evidence for the use of the MR in vaccine therapy for TB and other infectious diseases [43].

## Concluding Remarks

The majority of strategies for imaging, diagnostic or therapeutic agents that target the macrophage MR have used mannosylation of their carriers rather than modifying the agents themselves. Incorporation of polymannoses in the structures of the agents could be an effective approach for their faster, more penetrable delivery to target sites, assuming the compounds remain non-toxic and do not lose their efficacy upon mannosylation. For example, the FDA recently approved technetium (<sup>99</sup>Tc)-labeled tilmanocept from Navidea Pharmaceuticals as the first receptor-targeted poly mannosylated radiopharmaceutical [44] used for precision imaging of sentinel lymph nodes in solid tumor staging. Targeting the MR by tilmanocept and derived compounds has the potential to enhance imaging agents, particularly within TB granulomas, as well as the delivery of chemotherapeutic agents (Figure 1 and Figure 2). Innovative strategies for targeting the MR pathway for the rational design and effective delivery of agents into uniquely located tissue macrophages hold promise for the diagnosis and treatment of a variety of infectious diseases.

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## Acknowledgements

The work discussed from our lab in this review was supported in part by the NIH grants awarded to LSS (R01 AI059639 and R01 AI052458).