Chapter 3
Infectious Diseases: Need for Targeted Drug Delivery

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3.1 Infectious Diseases in the Modern World

Infectious diseases are among the leading cause of death worldwide, even in the twenty-first century. Developing nations are more susceptible due to lack of proper sanitation, uneducated population and increasing pollution and the booming population explosion. Tuberculosis, HIV/AIDS, malaria are infectious diseases that have become epidemic in a true sense. According to a 2004 World Health Organization (WHO) report, infectious diseases are a major cause of morbidity in developing countries. A more recent report in 2012 records the death of more than 8.7 million people worldwide in 2008, due to infectious diseases. Diseases earlier confined to particular territories have changed face as global epidemics, due to globalisation and cross movement of people across geographical boundaries. A classic example is swine flu which originated in Asia and rapidly spread to the west.

3.1.1 Extracellular and Intracellular Infectious Diseases

Several microorganisms survive in the extracellular spaces within the body, or on epithelial surfaces, to cause extracellular infections. Extracellular pathogens release specific toxins or proteins which triggers the production of antibodies. On the other hand, intracellular infections reside within the cells of the body's defence system the reticuloendothelial system (RES). The normal body response to a pathogen is rapid opsonisation followed by phagocytosis, which results in killing and clearing

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Table 3.1 Infectious diseases and causative organisms

Intracellular diseases	
Infectious diseases	Causative organisms
AIDS/HIV	Human immunodeficiency virus
Cholera	*Vibrio cholerae* (bacteria)
Dengue	Dengue (RNA) virus
Hepatitis A/B/C	Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV)
Influenza	RNA viruses (Influenza A/B/C viruses)(e.g. H1N1)
Legionellosis	Legionella
Leishmaniasis	*Leishmania donovani*
Listeriosis	*Listeria monocytogenes*
Malaria	*Plasmodium* sp.
Shigellosis	*Shigella*
Tuberculosis	*Mycobacterium tuberculosis*
Typhoid	*Salmonella typhi*
Tularemia	*Francisella tularensis*
Extracellular diseases	
African trypanosomiasis	*Trypanosoma brucei gambiense, Trypanosoma brucei rhodesiense*
Pneumonia	*Streptococcus pneumonia, Haemophilus influenza, Chlamydophila pneumonia, Mycoplasma pneumonia, Staphylococcus aureus, Moraxella catarrhalis, Legionella pneumophila, Klebsiella pneumonia; rhinoviruses, coronaviruses, influenza virus, respiratory syncytial virus (RSV), adenovirus, and parainfluenza*
Schistosomiasis	*Schistosoma mansoni, Schistosoma intercalatum, Schistosoma haematobium, Schistosoma japonicum, Schistosoma mekongi*

of the microorganism. Intracellular infections result when the organisms cleverly evade destruction following phagocytosis. The intracellular location of these microorganisms protects them from the host defence mechanisms, such as antibodies or complement, and from the action of drugs that are unable to penetrate the cell efficiently. Hence, while adequate drug concentrations are readily achieved at extracellular infection sites to enable efficient therapy, intracellular infections are more difficult to treat. Some common intracellular and extracellular infectious diseases and their causative organisms are listed in Table 3.1.

3.2 Reticuloendothelial System and Intracellular Infections

The RES also known as the mononuclear phagocytic system (MPS)/macrophage system is the primary defence mechanism of the human body and hence the site of intracellular infections. The macrophages constitute the major defence cells of the RES. Derived from the bone marrow the RES also contributes to both non-specific and specific immunity. Recognition by the RES is facilitated by opsonins, with the step of opsonisation being a precursor to phagocytosis.

3.2.1 Opsonisation

Opsonisation is the process by which bacteria are altered by opsonins so as to become more readily and efficiently engulfed by phagocytosis. Opsonisation is mediated by the complement system: C3b, C4b, and iC3b, antibodies IgG and IgM and mannose-binding lectin. Mannose binding lectin initiates the formation of C3b. Opsonisation of particles enables recognition by the Fc receptors, complement receptors or specific receptors for phagocytosis. Opsonins are generally proteins which can bind to pattern-recognition receptors (PRRs) or other specific receptors expressed on the surface of macrophages. Pentraxins [C-reactive protein and serum amyloid P] [1], mindin, collectins [2] and ficolins [3] are such opsonins. The function of pattern-recognition receptors (PRRs) is to recognise and enhance phagocytosis of pathogen-associated molecular patterns (PAMPs), specific patterns present on microbial pathogens like lipopolysaccharide (LPS) in Gram-negative bacteria, lipotechoic acid (LTA) in Gram-positive bacteria and mannans in yeast. Toll-like receptors (TLRs) are PRRs essential for recognition of microbial components such as TLR4 (LPS) [4–6], TLR3 [double-stranded RNA] [7], TLR6 [mycoplasmal macrophage-activating lipopeptide—2 kDa] [8], TLR9 [CpG bacterial DNA] [9], TLR5 [bacterial flagellin] [10], and TLR2 [peptidoglycan]. However, the exact mechanisms of TLR recognition of microbial components remain unclear.

3.2.2 Phagocytosis

Opsonisation facilitates adherence of pathogens to macrophages, and is facilitated by integrins. Adherence induces membrane protrusions, called pseudopodia, to extend around the attached material. Following fusion with the macrophage, the pseudopodia forms a phagosome that encloses the pathogen within a membrane, which then enters the endocytic process. Phagosomes coalesce with intracellular organelles to mature into phagolysosomes, which have an acidic environment with many digestive proteins which finally degrades the internalised material. Phagocytised material is eliminated by exocytosis. The process of phagocytosis is mediated by several proteins such as actin, dynamin and cortactin. While actin is connected to the lipidic membrane and responsible for invagination of the membrane to form the endosome, cortactin is an actin-binding protein which stimulates its polymerisation. Dynamin hydrolyses guanidine triphosphate and uses the resulting energy for the contraction of actin and formation of endosome. Particulates that cannot be digested remain sequestered in residual bodies within the cell. Other cells such as fibroblast, endothelial and epithelial cells also exhibit phagocytic activity and can engulf microbes like Shigella, Listeria and Yersinia [11]. Such phagocytosis is mediated by laminin and fibronectin receptors/heparan sulfate present on the membrane surface [11]. However, the major cells responsible for phagocytosis are macrophages.

3.2.3 Macrophages

Macrophages (Greek: makros means large and phagein means eat) are cells formed by the differentiation of monocytes in tissues. Macrophages play an important role in both innate and adaptive immunity in vertebrates. These specialised phagocytic cells engulf and destroy infectious microbes, foreign particles and cancer cells [12]. The macrophages also regulate lymphocyte, granulocyte populations and important tumor growth modulators [13]. Macrophages act by both oxygen-dependent killing and oxygen independent killing mechanisms. The mediators for oxygen-dependent killing are reactive oxygen intermediates (ROIs) (superoxide anion, hydroxyl radicals, hydrogen peroxide and hypochlorite anion), reactive nitrogen intermediates (RNIs) (nitric oxide, nitrogen dioxide and nitrous acid) and monochloramine, while the mediators for oxygen independent killing are defensins, tumor necrosis factor (macrophage only), lysozyme and hydrolytic enzymes. Floating macrophages predominate in the vascular system, while tissue macrophages are localised in specific tissues. Based on the tissue of residence they have specific nomenclature (Fig. 3.1).

Macrophages can be classified mainly into two groups: (1) pro-inflammatory or classically activated macrophages (M1) and (2) anti-inflammatory or alternatively activated macrophages (M2).

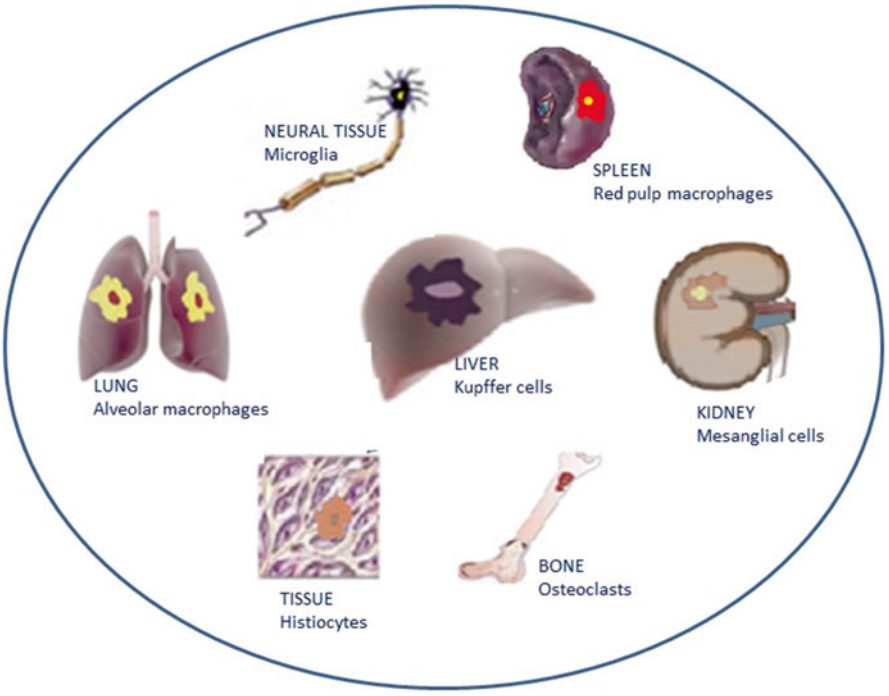

Fig. 3.1 Tissue macrophages and their organs of residence

3.2.3.1 Activated Macrophages (M1)

M1 macrophages are immune effector cells that aggressively work against microbes and cause their destruction much more readily. M1 is mainly associated with gastrointestinal infections (e.g. typhoid fever and *Helicobacter pylori* gastritis) and active tuberculosis. M1 macrophages are stimulated by interferon (IFN)-g or lipopolysaccharide (LPS) to release nitric oxide (NO), important for killing intracellular pathogens. Activated macrophages are characterised by expression of major histocompatibility molecule like MHC class II and CD86 and their ability to secrete proinflammatory cytokines such as tumor necrosis factor (TNF)-a, IL-1b, IL-12, IL-18 and the chemokines CCL15, CCL20, CXCL8-11 and CXCL13 [14]. Activated M1 macrophages facilitate killing of microorganisms by endocytosis, synthesising reactive oxygen intermediates (ROI), limiting the uptake of nutrients and iron essential for the growth of bacteria and replication of viruses, or production of nitric oxide facilitated by IFN-g-inducible NO synthase (iNOS).

3.2.3.2 Alternative Activated Macrophages (M2)

M2 macrophages are important for killing extracellular parasites, wound healing, tissue repair, and to turn-off immune system activation. M2 macrophages are activated by interleukin (IL)-4 or IL-13 (M2a) to produce IL-10, transforming growth factor (TGF)-b and arginase-1 (Arg1), to enable this function [14]. M2 macrophages are mostly observed in lepromatous leprosy, Whipple's disease and localised infections (keratitis, chronic rhinosinusitis).

A number of infectious organisms which manage to overcome the RES defence develop unique adaptive mechanisms which enable them to survive within the cell for prolonged periods of time. Eradication of such intracellular organisms poses immense challenges.

3.2.4 Survival Mechanisms Adapted by Pathogens

Many pathogens have an innate ability to develop adaptive mechanisms under stress conditions to fight for their survival. Such adaptive mechanisms or protective strategies, enables them to exhibit greater defence to the host and there by prolong survival. The different adaptive mechanisms employed by pathogens are discussed below.

3.2.4.1 Inhibition of Phagolysosome Formation

Strategies adopted by microorganisms to inhibit phagolysosome formation include interference with the transformation of primary endosomes into late endosome, fusion with lysosomes and or phagosome acidification. This delays the fusion of

Table 3.2 Mechanisms of inhibition of phagolysosome formation

Mechanism	Pathogens	Diseases	References
Enzymatic breakdown	*Mycobacterium tuberculosis*	Tuberculosis	Sturgill-Koszycki et al. [18]
	Mycobacterium leprae	Leprosy	Frehel and Rastogi [19]
	Listeria monocytogenes	Listeriosis	Alvarez-Dominguez et al. [20]
	Salmonella enteric	Salmonellosis	Buchmeier et al. [21]
	Leishmania spp.	Leishmaniasis	Desjardins et al. [22]; Mosser et al. [23]
	Toxoplasma gondii	Toxoplasmosis	Sibley [24]
	Helicobacter pylori	GIT infections	Borlace et al. [25]
	Trypanosoma cruzii	Trypanosomiasis	Ochatt et al. [26]
Lack of acidification	*Yersinia pestis*	Pneumonia, septicemia	Pujol et al. [17]
Disturbs the formation of lipid rafts by producing beta-1,2 glucans	*Brucella* spp.	Brucellosis	Roy [27]
Alteration of host cell signaling by dephosphorylation of signal regulated kinase	*Leishmania* spp.	Leishmaniasis	Ghosh et al. [28]

endosomes with lysosomes [15] or blocks the same [16]. The strategies to inhibit phagolysosome formation and the pathogens which exhibit the same [17] are summarised in Table 3.2.

3.2.4.2 Fusion of Endosome with Cell Organelles Other than Lysosome

Pathogens which exhibit this adaptation survive and multiply in vesicles formed by fusion of endosomes with cell organelles other than the lysosome, such as the rough endoplasmic reticulum, ribosome or mitochondria [29] and thus avoid phagolysosome formation. They thereby bypass destruction due to the enzymatic activity in the lysosome [30].

3.2.4.3 Disruption of the Phagolysosome

Escape from endocytosis is a crucial step for intramacrophagic survival. Pathogens from this category contain lytic enzymes which enable them to break the endosomes membrane and disrupt membrane of the vacuole [31], and hence evade degradation in the phagolysosome, and enter the cytosol rich in nutrients [32]. Specific enzymes are produced by the microorganisms for instance, *L. monocytogenes*

produces listeriolysin O (LLO) [33] and haemolysin C [34] while phospholipases are produced by the *Rickettsia* spp. [35].

3.2.4.4 Survival in the Late Phagolysosomes

The microbes in this category exhibit virulence factors which allow them to survive in lytic enzymes, acidic conditions and oxidants, the harsh conditions in the phagolysosome environment. Intramacrophagic resistance employing multiple virulence factors enables alternative pathways for survival and multiplication [36].

3.2.4.5 Internalisation by Non-phagocytic Pathways or by Parasitophorous Vacuole

Pathogens are internalised into macrophages by alternate routes. They traverse inside the cell by receptor mediated pathways like clathrin [37] and lipid rafts [38]. Formation of vesicles with new properties after fusion between the pathogen and membrane of the cell, like the parasitophorous vacuole formed by *Toxoplasma gondii* [38] also provides protection. In certain infections successful fusion of microorganisms with the macrophage is followed by secretion of antiapoptotic molecules (e.g. Bcl2). This results in impairment of apoptosis of the infected cells. Table 3.3 summarises illustrative examples of pathogens and their adaptive mechanisms for survival.

In addition to the adaptive mechanisms certain microbes employ highly specific strategies for persistence inside the cell. Such strategies are discussed with reference to some important diseases.

3.2.5 Specific Approaches of Some Important Pathogens for Persistence Inside the Cell

3.2.5.1 Tuberculosis

The adaptive mechanisms of *Mycobacterium tuberculosis* to survive inside the macrophages are prevention of fusion of the phagosome with lysosomes by producing tryptophan–aspartate-containing coat protein (TACO). Transformation of primary endosomes into phagolysosomes is prevented by a number of actions that occur simultaneously. These include reduced levels of proton ATPase inside the endosomes [18] removal of the Phosphatidylinositol 3-phosphate (PI3P) [16] and coupling of the inducible nitric oxide synthase (iNOS) [53]. The *M. tuberculosis* cell envelop comprises mycolic acid which can interact with cholesterol in the plasma membrane [50]. Further, mycobacteria are taken up inside macrophages by multiple receptors. The complement receptors are among the most widely used

Table 3.3 Other adaptive mechanisms of pathogens for persistence in macrophages

Adaptive mechanisms	Pathogens	Disease	References
Fusion of endosome with cell organelles other than lysosome	*Legionella pneumophila*	Legionellosis	Sibley et al. [38]
	Toxoplasma gondii	Toxoplasmosis	Sibley et al. [38]
Disruption of phagolysosome	*Listeria monocytogenes*	Listeriosis	Dabiri et al. [39]
	Shigella spp.	Shigellosis	Van der Wel et al. [40]
	Mycobacterium tuberculosis	Tuberculosis	Schroeder et al. [41]
	Mycobacterium leprae	Leprosy	Schroeder et al. [41]
	Francisella tularensis	Tularemia	Santic et al. [42]
	Trypanosoma cruzi	Trypanosomiasis	Andrews et al. [43]
	Rickettsia spp.	Typhus fever	Winkler et al. [44]
Survival in the late phagolysosomes	*Leishmania* spp.	Leishmaniasis	Alexander et al. [45]
	Legionella pneumophila	Legionellosis	Clemens et al. [46]
	Coxiella burnetii	Q fever	Burton et al. [47]
	Yersinia pestis	Pneumonia	Straley et al. [48]
	Staphylococcus aureus	Septicemic Endocarditis Bacteremia	Miller et al. [49]
Internalization by non-phagocytic pathways or parasitophorous vacuole	*Escherichia coli*	GIT infections	Shin et al. [37]
	Mycobacterium tuberculosis	Tuberculosis	Gatfield et al. [50]
	Salmonella spp.	Salmonellosis	Catron et al. [51]
	Clostridium spp.	Q fever	Simons et al. [52]
	Streptococcus spp.	Meningitis, bacterial pneumonia, endocarditis	Simons et al. [52]
	Toxoplasma gondii	Toxoplasmosis	Sibley et al. [38]

receptors for mycobacteria, for both opsonised and non-opsonised entry [54–56]. Other receptors are mannose receptors that bind glycosylated structures on the bacterial surface [57]. Fc receptors that can internalise IgG-opsonised bacteria [58] and scavenger receptors [59, 60] have also been implicated in mycobacterial uptake. Uptake of mycobacteria by the complement receptor pathway protects it from the aggressive lysosomal compartment ensuring relatively hospitable conditions.

3.2.5.2 Salmonellosis

Salmonella specifically forms a glycolipid capsule or biofilm. Biofilm formation in salmonella is related to the multicellular and aggregative response of rdar [61], rugose [62], or lacy [63]. This multicellular behavior is a property of salmonellae [64]

and is responsible for elaboration of thin fimbriae like Tafi, curli [65], cellulose [66], and other uncharacterised extracellular polysaccharides. Together, these components form the extracellular matrix that confers resistance to acid and bleach and facilitates environmental persistence [62, 64, 67–70].

3.2.5.3 Fungal Infections

Pathogens which cause fungal infections adapt various mechanisms to increase their pathogenesis and survive inside macrophages. *C. albicans* contains superoxide dismutases (SOD) and catalase enzymes which are able to convert O^{2-} into molecular oxygen and hydrogen peroxide, thereby decreasing the scavenging and toxic effects of O^{2-} and H_2O_2 levels by certain reactions [71]. Further, *C. neoformans* evade phagocytic uptake by phenotypic switching. This mechanism is observed in yeast cells that express glucuronoxylomannan mucoid capsule that resist phagocytic uptake and cause high lethality in mice [72]. In case of *Aspergillus conidia* infection collectins, pentraxin proteins are essential for opsonisation, but their deficiency is responsible for high susceptibility to infection in immunocompetent mice. Furthermore, several enzymes such as elastases and proteases released by the fungus enable conidia to escape from phagocytic uptake by alveolar macrophages.

3.2.5.4 HIV Infection

In HIV-1-infected macrophages, the viral envelope protein induces macrophage colony-stimulating factor (M-CSF). This pro-survival cytokine down regulates the TRAIL (tumor necrosis factor-related apoptosis-inducing ligands) receptor and up regulates the anti-apoptotic genes Bfl-1 and Mcl-1 enabling HIV to survive inside the macrophages. HIV invades the macrophage through CCR5 a chemokine receptor and through binding of gp120 to CD4 [73]. Macropinocytosis as a route of entry of HIV-1 into macrophages [74] also enables intracellular protection.

3.2.5.5 Leishmaniasis

Leshmania prevent activation of macrophages by inhibiting secretion of cytokines such as the inflammatory response IL-1 and tumor necrosis factor beta (TNF-beta) or T-lymphocyte activation (IL-12) and produce various immunosuppressive signaling molecules, such as arachidonic acid metabolites and the cytokines TNF-beta and IL-10. *L. chagasi* induces TNF-beta production in the immediate environment of the infected human macrophage, and this may lead to inhibition of immune responses [75]. Further, this pathogen induces alteration of host cell signaling. Macrophages infected with *L. donovani* or *L. mexicana* have shown altered Ca^{2+} dependent responses, such as chemotaxis and production of ROI [76, 77].

3.3 Intracellular Targets

Based on the adaptive mechanisms microorganisms reside in different cells and at different locations in the cells. Treating diseases therefore, necessitates an understanding of both the resident cells and target organelles. Illustrative examples of microorganism and their cellular/organelles targets are listed out in Table 3.4.

3.4 Other Reticuloendothelial System Cells

The granulocytes are classified as neutrophils, eosinophils, or basophils on the basis of cellular morphology. Neutrophils play the major role in the body's defence.

3.4.1 Neutrophils

Neutrophils are produced in the bone marrow by hematopoiesis. They are released into blood where they circulate for 7–10 h and migrate into tissues where they have a life span of a few days. During infection the bone marrow releases more than usual

Table 3.4 Diseases and intracellular targets of pathogens

Intracellular diseases	Target Cell	Target organelle	References
AIDS/HIV	T cells, epithelial cells	Phagosome, nucleus	D'Orsogna [78]
Brucellosis	Macrophage	Phagosome/lysosome or vacuole, endoplasmic reticulum	Roop [79]; Celli [80]
Dengue	WBCs, hepatocytes, vascular endothelial cells	–	Libraty et al. [81]
Hepatitis B, C	Hepatocytes	Endoplasmic reticulum	Moradpour [82]
Herpes Simplex virus (HSV-2)	Epithelial cells, neural ganglion	Nucleus	Heinz et al. [83]
Influenza	Respiratory epithelial cells	–	Arnheiter et al. [84]
Legionellosis	Macrophages	Phagosome/lysosome or vacuole, endoplasmic reticulum	Tilney et al. [85]
Leishmaniasis	Macrophages	–	Handman et al. [86]
Listeriosis	Macrophages	Cytosol	Collins, [87]
Malaria	Hepatocytes, red blood cells	–	Moulder [88]
Salmonella infection	Macrophages	Phagosome/lysosome or vacuole	Trebichavsky [89]
Tuberculosis	Alveolar macrophages, dendritic cells	Phagosome/lysosome or vacuole	Skvortsov [90]
Tularemia	–	Cytosol	Al-Khodor [91]

number of neutrophils, which migrate to the site of the infection. They act by both oxygen-dependent and oxygen-independent pathways to kill microbes. Neutrophils exhibit a larger respiratory burst than macrophages and consequently are able to generate more reactive oxygen intermediates and reactive nitrogen intermediates. In addition, neutrophils express higher levels of defensins than macrophages do. Hence, neutrophils are more active than macrophages in killing ingested microorganisms.

3.4.2 Dendritic Cells

Dendritic cells are antigen-presenting cells and constitute 0.5–1 % of the leukocyte population in the peripheral blood mononuclear cells. They are found mostly in non-lymphoid tissues and organs such as skin, heart, liver, lungs, and mucosal surfaces. The function of these cells is to initiate, stimulate and regulate a T cell response which includes antigen-specific T lymphocytes, Th1/Th2 modulation, regulatory T cell induction and peripheral T cell deletion. There are four types of dendritic cells, i.e. Langerhans cells, myeloid dendritic cells, plasmacytoid dendritic cells and infiltrating inflammatory dendritic epidermal cells. CD1b, CD11a, CD11b and CD11c, the thrombospondin receptor (CD36), and the mannose receptor (CD206), present on inflammatory dendritic epidermal cells, are known to be involved in the uptake of bacterial components. In case of *Mycobacterium tuberculosis* infection, alveolar macrophages (dust cells), along with dendritic cells engulf bacteria and exhibit innate as well as an adaptive immune response. Combined efforts by macrophages and dendritic cells establish protective immunity in 90 % of infected individuals.

3.4.3 Natural Killer Cells

Natural killer cells (NKC) are non-phagocytic cells present mostly in mammalian and avian species [92]. NKC express surface receptors for the Fc portion of IgG and their function is to mediate antibody-dependent cytotoxicity against tumor target cells [93]. It is also suggested that NKC play a role in resistance against some microbial infections. NKC also play a role in natural genetic resistance to infections caused by *cytomegalovirus* and *herpes simplex type I* [94, 95]. However, there is also evidence against the role of NKC in resistance to some other viruses [96].

3.4.4 Lymphoid Cells

Lymphocytes are cells present 99 % in the lymph and constitute 20–40 % of the body's white blood cells. There are approximately ~10^{10}–10^{12} lymphocytes in the human body, and this can vary with body weight and age. They circulate in the lymph and

blood, and can migrate into tissue spaces and lymphoid organs, enabling integration with the immune system. The two main categories of lymphoid cells that can recognise and react against a wide range of specific antigens are B lymphocytes or B cells and T lymphocytes or T cells.

3.4.4.1 B Lymphocytes

The main function of B cells is to produce antibodies against antigens [97]. Each of the approximately 1.5×10^5 molecules of the antibody on the membrane of a single B cell has identical binding sites for antigen. B cells express various receptors on the surface and exhibit following function for instance, Class II MHC molecules permit the B cell to function as an antigen-presenting cell (APC), CR1 (CD35) and CR2 (CD21) are receptors for certain complement products, while the FcRII (CD32) is a receptor for IgG, a type of antibody. Interaction of the membrane-bound antibody present on mature B cells with the antigen, as well as the interactions of the antigen with macrophages and T cells, results in B-cell clones of corresponding specificity. Repeated division of the B cell over 4–5 days generates a population of memory cells and plasma cells. Further plasma cells, are responsible for synthesis and secretion of antibody.

3.4.4.2 T Lymphocytes

Natural T lymphocytes mature in the thymus region and survive in the periphery. The chief function of T cells is to respond to signals associated with tissue destruction and to minimise the collateral tissue damage they cause [98]. T cells express T-cell receptors (TCR) which are a composite of polypeptides including CD3 and either of one of the two membrane molecules, CD8 and CD4. TCR recognises virus infected cells and cancer cells. However, unlike B cells, TCR does not recognise free antigen, unless it is bound to MHC molecules on the membrane of antigen presenting cells. The main function of T cells is to induce death of virus infected cells by secretion of cytotoxins and cytokines which activates B cells, macrophages and cytotoxic T cells. T cells also play role in infectious diseases such as Leishmaniasis [99], infection by hepatitis C virus (HCV), etc. Their ability to confine exuberant immune reactivity, associated with many chronic infections is beneficial the host due to limited tissue damage [100].

3.5 Non-specific Immune System Cells

Infectious diseases are also located in cells other than cells of the RES. Such cells include hepatocytes, epithelial cells and erythrocytes. Hepatocytes are located in the liver and are major site for infections such as hepatitis B/C and malaria.

The hepatocytes are discussed in greater detail in Chapter 6 of this book. Epithelial cells bind together to form the epithelial tissue which is held together by adherens, tight junctions, gap junctions and desmosomes. The functions of epithelial cells are boundary and protection of vital organs, transportation, absorption, secretion, lubrication and movement. These epithelial cells can be readily attacked by microbes such as HIV virus, influenza, Herpes Simplex virus (HSV-2) and cause infections. Furthermore, erythrocytes are infected and act as hosts for plasmodium causing malaria, one of the current fatal infections posing serious challenges.

3.6 Limitations of Conventional Therapy for Infectious Diseases

The introduction of antimicrobial agents such as penicillin resulted in a major breakthrough to decrease morbidity and mortality caused by infectious diseases. Antibiotics represented one of the greatest discoveries. This euphoria was short lived due to adverse effects and the emergence of drug resistance. Conventional therapy when associated with side effects or necessitates long term treatment, results in low patient compliance. Further inadequate drug concentration within cells is a major barrier for effective treatment of intracellular diseases. Increasing the dose, however, resulted in enhanced toxicity. Mono-drug therapy evolved into multi-drug therapy, and enabled a good degree of success and continues to form standard therapy, even today. Classic examples include the multi-drug combination for tuberculosis AKT2, AKT3, AKT4 comprising 2, 3 or 4 drugs, respectively. The HAART combination for AIDS and two drug combinations for malaria are also examples of successful therapy. Nevertheless, the alarming rate at which drug resistance is occurring, and more so the emergence of multi-drug resistance are a matter of great concern. Tuberculosis is one such major disease which has evolved from Resistant to Multi-drug Resistant(MDR) to total drug resistant (TDR), the latest being extremely drug resistant tuberculosis (XXDR), wherein, resistance is seen to almost all known antitubercular drugs.

3.6.1 Multi-drug Resistance (MDR)

The emergence of multi-drug resistance is attributed to a number of factors. Pathogens resort to different mechanisms to avoid intracellular killing. Some pathogens secrete exotoxins which destroy phagocytes and prevent phagocytosis. Bacteria with pore forming cytolysins avoid the phagosome and also escape lysosomal destruction [101–105]. Certain bacteria interfere with the production of cytotoxic metabolites of phagocytes or contain the antioxidant proteins, thereby overcoming the effects of RNIs or ROIs and cause obstruction in phagocytosis [106, 107].

3.6.2 Microbial Biofilms

Bacteria adhere to surfaces, aggregate and form a hydrated polymeric matrix comprising of exopolysaccharide known as biofilms [108]. Biofilms are developed by various bacteria such as *Salmonella*, *Streptococcus, Vibrio cholerae, Klebsiella pneumonia and Haemophilus influenzae.* Further some cells in the biofilm experience nutrient limitation and therefore survive in the starved state. Such cells are slow growing cells and less susceptible to antimicrobial agents [109]. Certain cells in a biofilm adapt a different and protected phenotype. Biofilms are resistant to antibodies, phagocytes, and antibiotics. Although *p*hagocytes reach the biofilms, they become frustrated and release their enzymes, which cause damage to the tissue around the biofilm. Release of bacteria through the damaged biofilm results in dissemination of the infection, leading to acute infection in the surrounding tissues [110, 111].

3.6.3 Efflux Pumps

Efflux pump genes and proteins are present in almost all organisms. Efflux pumps thwart the entry of an antibiotic in the bacterial cell and export an antibiotic from the cell. As efflux pumps can be specific for one substrate or for drugs of dissimilar structure, they can be associated with multi-drug resistance. Multi-drug-resistance efflux pumps are a known cause for the development of bacterial resistance against antibiotics. Bacterial efflux-pump proteins related with MDR are divided into five families namely *the ATP binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the multi-drug and toxic-compound extrusion (MATE) family, the small multi-drug resistance (SMR) family and the resistance nodulation division (RND) family* [112]. Multi-drug resistance occurs, when efflux proteins are overexpressed on the cell, and easily identify and efficiently expel a broad range of antibiotics from the cells [113]. Gram-negative bacteria express several families of transporters which cause resistance [114]. Gram-positive bacteria mainly *Staphylococcus aureus* and *Streptococcus pneumoniae* express MDR efflux pumps. *S. aureus* (responsible for skin and soft-tissue infections) overexpress MFS efflux pump NorA which enables resistance to chloramphenicol and fluoroquinolones. The *S. pneumoniae* MFS efflux pumpPmrA exports the fluoroquinolones ciprofloxacin, norfloxacin, and also expels the dyes acriflavine and ethidium bromide [115–117] *Escherichia coli* EmrE express a member of the small multi-drug resistance (SMR) superfamily and AcrAB–TolC, a member of the resistance-nodulation-cell division (RND) superfamily. *Vibrio parahaemolyticus* overexpress NorM, a member of the multi-drug and toxic compound extrusion (MATE) superfamily.

Multi-drug-resistant tuberculosis (MDR-TB) is appearing as a ghost among the MDR bacteria because TB patients are at high risk of death due to failure of treatment. It is evident that MDR exhibits p55 efflux pumps which play a crucial

role in the pathogenicity of the microorganisms, and is responsible for the efflux of tetracycline and aminoglycosides. This has opened a vast array for research in identifying mutants which are responsible for overexpressing these protein pumps in cases of elevated virulence [112].

3.6.4 Enzymatic Drug Degradation and Chemical Modification

Chemical modification of antibiotics resulting in their inactivation and hence, ineffective dug concentration can be a cause of bacterial resistance. The inactivation reactions include hydrolysis, redox, and group transfer. Hydrolysis is the major cause of degradation of beta lactam antibiotics. The group transfer approach is the most varied and includes modification by thiol transfer, glycosylation, acyl transfer, ribosylation, nucleotidylation and phosphorylation transfer. Drugs which are degraded by group transfer are aminoglycoside, chloramphenicol, rifamycin, macrolides, etc. [118].

3.7 Strategies to Overcome Limitations of Conventional Drug Delivery

One important strategy to overcome the limitation of conventional drug delivery is to deliver high therapeutic payloads intracellularly. This could ensure high efficacy, coupled with low toxicity to provide major advantages. Targeted nanocarriers provide high promise as potential drug delivery systems with the capacity to address this specific challenge. Targeted nanocarriers could therefore prove to be the magic wand.

Passive and active targeting approaches could be relied on to achieve organ based targeting (first order), specific cell based targeting in an organ (second order) and cell organelle based targeting (third order) [119]. A major requirement, however, besides reaching the targeting site is to ensure adequate concentration and adequate retention at the site.

3.7.1 Passive Targeting

Passive targeting can be described as deposition of drug or drug-carrier systems at a particular location due to pharmacological or physicochemical factors [120]. Passive targeting can be achieved by exploiting pathophysiological and anatomical opportunities. Introduction of drugs directly into various anatomical sites for example

lungs and the eye by using non-invasive or invasive methods such as catheters or direct injections can enable local targeting. These site specific drug delivery methods limit systemic toxicity of the drug thus reducing adverse effects of drugs in the non-target tissues [121]. Exploiting altered pathological conditions in diseased tissues are strategies that can be adopted for passive targeting for example chemotactic factors released in infected or inflamed tissues increased permeability of vascular tissues, decreased pH and/or increased temperature [122, 123]. Increased vascular permeability specifically in cancers has enabled passive targeting of nanocarriers and is cited as the enhanced permeation and retention effect (EPR) effect [124]. Surface properties such as particle size, shape, hydrophobicity and surface charge have great impact on macrophage activation and phagocytosis.

3.7.1.1 Size

Particle size plays essential role in distribution and elimination of nanocarriers [125]. Particles size can influence attachment, adhesion, phagocytosis, distribution, circulation half-life and endocytic pathways [126, 127]. The opsonisation and phagocytosis of particles is strongly affected by size of nanocarriers. Although macrophages engulfed 0.2 versus 2 μm IgG-coated spherical particles by different mechanisms, they followed similar kinetics [clathrin endocytosis versus Fc-receptor mediated phagocytosis]. Phagocytic uptake is generally observed with polymeric particles and liposomes with high particle size [>200-microns] [128]. Table 3.7 highlights the size of a number of nanocarriers evaluated for targeted delivery in infectious diseases.

3.7.1.2 Shape

A broad range of non-spherical shaped particles studied including cylinders, cubes, hemispheres, ellipsoids, cones and complex shapes like filamentous, biconcave discoid showed varying effects on phagocytosis [169]. Non-spherical shaped particles bypassed phagocytosis due to incomplete actin structure formation. Particle shape affected attachment and internalisation during phagocytosis [170]. For instance oblate ellipsoids show best attachment and internalisation by phagocytosis, while prolate ellipsoids showed good attachment but poor internalisation. Champion et al. reported that worm-like particles showed low phagocytosis as compared to spherical particles of the same volume [169]. Asymmetric polymer lipid nanostructures (LIPOMER) of Doxycycline hydrochloride (DH) in the range of (250–400 nm) [171] revealed enhanced splenic delivery. The irregular shape of the LIPOMER coupled with rigidity resulted in filtration and non-phagocytic accumulation to reveal splenotropy in sinusoidal spleen models, rat, rabbit and dog. A high spleen liver ratio of 6.7:0.53 was seen in the dog model (Fig. 3.2) [172].

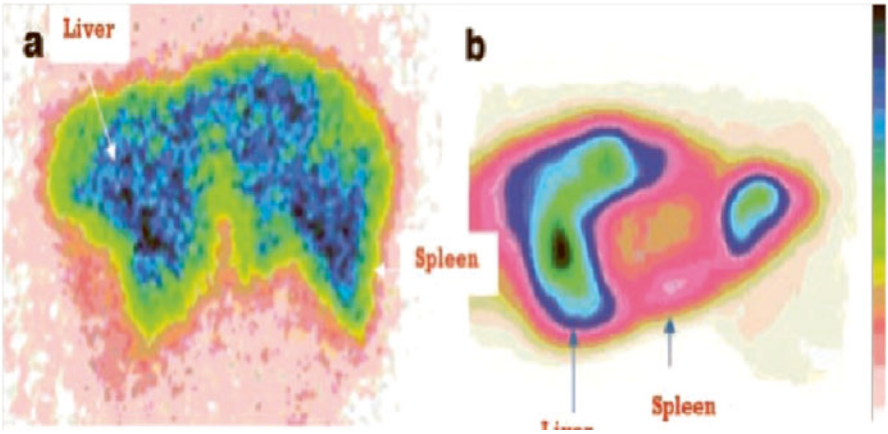

Fig. 3.2 Gamma scintigraphic images—biodistribution of LIPOMER in dog: (**a**) irregular, (**b**) spherical. Reprinted with permission from John Wiley and Sons

3.7.1.3 Surface Properties

Surface properties like hydrophobicity and surface charge also impact opsonisation, phagocytosis and biodistribution of nanoparticles [173]. Hydrophobic nanocarriers are readily coated by complement proteins, albumin, and immunoglobulin and scavenged by RES [174]. Surface charge of particles also influences interaction and stability with cells [175]. Reports suggest that positively charged particles showed high phagocytic uptake over negatively charged particles probably due to better interaction with the negatively charged cell membrane. Cationic and neutral nanocarriers are less taken up by RES as compare to negatively charge [176–178]. However, negatively charged nanoparticles can potentially attach to cationic sites on the macrophages namely the scavenger receptors, which facilitate their uptake by RES [179].

For details on influence of particle size, shape and charge readers are directed to the following reviews [126, 180].

3.7.2 Active Targeting

Active targeting, defined as specific targeting of drugs or drug containing nanocarriers by anchoring active agents or ligands, provides selectivity, recognisability and potential to interact with specific cells and tissues in the body [181]. Targeting by attaching ligands has been investigated as an additional strategy to enhance translocation of antimicrobials inside cells. Attaching ligands facilitates greater uptake and can be mediated by various mechanisms.

3.7.3 Receptor Mediated Endocytosis (RME)

The membrane of macrophages expresses various receptors to facilitate the internalisation of cargoes inside the cell and their degradation. Receptor mediated endocytosis (RME) permits the rapid internalisation of ligand attached particles as compared to untargeted particles [182]. The common RME mechanisms are macropinocytosis, clathrin dependent endocytosis (CDE), caveolae-mediated endocytosis and clathrin independent endocytosis (CIE). Each approach exhibits different binding and internalisation mechanisms. Further, the predominant uptake mechanism is often dictated by the nature of the ligand. Receptor mediated processes are relatively slower than phagocytic processes, with the ligand playing an important role. The sizes, geometry, charge and density of the ligand significantly influences receptor mediated endocytosis [183]. For more references readers can refer to [182, 184, 185]. Table 3.5 lists the endocytic pathways, endosome morphology and the proteins involved in the endocytic pathways.

Table 3.5 Endocytic pathways, endosome morphology and the proteins involved in the endocytic pathways

Endocytic mechanism	Proteins	Morphology	References
Clathrin mediated endocytosis	Dynamin, AP180, adaptin, Clathrin, AP2, epsin, SNX9, synaptojanin, actin, amphiphysin, Rab5, Arf6 plus many others	Vesicular	Ford et al. [186]; Roth et al. [187]
Caveolae mediated endocytosis	Caveolins, Cavins, PTRF, src, PKC, actin	Vesicular/ tubulovesicular	Parton et al. [188]; Rothberg et al. [189]; Krajewska et al. [190]
Flotillin-dependent endocytosis	Flottilin-1 and -2	Vesicular	Glebov et al. [191]; Frick et al. [192]
Clathrin-independent carrier (CLIC)/GPI-AP-enriched early endosomal compartment (GEEC)	ARHGAP10, actin, GRAF1, other GRAFs, Cdc42, Arf1	Tubular/ring	Lundmark et al. [193]; Naslavsky et al. [194]
ADP-ribosylation factor 6 (Arf6) mediated CIE	Arf6	Vesicular/ tubular	Donaldson et al. [195]
Macropinocytosis	Phosphoinositide 3-kinase, Rac1, Brefeldin A-ADP ribosylated substrate (BARS), Actin, PAK1, PI3K,Ras, Src, HDAC6	Highly ruffled	Kirkham et al. [196]; Marbet et al. [197]
Circular dorsal ruffle	Cortactin, actin	Highly ruffled	Krueger et al. [198]
IL2Rβ pathway	RhoA, Rac1, PAK1, PAK2	Vesicular?	Grassart et al. [199]; Lamaze et al. [200]

Table 3.6 Receptors expressed by macrophages and their specific ligands

Receptor	Ligands	References
Mannose	Mannose, fucose, N-acetyl glucosamine, glucose, collagen, mannan, mannosyl lipoarabinomanan	Ezekowitz et al. [201]
Tuftsin	Tuftsin	Agrawal and Gupta [202]; Tzehoval et al. [203]
Scavenger	Modified LDL, lipopolysaccharides (LPS), lipoteichoic acid (LTA)	Wilkinson and Khoury [204]; Graversen et al. [205]
Fc	Monoclonal Antibody	Guilliams et al. [206]
Fibronectin	Fibronectin, laminin, serum amyloid P	Taylor et al. [207]; Schett 2008
Folate	Folic acid	Kroger et al. [208]; Van Der Heijden et al. [209]
Transferrin	Transferrin	Qian et al. [210]
Toll-like receptor	Lipopolysaccharides (LPS), lipoproteins, lipopeptides and lipoarabinomannan	Kawai and Akira [211]
Complement receptors CR3 and CR4	C3b, iC3b, C3c and C3d	Campagne et al. [212]

3.7.4 Receptors for Macrophage Targeting

Macrophages possess large number of surface receptors which help in the process of recognition and endocytosis of engineered particulate carriers. Infection of macrophages leads to changes in the expression pattern of the concerned receptors, which can be exploited for targeted drug delivery employing nanocarriers. Table 3.6 is a summary of the important receptors on macrophages and illustrative examples of ligands for the same that could play a role in designing targeted nanocarriers for infectious disease therapy.

CD14 [213], Decay accelerating factor (CD55), Endo180 [214] are also receptors which could be targeted. Nevertheless, ligands for the same need to be explored.

3.8 Nanocarriers for Targeted Delivery in Infectious Diseases

All known nanocarriers can be effectively employed for targeted delivery in intracellular infections. Both passive and active targeting approaches have been evaluated. The following Tables 3.7 and 3.8 illustrate examples of nanocarriers, limited to major anti-infective agents for active and passive targeting, respectively. Size being a major parameter influencing targeting to RES. Table 3.7 also highlights the size of nanocarriers, which is a primary factor in passive targeting.

Table 3.7 Nanocarriers containing anti-infective agents and their particle size for passive targeting

Nanocarriers	Drug	Particle size	Diseases	References
	Polymyxin B	343 ± 28 nm	*Pseudomonas aeruginosa*	Alipour et al. [129]
Liposomes	Clofazimine	–	Tuberculosis	Mehta et al. [130]
	Pyrazinamide and rifabutin	0.1 μm	Tuberculosis	El-Ridy et al. [131]; Gaspar et al. [132]
	Ampicillin	208 + 70 nm	Salmonellosis	Fattal et al. [133]
	Gentamicin and streptomycin	–	Brucellosis	Fountain et al. [134]
	Ciprofloxacin	–	Salmonellosis	Magallanes et al. [135]
	Antimonials	–	Leishmaniasis	Date et al. [136]
	Dideoxycytidine	0.3 μm	HIV	Oussoren et al. [137]
Polymeric nanoparticles	Rifampicin gelatin NPS	264 ± 11.2 nm	Tuberculosis	Saraogia et al. [138]
	Guar gum	895.5 ± 14.73 nm	Tuberculosis	Kaur et al. [139]
	Rifampicin and isoniazid	382 ± 23 nm	Tuberculosis	Booysen et al. [140]
	Rifampicin	200-260 ± 10.24 nm	Tuberculosis	Esmaeili et al. [141]
	Indinavir	1.6 μm	HIV-1 encephalitis (HIVE)	Dou et al. [142]
	Rifampin and azithromycin antibodies	260 nm	Chlamydia infection	Toti et al. [143]
	AMB	250 nm	Leishmaniasis	Tyagi et al. [144]
	Gentamicin	245 ± 45 nm	Leishmaniasis	Zhang et al. [145]
	Rifampicin	–	*Staphylococcus aureus* and *Mycobacterium avium*	Azrami et al. [146]
	Moxifloxacin	418 ± 90.2 nm	Tuberculosis	Kisich et al. [147]
	Quinine	176 nm	Malaria	Hass et al. [148]
	AMB	358 ± 62 nm	Leishmaniasis	Espuelas et al. [149]
Gelatin NPS	Rifampicin	264 ± 11.2 nm	Tuberculosis	Saraogi et al. [138]
Microparticles	Isoniazid and rifabutin	–	Tuberculosis	Yadav et al. [150]
	Isoniazid	1 μm	Tuberculosis	Zhou et al. [151]
Solid lipid nanoparticles	Lopinavir	230 nm	HIV	Alex et al. [152]
	Tobramycin	855 nm	Bacterial	Bargoni et al. [153]
	Zidovudine	294 + 32 nm	HIV	Heiati et al. [154]
	Atazanavir	167 nm	HIV	Chattopadhay et al. [155]
	Isoniazid	131.7 nm	Tuberculosis	Bhandari and Kaur [156]

(continued)

Table 3.7 (continued)

Nanocarriers	Drug	Particle size	Diseases	References
Metallic nanoparticles	Rifampin, Isoniazid	100 nm	Tuberculosis	Clemens et al. [157]
Niosomes	Isoniazid	450 nm	Tuberculosis	Singh et al. [158]
Nanoemulsions/ nanosuspension	Primaquine	10–200 nm	*Plasmodium berghei*	Singh et al. [159]
	AMB	–	Leishmaniasis	Falk et al. [160]
Dendrimer	Lamivudine	–	HIV	Dutta and Jain [161]
	Primaquine phosphate	–	Malaria	Bhadra et al. [162]
Carbon nanotubes	AMB	100–400 nm	Leishmaniasis	Prajapati et al. [163]
Cu oxide		20–95 nm	*Meticillin-resistant Staphylococcus aureus (MRSA); Escherichia coli (E.coli)*	Ren et al. [164]; Raffi et al. [165]
Zn oxide		50–70 nm	*Staphylococcus aureus*	Jones et al. [166, 167]
Iron nanoparticles		3–9 nm	*E. coli*	Chatterjee et al. [168]

Table 3.8 Nanocarriers containing anti-infective agents and ligands for active targeting

Nanocarriers	Targeting Ligands	Drug	Diseases	References
Liposomes	Mannose	Pentamidine isethionate	Pneumocystis pneumonia	Banerjee et al. [215]
	Mannose	Ciprofloxacin	Respiratory intracellular parasitic infections	Chono et al. [216]
	Hyaluronan	Anti-inflammatory drug	Inflammatory sites	Glucksam-Galnoy et al. [217]
	Apolipoprotein E	–	Hepatic diseases	Kim et al. [218]
	Polyinosinic acid and phosphatidylserine	Antimony-lipopolysaccharide (Sb-LP)	Leishmaniasis	Tempone et al. [219]
	Ostearoylamylopectin (O-SAP)	Rifampicin and Isoniazid	Tuberculosis	Deol et al. [220]
	O-palmitoyl amylopectin (OPA)	Amphotericin B	Pulmonary candidiasis	Vyas et al. [221]
	IgG	–	Liver disease	Derksen et al. [222]

(continued)

Table 3.8 (continued)

Nanocarriers	Targeting Ligands	Drug	Diseases	References
	Tuftin	AMB	Leishmaniasis	Agrawal et al. [202]
	Immunoliposomal	AMB	HIV-1	Bestman-Smith et al. [223]
	Antibodies against human and murine HLA-DR and CD4 antigen	Indinavir	HIV	Gangne et al. [224]
Nanoparticle	Mannose	Rifampicin	Visceral leishmaniasis	Chaubey et al. [225]
	Folate	Rifampicin	Tuberculosis	Date et al. [226]
	Folate	Vancomycin	*Staphylococcus aureus*	Chakraborty et al. [227]
	TAT (trans-activating transcriptor) peptide	Ritonavir	HIV	Rao et al. [228]
	Transferrin anchored pegylated albumin nanoparticles (Tf-PEG-NPs)	Azidothymidine	HIV	Mishra et al. [229]
	Transferrin	Saquinavir	HIV	Ulbrich et al. [230]
	Mannan	Diadanosine	HIV	Kaur et al. [231]
SLN	Mannan	Gene delivery	Alveolar macrophages	Yu et al. [232]
	Mannose	Rifabutin	Alveolar macrophages	Nimje et al. [233]
	Transferrin	Quinine HCl	Malaria	Gupta et al. [234]
Dendrimers	Mannose	-	Macrophages	Gao et al. [235]
	Tuftsin	Efavirenz (EFV)	HIV	Dutta et al. [236, 237]
	Mannose	Rifampicin	Tuberculosis	Kumar et al. [238]
Carbon Nanotubes	Mannose	Amphotericin B	Macrophages	Pruthi et al. [239]

3.9 Specialised Targeting Approaches for Important Infectious Diseases

3.9.1 Tuberculosis

Tuberculosis is persistent and deadly infectious disease, caused *Mycobacterium tuberculosis* which is non-specifically phagocytosed by alveolar macrophages. The emergence of various resistance forms of tuberculosis has accelerated research in specific approaches to target the *M. Tuberculosis*. Date et al. developed folate

anchored polymeric nanoparticles of rifampicin and demonstrated 480 % enhancement in rifampicin uptake as compared to 300 % in the absence of folate in the human macrophage cell line U-937 [226]. Folate receptors enable flotillin-1 and caveolin receptor mediated endocytosis, thereby bypassing normal phagolysome formation to deliver the nanocarriers into the cytoplasm [191]. Lemmer et al. developed mycolic acid (MA) anchored nanoparticles (NP) of isoniazid. MA nanoparticles exhibited macrophage uptake, possibly localising in the cytoplasm. Verma et al. developed inhalable microparticles containing NO donors for the treatment of *Mycobacterium tuberculosis*. Such inhalable microparticles specifically delivered NO donors inside macrophages and showed sustain release in the cytosol. The antimycobacterial activity of microparticles was confirmed by the decrease in the *M. tuberculosis* CFU by up to 3-log in 24 h. The activity could be attributed to interaction of NO with bacterial DNA, lipids and protein. This strategy could be considered practical as the doses of NO donors (isosorbide nitrate) were much lower than those required for cardiovascular effects [240].

3.9.2 Malaria

Malaria is a complex disease caused by plasmodium and majorly resides in non-RES cells like red blood cells (RBCs) and hepatocytes. Entry of the parasite into the brain causes cerebral malaria. Malaria can be targeted at the exoerythrocytic stage by targeting RBCs, or targeting the hypnozoites to tackle malarial relapse and further in case of cerebral malaria targeting the brain. Increased permeability of infected RBCs is seen after 12–16 h of plasmodium invasion through formation of channels. These channels are "new permeability pathways" (NPPs) which allow entry of molecules such as dextran, protein A and IgG2a antibody thereby differentiating the non-infected and infected RBCs. Such pathways could be targeted to enable high drug loading in the erythrocytes specifically through design of nanocarriers of <80 nm [241]. This could be supported through design of stealth nanocarriers which could enable long circulation, using various stealth agents like poly(ethyleneglycol) (PEG), Pluronic, etc. [242]. Chloroquine liposomes anchored with anti-erythrocyte F (ab′)2 were studied for targeting to erythrocytes [243]. Hepatocytes the residence of hypnozoites expresses the asialoglycoprotein receptor (ASGPR), which is overexpressed in infections. Targeting this receptor using nanocarriers anchored with ASGPR ligands is a strategy for hepatocyte targeting. Joshi et al. prepared in situ primaquine nanocarboplex of primaquine phosphate anchored with pullulan as the ASGPR ligand for specific targeting to hepatocytes. Significantly, enhanced hepatic accumulation with preferential accumulation in the hepatocytes and a high hepatocytes/nonparenchymal cells ratio of 75:25 confirmed hepatocyte targeting [244]. Transferrin (Tf)-anchored solid lipid nanoparticles (SLNs) were intravenously administered for targeting quinine dihydrochloride to the brain, in cerebral malaria. Compared to conventional SLNs or drug solution the Tf-SLNs significantly enhanced the brain uptake of quinine [234].

3.9.3 HIV

A major feature of HIV that complicates therapy is the existence of HIV in multiple reservoirs, which include various cellular and anatomical sites [245]. The typical reservoirs are the liver, spleen, lungs, GIT and genital tract with the brain and bone marrow representing remote sites [246]. Targeted delivery for HIV therefore needs to address delivery to maximum sites simultaneously to achieve remission. One strategy that we propose is a combination of nanocarriers of size <100 nm to target remote sites and size >200 nm target major RES organs (Unpublished data). Viral replication is inhibited by the antioxidant glutathione. Erythrocytes containing glutathione (GSH) in combination with azidothymidine (AZT) and didanosine (DDI) showed higher reduction in viral DNA in bone marrow and brain as compared to DDI+GSH alone [247]. Immunoliposomes containing siRNA for targeting the lymphocyte function-associated antigen-1 (LFA-1) integrin, which is expressed on all leukocytes, was selectively taken up by T cells and macrophages, the primary site of HIV. Further, in vivo administration of anti-CCR5 siRNA resulted in leukocyte-specific gene silencing that was sustained for 10 days [248]. Nanogels comprising non-reverse transcriptase inhibitors (NRITs) decorated with a peptide for brain specific apolipoprotein E (apoE) receptors, showed tenfold suppression of retroviral activity and decrease inflammation in humanised mouse model of HIV-1 infection in the brain [249].

3.10 Veterinary Applications of Targeted Drug Delivery Systems

Targeted drug delivery for the therapy of veterinary infections assumes immense importance not only for improved animal health but due to the challenges posed by zoonotic diseases. About 13 zoonotic diseases including brucellosis, tuberculosis, trypanosomiasis, cysticercosis and others are related to 2.4 billion cases of infection in humans and over two million deaths annually [166, 167]. Such infections exist both in domestic animals and wild life. The close proximity of humans especially with such domestic animals is a cause of global concern. The WHO policy of "Cull and Kill" results not only in the loss of lives but also heavy monetary losses to the farmer. Targeted treatment strategies using nanodrug delivery systems could provide a revolutionary strategy to benefit both the animals and man. The benefits of targeted nanomedicine strategies are slowly gaining recognition as evident from a number of reported studies. Liposomes have been used by many researchers for treating various veterinary diseases such as Leishmaniasis [250, 251], Brucellosis [252], Blastomycosis [253], Babesiosis [254], etc. Patil et al. [171] developed an asymmetric lipomer. This is a combination of polymer–lipid containing doxycycline which could have application in the treatment of intracellular infections that are primarily resident in the spleen like brucellosis, ehrlichiosis, etc. A number of studies are reported on horses infected with babesiosis, *Streptococcus equi, T. gondii*

and *Strongylus vulgaris* infections using liposomes [254], polymeric nanospheres [255], dendrimers [256] and micelles [257] respectively. A recent study revealed the improved therapy of theileiriosis in cattle with solid lipid nanoparticles (SLN) of buparvaquone [258]. SLN revealed comparable effect with the intramuscular injection at significantly lower doses. Nanodrug delivery systems have also been evaluated in dogs, sheep and pigs. For details on nanodrug delivery applications in targeted delivery in veterinary infections, readers are directed to the following reference [259].

3.11 Future Scope

Targeted delivery for infectious diseases has immense scope. Tackling infections using nanodrug delivery systems could provide a practical alternative as a short term strategy. A rate-limiting factor however would be the serious concerns of toxicity. Nanodrug delivery systems due to their high intracellular delivery could precipitate new and unknown toxicities. Evolving strategies to predict the same is an important path forward. While vaccines could probably provide the ultimate cure and control, vaccine development is a complex process and not yet easily attained as evident from the limited success stories. However, designing nano-vaccines targeted to exhibit greater cellular response is also a near future prospect.

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