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Targeted Drug Delivery to Macrophages in Parasitic Infections

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Abstract: Successful homing of drugs to the desired biological compartment of the host usually depends on the intrinsic properties of the drug molecules. However, it can always be manipulated by appropriate designing of the carrier/delivery system, as little can be done to influence the target and its surroundings. Various carrier systems have emerged to deliver drugs to macrophages, albeit the efficacy, reliability and selectivity of these carriers are still in question. To date, the most extensively studied carriers are liposomes and microspheres. In fact, physicochemical properties of these carriers can alter their efficacy and specificity to a great extent. These properties include hydrophilicity, surface charge, composition, concentration, and presence of various target specific ligands on their surface. Incidentally, the particulate nature of these vehicles may facilitate passive homing of the entrapped drug molecules to the macrophages, which may harbour many of the important pathogens in their intracellular compartments, such as *Mycobacterium* sps, *Leishmania* and dengue virus etc., belonging to three different major classes of microbes. Moreover, macrophages upon interaction with particulate drug delivery vehicles may act as secondary drug depot, thus helping in localized delivery of the drug at the infected site. In the present article, a comprehensive review of literature is presented on the suitability of some lipid-based and polymeric materials as vehicles in delivery of drugs to macrophages in parasitic infections.

Keywords: Macrophages, infections, mycobacteria, leishmania, drugs, liposomes, microspheres, targeting.

1. INTRODUCTION

Design and development of potential carriers for cell specific delivery of therapeutics are immensely dependent on the selectivity of the carrier to the cellular receptors distributed variably at both the intracellular compartments and the surface of the cellular systems. Other crucial factors include the anatomical and pathological barriers that have to be circumvented, en route before access to the recognition sites. Most of the drugs introduced to clinical medicine exert their pharmacological action by interactive crosstalk with cell membrane through concentration- dependent reversible interactions with specific receptor, or active sites of crucial metabolic enzymes. Obviously, to obtain a desirable therapeutic response, the optimum amount of the drug should be transported and delivered to the site of action with subsequent control of drug input rate. The distribution of administered drug to other tissues therefore seems unnecessary, wasteful and potential cause of toxicity. Keeping into consideration the innate toxicity and undesirable manifestations that are common features of the available medicaments, last few decades have witnessed tremendous advancements in the field of controlled and targeted delivery of pharmacologically active therapeutic agents. In this regard, the cell related biological events occurring in high order of specificity and precision, offer basis for quantitative targeted drug delivery.

Selective drug delivery or targeting seeks to improve upon the benefit versus risk ratio associated with the therapeutic agents. Ideally, a drug intended for clinical use should have a high therapeutic index, which is a ratio of drug efficacy (therapeutic effect) and drug toxicity (untoward effects). The drug delivery technology has certainly infused new interest in seemingly traditionally old drugs by providing them new bio-distribution pattern with the help of novel drug delivery system. In general, targeted drug delivery can be achieved by using carrier systems, where reliance is placed by exploiting both, intrinsic pathways that these drug carriers follow, and bioprotection that can be offered to the therapeutic molecules during transit through various body compartments. This led to conclusion that desired pharmacological action of the administered drug is required at the desired site only. In this regard, mononuclear cells of the host can play important role in effective delivery of the therapeutic agents, provided drug molecules are delivered as particulate carrier/delivery system. The carrier-mediated delivery becomes more pertinent in situations when these cells provide shelter to certain intracellular organisms. In normal course, ingested microorganisms are killed by the lysosomal contents that are released into the phagosomes. Some microorganisms however can survive and multiply within the macrophages. These intracellular pathogens include *Listeria monocytogens, Salmonella typhyimurium, Neisseria gonorrhoeae, Mycobacterium avium, Mycobacterium tuberculosis, mycobacterium leprae, Brucella abortus, Leishmania spp. and Candida albicans* [1-3].

In order to eliminate pathogens that find shelter inside the macrophages, it is essential to deliver active drug molecules inside these cells. Since these cells scavenge and destroy most of the foreign particulate material that enters the body, it is always advantageous to deliver the chemotherapeutic agents to the macrophages by encapsulating them in a

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biodegradable particulate material. Such a delivery would not only help in concentrating the drug into these cells but should also result in controlled intracellular drug release. Various lipid and polymeric carriers, such as liposomes and microspheres, have been used for delivery of drugs to the macrophages [1-5]. In the present article we have attempted to review the application of these carriers in selective drug delivery to these cells. However, for better understanding of the macrophage-liposome (or microsphere) interactions, we considered it necessary to first briefly describe the origin and properties of the mononuclear phagocytic system.

2. MONONUCLEAR PHAGOCYTIC SYSTEM

The macrophages as well as other related cells, such as monocyte, promonocyte, and monoblast, have been reported to originate from a common progenitor, called the granulocyte-macrophages colony-forming cells. Monoblasts, the least mature cells of the mononuclear phagocyte system, firstly differentiate into monocytes that remain in the bone marrow for 24 h and subsequently appear in the peripheral blood. From the peripheral blood, monocytes migrate to the extravascular tissue where they differentiate into macrophages [6]. In fact, macrophages occupy all possible places of the body that can be accessed from outside, thus they are found in the liver (kupffer cells), lungs (alveolar-interstitial macrophages), spleen, lymph nodes, thymus, gut, marrow, brain, connective tissue and serous cavities [7]. They scavenge foreign substances as well as those materials or cells that have undergone significant changes in their native phenotype. Moreover, they also play a critically prominent role in host defense against many infectious agents of bacterial, viral, protozoal and parasitic origin. Upon invasion of microorganisms, as a first line of defense, various chemotactic cytokines (chemokines) send signals for accumulation of the mononuclear cells, such as monocytes and macrophages, at the site of pathogen attack. Besides, chemokines, a variety of other substances, including bacterial components and endotoxins, complement components, immune complexes, etc. can also attract macrophages. These cells may then phagocytose and kill the infectious agents by a variety of mechanisms [8]. In addition to the physical killing/elimination of the accumulated foreign substances or microbes, macrophages can activate immune system of the host as well [9]. In fact besides the dendritic cells and B lymphocytes, macrophages also play a central role in activation of the host's immune system. Moreover, tumour mass can also be infiltrated by the macrophages where they form an important mechanism of host defense against tumour cells, either by inhibiting the tumour cell division or killing the cells following secretion of soluble mediators or by other means [10,11]. Macrophages can also synthesize a large number of other substances involved in functions of diverse nature [12]. The secreted molecules might induce acute phase response [13], regulate haematopoiesis [14], help in cleansing and healing of injured tissue [15] and regulate the homeostasis [16]. Some of the molecules expressed in macrophages are supposed to be involved in regulation of atherosclerosis, diseases affecting nervous system or certain autoimmune disorders [17,18]. Interestingly, a variety of infectious diseases originating from facultative or obligate intracellular parasites rely on the intracellular parasitism as a strategy to defer immune onslaught [1-3].

Although, normally in the resting state, macrophages are activated by a range of stimuli in the course of their interaction with foreign substances. Phagocytosis of foreign substances (*cf.* Antigen) serves as an initial activating stimulus. In natural course, macrophages are activated by cytokines released by T helper cells, or by the molecules that are either secreted or are integral components of the external surface of the microbes. Among various potent activators of the macrophages, interferon gamma (IFN-γ) secreted by activated T_H cells, and tuftsin, a split tetra peptide from a leucophilic IgG, are the major ones.

Activated macrophages are more effective than the resting cells in eliminating the potential pathogens, because of their increased ability to kill the ingested microbes. In fact activation of macrophages is usually accompanied with an increased secretion of inflammatory mediators, and an increased ability to activate T cells. In addition, activated macrophages but not the resting cells, express various cytotoxic proteins that help them in eliminating a broad range of pathogens, including virus-infected cells, tumour cells and intracellular bacteria. Activated macrophages also express higher levels of class II MHC molecules, allowing them to function more effectively as antigen-presenting cells. In fact, macrophages and T_H cells during the immune response facilitate each other's activation.

Macrophages are capable of ingesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matters, such as injured or dead host cells, cellular debris, and activated clotting factors. In the first step in phagocytosis, macrophages are attracted by and move towards a variety of substances generated in an immune response; this process is called chemotaxis. The next step in phagocytosis involves adherence of the antigen to the macrophage cell membrane. Complex antigens, such as whole bacterial cells or viral particles, tend to adhere well and are readily phagocytosed, whereas isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed. Adherence induces membrane protrusions, called pseudopodia, to extend around the attached material. Fusion of the pseudopodia encloses the material within a membrane-bounded structure called phagosome, which upon entering the endocytic processing pathway, moves towards the cell interior, where it fuses with a lysosome to form a phagolysosome. Lysosomes basically are a collection of lysozyme and various other hydrolytic enzymes, which digest the ingested substances. The digested contents of the phagolysosome are then eliminated in a process called exocytosis.

The macrophage membrane has been reported to possess repertoire of receptors including those for Fc region of the antibody and certain complement factors. Incidentally, both of which also bind to antigen. If an antigen (e.g., a bacterium) is coated with an appropriate antibody or complement factor, it binds more readily to the macrophages membrane; as a result, phagocytosis is enhanced. The process by which particular antigens are rendered more susceptible to phagocytosis is called opsonization.

3. ANTIMICROBIAL ACTIVITY OF MONONUCLEAR PHAGOCYTES

A number of antimicrobial and cytotoxic substances produced by activated macrophages can destroy phagocytosed microorganisms. Many of the mediators of cytotoxicity are reactive forms of oxygen. In fact, macrophages are known to produce various reactive oxygen and reactive nitrogen intermediates that have potent antimicrobial activity. For example, phagocytosis is usually followed by a respiratory burst that play a major role in killing of the pathogens. This process results in the activation of a membrane-bound oxidase that catalyses the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is extremely toxic to the ingested microorganisms. The superoxide anion also generates other powerful oxidizing agents, including hydroxyl radicals and hydrogen peroxide. As the lysosome fuses with the phagosome, and the activity of the myeloperoxidase produces hypochlorite, the active agent of the household bleach, which is toxic to the ingested microbes.

A range of substances can activate macrophages. For example, bacterial cell-wall components, such as lipopolysaccharide (LPS) or muramyl dipeptide (MDP), together with a T-cell-derived cytokine (IFN-γ) or split tetrapeptide product of IgG known as tuftsin, can upregulate expression of nitric oxide synthetase (NOS), an enzyme that oxidizes L-arginine to L-citrulline and nitric oxide (NO), which have potent antimicrobial activity. The nitric oxide yields potent antimicrobial substances in combination with the superoxide anion. Recent evidences suggest that much of the antimicrobial activity of the macrophages against bacterial, fungal, helminthic, and protozoal pathogens is due to nitric oxide and substances derived from it.

Interestingly, macrophages can execute killing of the organisms in non-oxygen dependent manner as well. In fact, they can synthesize various hydrolytic ezymes which can hydrolyse a range of biological molecules without active involvement of oxygen. In addition, activated macrophages can also produce cysteine rich cationic peptides known as defensins; the molecules that contain six invariant cysteines, form a circular structure which is stabilized by the intramolecular disulphide bonds. The circularized defensin peptides have been shown to form ion-permeable channels in bacterial cell membranes, the feature that helps these peptides to kill a variety of bacteria, including *Staphylococcus aureus, Streptococcus pneumoniae, Escheri-chia coli, Pseudomonas aerugenosa and Haemophilus influenzae.* Activated macrophages also secrete tumour necrosis factor α (TNF-α), a cytokine that has a variety of effects and is cytotoxic to some tumour cells.

4. DRUG HOMING TO MONONUCLEAR PHAGOCYTES

Amongst the two most widely acclaimed delivery systems, viz. microspheres and liposomes that may specifically deliver entrapped material to macrophages, liposomes have been documented as microscopic vesicles composed of phospholipid bilayers surrounding aqueous compartments. The details of the prèparation methods of liposomes and their characteristics are described elsewhere [19,20]. Liposomes have been used extensively as drug carriers, and their potential applications in cancer chemotherapy, enzyme therapy, immuno-modulation, antimicrobial therapy, metal detoxification, diagnostics, and topical therapy have been reviewed elsewhere [20-24].

Injecting drugs encapsulated in liposomes always have an advantage as being colloidal in nature they are recognized as foreign particles and can readily be taken up by the phagocytic cells (cf. macrophages) and consequently, rapidly accumulate in the mononuclear phagocyte system (MPS). The inherent tendency of the liposomes to concentrate in MPS can be exploited to enhance the non-specific host defense against various intracellular pathogens by entrapping chemotactic or immunomodulatory molecules in them besides using them as carriers of antibiotics against various intracellular infections. Liposomal systems have been optimistically considered as "magic bullets" for more than three decades. Drug delivery with liposomes as a carrier system provides options and opportunities for designing bio-stable and/or site specific drug therapy. The engineered or tailored versions of liposomes that offer potential of exquisite levels of target specificity are of major concern these days. Depending on the site of targeting, liposomes may be modified by either grafting chemotactic ligands, such as peptides, polysaccharides or affinity ligands like antibodies, on the liposomes surface directly or by coating their surface with polyethylene glycol [25-27].

For treatment of diseases with lymphatic involvement it is desirable to develop approaches to deliver diagnostic, therapeutic and immunomodulatory agents to the lymph nodes [28-30]. Liposomes carrying saccharides on their surface showed enhanced absorption from the injection site and also an enhanced lymph node uptake compared to the control liposomes [31]. Further, liposomes coated with the non-specific human antibodies upon injecting subcutaneously exhibited a modestly increased lymphatic absorption and lymph node uptake, compared to liposomes without the antibody [32]. Moreover, an administration of anti-HLA-DR immunoliposomes resulted into their 3-fold higher accumulation in regional lymph nodes, as compared to the conventional liposomes [33]. Furthermore, poly ethylene glycol (PEG) coated liposomes were found to avoid uptake by the lymphatic system as compared to the plain liposomes [34,35]. This apart, a novel method to enhance the lymph node uptake of biotin coated liposomes has been explored recently [36].

It has been shown that PS-exposure on the cell surface serves as a signal for triggering recognition by the macrophages [37]. Also the macrophages have been shown to phagocytose the large size liposomes more efficiently than the smaller ones [38]. In addition, grafting of the tetrapeptide tuftsin on the liposomes surface would enable the liposomes not only in homing the liposomised drug to the macrophages but also it would stimulate these cells non-specifically against infections [39-44].

Analysis of the intracellular trafficking patterns of the liposomal antigens reveals that after being phagocytosed by the macrophages, liposomal antigen readily escapes from the endosomes into the cytoplasm of the macrophages [45]. Moreover, liposomes made up of lipids with fusogenic properties have been shown to deliver their entrapped molecules

in the cytosol of the target cells more efficiently than the conventional forms of liposomes. On the other hand, the membrane lipid composition of microorganisms exhibits a great majority of anionic phospholipids that play a pivotal role in membrane-membrane fusion [46]. As a result, liposomes derived from the membrane lipids of various microorganisms have been used for targeting to macrophages as well as dendritic cells [47-50]. Recently, it has been demonstrated that antigens entrapped in liposomes made up of lipids from *E. coli* and edible yeast generate a strong humoral as well as cell-mediated immune responses [51-53]. Moreover, sterically stabilized liposomes have been found to possess increased stability and play major role in $CD8^+$ T-cell response upon their targeting to the mature as well as immature dendritic cells. In a more recent study, PorA, a major antigen of *Neisseria meningitidis*, was purified and reconstituted in different types of liposomes, such as the liposomes having mannose or phosphatidylserine on their surface for targeting to dendritic cells (DCs). The use of targeted PorA liposomes resulted in an improved uptake by and activation of dendritic cells and also in an increased localization in the draining lymph nodes [54].

The complex interaction of liposomes with phagocytic cells is described in different steps that include stable adsorption to the cell surface, cellular uptake of intact vesicles by an energy-dependent mechanism and lysosomal degradation of the liposomes and their contents. Liposome adsorption to the cell surface seems to be the rate limiting step, since it can be assumed that stably adsorbed vesicles are more susceptible to subsequent uptake than the vesicles that are loosely attached with the cell surface. Several prerequisites have to be successfully met that eventually enable liposomes to deliver biologically active agents to the macrophages: (I) liposomes must readily bind to and be phagocytosed by free and fixed phagocytes, (II) they must prevent degradation of the entrapped drug, (III) they must retain the encapsulated agent for delivery to the intracellular compartment of the RES cells, and (IV) they must localize to the macrophages in organs where metastasis or macrophage-associated disorders occur [22].

In general, liposomes are the most widely studied carrier used for macrophage-specific drug delivery. However, the extent of the liposomes binding and subsequent ingestion by macrophages depends on a number of features of the lipid vesicles. These include composition, type, size and surface properties of liposomes. For example, negatively charged liposomes associate more effectively and deliver their content more efficiently to the macrophages than the neutral liposomes [55-58]. Similarly, smaller liposomes deliver drugs more effectively than larger one; presumably due to their efficient internalisation [59]. Further, it has been shown that positively charged [60] and large sized liposomes [61] can improve the liposome uptake, as compared to their counterparts.

Systematic evaluation of multilamellar vesicles (MLV) with different phospholipid composition reveals that certain classes of phospholipids are recognized preferentially by the macrophages [35,37]. For example, inclusion of the negatively charged phospholipids, such as phosphatidylserine (PS), phosphatidylglycerol in MLV consisting of phosphatidylcholine (PC), greatly enhances their binding to and phagocytosis by the macrophages. In contrast, neutral MLVs composed exclusively of PC are internalized by these cells with significantly lesser efficiency. In addition, it was observed that liposome uptake increased linearly with the incubation time and concentration. Higher uptake was observed with smaller and negatively charged liposomes. And inclusion of increasing amounts of cholesterol and sphingomyelin resulted in a decreased uptake in the macrophages [55-58].

The efficacy of the liposomal drug formulation is influenced also by the melting phase transition temperature of the liposomal phospholipids [62,63]. Thus, encapsulation of ampicillin in liposomes prepared from distearoylphosphatidyl-choline (DSPC), and dipalmitoylphosphatidylglycerol (DPPG) resulted in delayed intracellular killing of *L. monocytogenes,* as compared to the liposomes composed of cholesterol, unsaturated phosphatidyl cholines and phosphatidyl serines. Like the lipid composition, the lamellarity and size of the liposomes may also affect significantly the efficacy of the encapsulated agents [64]. This apart, polyethylene glycol-coated liposomes, known as stealth liposomes, are not readily taken up by the macrophages in the reticuloendothelial system and thus, stay in the circulation for relatively longer periods of time [65-67]. In addition, incorporation of different polymers into liposomes has been shown to enhance the half-life of the liposomes in blood circulation in a concentration-dependent manner [67]. On the other hand, grafting of some specific peptides on the liposomes surface has been reported to significantly enhance their uptake by the macrophages, and also the anti-microbial activity of these cells [68,69].

Macrophages possess a number of receptors, such as Fc receptors and receptors for complements, fibronectin, lipoprotein, mannosyl, galactosyl and many other ligands [6]. These macrophage surface receptors regulate various macrophage activities, such as activation, recognition, endocytosis, secretion etc. A useful approach for promoting the uptake of liposomal content by the macrophages is to incorporate on the liposomes surface the ligands that can interact with the macrophage surface receptors [70]. Numerous investigators have shown that uptake of the ligand-incorporated liposomes is significantly higher than the ligand-free liposomes [71-77]. For example, incorporation of neoglycoprotein or the ligands that contain terminal mannosyl residues on the liposomes surface leads to their selective uptake by the macrophages [77-79]. Similarly, liposomes composed of mannosylated myo-inositol (extracted from the cell wall of microorganisms) have been shown to be preferentially taken up by the peritoneal macrophages [78]. Further, both *in vitro* and *in vivo* uptakes of the liposomes have been shown to significantly increase in the macrophages by targeting (the liposomes) through Fc surface receptors [79-81].

The incidences of the life-threatening fungal infections, over the last several years, have dramatically increased particularly among cancer, diabetic, and immunocompromised patients [82]. This may, however, partly be attributed to the advancement in the field of medical sciences that have made possible the improved recognition and diagnosis of the fungal infections. Besides the prolonged survival of the patients with defects in their defense mechanisms, more invasive

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surgical procedures, development of resistant strains to currently available anti-fungal drugs, and the increased number of patients contracting AIDS are some of the reasons that provide ample opportunities to the opportunistic fungi for their establishment, leading to the full blown disease. The emergence of fungi as clinically important pathogen has been well documented, although their role in pathogenesis of human infection has only recently been appreciated [82,83].

Various antifungal chemotherapeutic agents available include polyenes, azoles, allylamines, morpholines, flucytosine, griseofulvin, iodides, hydroxy stibamine and imidazole classes of drugs [84,85]. However, the drug of choice for most systemic mycosis is a polyene antibiotic amphotericin B (Amp-B) that interacts with ergosterol present in the fungal cell membranes, creating transmembrane channels which permit the escape of vital ions and metabolites. In spite of its promising antifungal properties, the drug had limited use due to its systemic toxicity. The efforts for reducing toxic side effects of Amp-B by its chemical derivatization have remained elusive [86].

Reports from many groups demonstrate that liposome encapsulated Amp-B is effective against drug-sensitive as well as resistant fungal infections, e.g. aspergillosis, candidiasis, cryptococcosis, histoplasmosis and fusarium infections [39-41]. Further, it has been shown that incorporation of Amp-B in liposomes successfully reduces Amp-B nephrotoxicity, which results in improved therapeutic index [39]. Following the promising clinical results, three formulations containing Amp-B (Abelcet, Amphocil and AmBisome) are currently available for human use [87].

Keeping into consideration the fact that cell mediated immune response involving activation of the mononuclear phagocytes by sensitized T-cells play a key role to control fungal infections [88], treatment with antifungal drugs along with the agents that provoke macrophages/monocytes may have an advantage. That this indeed is the case has been demonstrated by the considerably high efficacy against systemic fungal infections of Amp-B encapsulated in the liposomes that contained tuftsin on their surface [39]. This Amp-B formulation (Tuft-lip-Amp-B) was effective against not only the sensitive but also the resistant strains of *Candida albicans* [41]. An evaluation of the effect of liposomised tuftsin on T-cell proliferation as well as antibody production reveals that tuftsin by itself elicits a strong immunopotentiating effect besides homing the liposomes to macrophages. Pre-treatment with tuftsin bearing liposomes prior to challenging the animals with drug-resistant *C. albicans* has been shown to render the animals resistant to the infection [41].

Nystatin encapsulated in tuftsin bearing liposomes has been evaluated for its potential use against isolates of *C. albicans* showing lesser susceptibility to Amp-B [89]. Although the liposomised Amp B in higher doses was found to be effective in elimination of less susceptible strain of *C. albicans* in BALB/c mice, but it cannot be recommended due to toxicity constraints. However, liposomal nystatin at a dose of 5mg/kg was shown to possess higher efficacy (survival 40 %), compared to Amp B, and could thus be used for treatment of drug-resistant *C. albicans* infections. In addition, nystatin encapsulated in tuftsin bearing liposomes has recently been evaluated against candidiasis in cylophosphamide-treated neutropenic BALB/c mice. The role of tuftsin in the activation and maturation of leukocytes was analysed by treating the animals with tuftsin after cyclophosphamide injection. A single peritoneal injection of tuftsin (50 μg/animal) for three consecutive days, significantly increased the numbers of leukocytes in the treated animals, as compared to the animals that did not receive such injections [41]. The neutropenic mice on challenging with *C. albicans* followed by treatment with nystatin loaded tuftsin bearing liposomes showed increased survival, as compared to Amp-B administered after its encapsulation in tuftsin-containing liposomes in identical conditions.

Besides the fungal infections, liposomisation also enhances anti-bacterial efficacy of some of the chemotherapeutic agents. For example, encapsulation of streptomycin, isoniazid and rifampicin in liposomes has been shown to increase their efficacy against *Mycobacterium tuberculosis* [90,91]. Further, the antimycobacterial activity of liposomised rifampicin has been demonstrated to considerably increase upon grafting tuftsin on the surface of liposomes containing rifampicin [92]. Moreover, other aminoglycoside classes of antimycobacterial drugs, such as amikacin and gentamycin, have also been shown to efficiently control mycobacterial infections after their encapsulation in liposomes. Since *Mycobacterium* sps are intracellular pathogens, the higher efficacy of antitubercular drugs can be directly correlated to the liposome mediated passive delivery of drug molecules to the macrophages.

Leishmania is a protozoan parasite which causes visceral, cutaneous, mucocutaneous and diffuse cutaneous types of leishmaniasis in humans. Amongst these, the most devastating clinical form, visceral leishmaniasis (Kala-azar), is caused by *Leishmania donovani*, which if left untreated is usually fatal. The major front line drugs available for treatment of this disease are usually very toxic, and to some of which, such as antimoniates, the parasite has developed resistance due to their indiscriminate use. It has, therefore, become essential to develop alternate strategies to combat this infection. Interestingly, some of the antifungal drugs like griseofulvin, Amp B and 5-fluorocytosine have been shown to exhibit an enhanced efficacy against leishmaniasis when used in the liposomal form [93].

Since *Leishmania* parasite, primarily colonizes the mononuclear macrophages, antiparasitic activity of antileishmanial drugs is expected to significantly increase by their encapsulation in liposomes. As a matter of fact, the very first use of liposomal drugs in treatment of infectious diseases was made in case of *Leishmania* only. It has been shown that liposomised antimoniates are 700-1800 times more effective than the free drug in controlling leishmaniasis [93]. Recently, antimonials entrapped in liposomes containing the negatively charged lipid, phosphatidylserine, which is known to selectively interact with the macrophage surface scavenger receptors, have been reported to have considerably higher efficacy, as compared to other liposomal formulations [94]. Further, Hamycin encapsulated in mannose coated liposomes has been used in treatment of the experimental leishmaniasis in hamsters [95]. Apart from this, primaquine-loaded liposomes after grafting a chemotactic peptide on

their surface have been reported to be quite effective against experimental leishmaniasis [68]. Such peptide-grafted liposomes were found to be rapidly cleared from the blood circulation (half-life ~2 min) and taken up by the cells of the reticuloendothelial system. Furthermore, doxorubicin encapsulated in parasite-specific antibody coupled liposomes has been used successfully against visceral leishmaniasis in BALB/c mice [96]. Besides, antileishmanial efficacy of liposomised sodium stibogluconate has been shown to further increase (up to 200 times) by grafting tuftsin on the liposomes surface [44].

In our recent studies, we examined chemotherapeutic efficacy of Amp-B after its encapsulation in tuftsin-free as well as tuftsin bearing liposomes against *L. donovani* infections that were resistant to the conventional chemotherapy with antimonials [97]. The antileishmanial activity of Amp-B was significantly increased ($P < 0.05$) upon delivering Amp B in tuftsin free liposomes. This activity was further increased ($P < 0.05$) by co-administration of liposomised Amp-B with tuftsin. These results clearly suggested that efficacy of the various antileishmanial drugs can be substantially improved by their delivery in liposomes and that the macrophage-activation by immunomodulators, like tuftsin, may significantly improve the activity of the liposomised drug preparations. In addition to tuftsin, uptake of liposomes by the macrophages can also be enhanced by incorporation of fibronectin or galactosyl residues on the liposomes surface [98-100].

Beside the liposomes, nanoparticles and microspheres have also been extensively examined for their use as carriers for delivering drugs to the macrophages. They may be polymeric-biodegradable or nondegradable and proteinaceous in nature. The term microsphere, is defined as a spherical particle with size range varying from 50 nm to 2 um, containing a core substance. Microspheres are monolithic or matrix-type microparticles. In contrast to microspheres, nanoparticles are in the size ranging between 10 and 1000 nm. Nanoparticles are collective names for nanospheres and nanocapsules. The former have a matrix structure, drug or tracers may be adsorbed at their surface, entrapped in the particle or dissolved. The latter have a polymeric shell and inner liquid core. Preparation and characterization techniques of these particulate carriers have been reviewed extensively [101-104].

In a manner similar to liposomes, size, surface property, composition, concentration, and hydrophilicity or lipophilicity of microspheres and nanoparticles play a significant role in their uptake by macrophages [105]. The events of phagocytosis include contact with pseudopods of macrophages followed by their engulfment into the cytoplasm by lamellipods [106]. Hydrophobic and relatively large microspheres are more susceptible to phagocytosis than their hydrophilic counterparts. Likewisely, nanoparticles with lipophilic coating are taken up by macrophages more readily as compared to their hydrophilic counterparts. The extent of phagocytosis can be improved by coating the particle surface with opsonic materials and activating macrophages with various activating factors. Incubation time and dose of the vehicles can also control the process of phagocytosis.

The influence of surface charge and size of microspheres on their phagocytosis by mouse peritoneal macrophages were studied by using polystyrene and phenylated poly-acrolein microspheres of different diameter as well as modified cellulose microspheres with different surface charge [107]. It was found that efficiency of uptake was maximum when size range of microsphere was of the order of 1-2 μm. For both negatively and positively charged particles, the extent of phagocytosis was increased with increasing zeta potentials, and was the lowest when zeta potential was zero [108]. However, there was no significant difference in the phagocytosis between the cationic and anionic surfaces when compared at a zeta potential of the same absolute value. Modified cellulose microspheres were allowed to incubate with macrophages in order to study the influence of the hydrophobicity on macrophage phagocytosis. Hydrophobic microspheres prepared from benzoyl cellulose were the most susceptible to phagocytosis and the non-ionic hydrophilic one was the least. It was observed that an optimal surface hydrophobicity is necessary for the microspheres to be phagocytosed [109].

Similarly, nanoparticles made from polyalkylcyanoacrylate, polymethylmethacrylate, and human serum albumin microspheres have been used to study the influence of various parameters that can regulate uptake by human macrophages. The incorporation of lipophilic polymethylmethacrylate in nano particles was found to result in better phagocytosis, when compared with polyalkylcyanoacrylate nanoparticles of similar size range [109]. Polybutylcyanoacrylate nanoparticles coated with lipophilic Pluronic F68, a biocompatible poloxamer, increased phagocytosis by nearly 50%, while Pluronic F108 had no influence. Nanoparticles of the same material were phagocytosed to a larger extent if they were of larger diameter. For example, phagocytosis of nanoparticles made from human serum albumin of 1.5 μm in diameter was higher than that of 200 nm in diameter. Recently, it has been found that polyethylene glycoldistearate incorporated microspheres can modify the extent of the phagocytosis depending on the concentration of the excipient used [110]. It seems that microsphere association or uptake by macrophages might be a saturable process [111]. For a given cell density, uptake of particles into macrophages was also dose-dependent. The most avid uptake was observed with a microsphere dose of 1 mg/l, however, there was a gradual reduction in the uptake as microsphere dose was increased further [111].

In a manner that is typical of particulate delivery system, coating of microspheres with opsonic materials and amphiphiles can modify significantly the extent of phagocytosis by macrophages depending on the state of macrophage activation. Several proteins such as gamma-globulin, human fibronectin, bovine tuftsin, and gelatin enhance the phagocytosis, while bovine serum albumin reduce the phagocytosis of cellulose microspheres [112]. It was also observed that the presence of fetal calf serum increases the phagocytosis of gelatin-grafted cellulose microspheres, while showing no affect on other protein grafted-microspheres. The influence of cross-linking and concentration of gelatin microspheres on the phagocytosis by mouse peritoneal macrophages have also been studied. Gluteraldehyde mediated cross linking of gelatin microspheres was found to facilitate interferon targeting to macrophages [113]. The study showed that phagocytosis of microspheres decreased with decreasing concentration of gelatin and glutaraldehyde, and was proportional to the

amount of microspheres added, until a saturation of phagocytosis was observed at higher doses of microspheres. It was also demonstrated that precoating or surface immobilization with gelatin was the most effective method to enhance the phagocytosis among all other opsonic proteins. Phagocytic uptake of polystyrene microspheres, coated with a series of perfluoroalkylated amphiphiles derived from phosphocholine and polyethylene glycol, was studied in the presence or absence of serum and peritoneal macrophages were used as phagocytic cells [114]. Phagocytic uptake tests carried out at 37 °C showed that microspheres coated with any surfactant cause a decrement in the phagocytosis in both the conditions, i.e. either in the presence or absence of serum. However, the extent of decrease varied among the nature of the surfactants, and in most instances, the presence of serum had no influence on the phagocytosis when microspheres were coated with the same surfactant.' Microspheres from some biodegradable substances such as copolymers of polylactic acid and poly-glycolic acid [115,116], cross-linked potato starch [117], hydroxyethyl starch [118], and cross linked starch, dextran, lichenan and mannan are found to be successfully phagocytosed by macrophages [119].

Finally, we can conclude that despite the fact that most of the important factors influencing the targeting of various carriers to macrophages have been studied extensively, very little attention has been paid to the factors relevant to drug materials themselves such as molecular weight, position in the particles, concentration, physicochemical parameters. Much work remains to be done on the environmental factors such as hydrogen ion concentration, polarity, ionic strength and presence of enzymes. It can be envisaged that this may require a lot more to decipher role of various yet unknown factors in targeting of drugs to this important cell of the immune system. It is quite heartening to note that a lot many liposome-based formulations have already been introduced in the market [87], and some lipid based vehicles having muramyl peptide have been successfully employed in treatment of retractable melanoma and relapsed osteosarcoma in human subjects [120-122]. However, no serious efforts have been made to investigate the relevance of tuftsin-based formulations against treatment of various macrophage related ailments. It would be imperative to emphasize, that the magic peptide that has potential to establish itself as panacea, ought to get due attention.

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