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
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.

### 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, Chlamyphilia 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* |

#### 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, lipoteichoic 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](image-url)
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)-γ 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)-α, IL-1β, IL-12, IL-18 and the chemokines CCL15, CCL20, CXCL8-11 and CXCL13. 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-γ-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)-β and arginase-1 (Arg1), to enable this function. 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
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*.

**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]                            |
|                                                | *Brucella spp.*               | Brucellosis               | Roy [27]                                     |
|                                                | *Leishmania spp.*             | Leishmaniasis             | Ghosh et al. [28]                            |

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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. Bel2). 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
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 O₂⁻ into molecular oxygen and hydrogen peroxide, thereby decreasing the scavenging and toxic effects of O₂⁻ and H₂O₂ 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

Leishmania 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 Ca²⁺ 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 | – | Library 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 $\sim 10^{10}$–$10^{12}$ 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 $1.5 \times 10^5$ 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 lyosomal 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 phagocytes 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 para-haemolyticus 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 drug 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].
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 compared to negatively charged [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.

| 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 lipoarabinomannan | 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                                                     | Guiliams 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                    | Saraogi et al. [138]        |
|                     | Guar gum                           | 895.5 ± 14.73 nm | Tuberculosis                    | Kaur et al. [139]          |
|                     | Rifampicin and isoniazid           | 382 ± 23 nm   | Tuberculosis                    | Booyisen 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     | \textit{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      | \textit{Meticillin-resistant Staphylococcus aureus (MRSA); Escherichia coli (E.coli)} | Ren et al. [164]; Raffi et al. [165] |
| Zn oxide                      |                       | 50–70 nm      | \textit{Staphylococcus aureus} | Jones et al. [166, 167]         |
| Iron nanoparticles            |                       | 3–9 nm        | \textit{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]           |
| Osteoarlyamylopectin (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)
3.9 Specialised Targeting Approaches for Important Infectious Diseases

3.9.1 Tuberculosis

Tuberculosis is a persistent and deadly infectious disease, caused by *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 nanocarriers to target tuberculosis.

**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 transcription) 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] |
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 phagolysosome 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(ethylene glycol) (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.

### References

1. Terry W, Clos D (2013) Pentraxins: structure, function, and role in inflammation. ISRN inflamm 1:101
2. Hoppe HJ, Reid KB (1994) Collectins – soluble proteins containing collagenous regions and lectin domains – and their roles in innate immunity. Protein Sci 3:1143–1
3. Ren Y, Ding Q, Zhang X (2014) Ficolins and infectious diseases. Virol Sin 29(1):25–32
4. Poltorak A et al (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 282:2085–2088
5. Hoshino K et al (1999) Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J Immunol 162:3749–3752
6. Qureshi ST et al (1999) Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). J Exp Med 189:615–625
7. Alexopoulou L, Medzhitov HA, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413:732–738
8. Takeuchi O et al (1999) Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 11:443–451
9. Hemmi H et al (2000) A Toll-like receptor recognizes bacterial DNA. Nature 408:740–745
10. Hayashi F et al (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor. Nature 410:1099–1103
11. Rabinovitch M (1995) Professional and non-professional phagocytes: an introduction. Trends Cell Biol 5:85–87
12. Krombach F et al (1997) Cell size of alveolar macrophages: an interspecies comparison. Environ Health Perspect 105(5):1261–1263
13. Lewis CE, Pollard JW (2006) Distinct role of macrophages in different tumor microenvironments. Cancer Res 66:605
14. Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8:958–969
15. Swanson MS, Fernandez-Moreira E (2002) A microbial strategy to multiply in macrophages: the pregnant traffic. Proc Natl Acad Sci U S A 102(11):4033–4038
16. Pujol C, Klein KA, Romanov GA, Palmer LE, Cirotta C, Zhao Z, Bliska JB (2009) Yersinia pestis can reside in autophagosomes and avoid xenophagy in murine macrophages by preventing vacuole acidification. Infect Immun 77(6):2251–2261
17. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J, Russell DG (1994) Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase. Science 263:678–681
18. Frehel C, Rastogi N (1987) Mycobacterium leprae surface components intervene in the early phagosome-lysosome fusion inhibition event. Infect Immun 55(12):2916–2921
19. Alvarez DC, Roberts R, Stahl PD (1997) Internalized Listeria monocytogenes modulates intracellular trafficking and delays maturation of the phagosome. J Cell Sci 6:731–743
20. Buchmeier NA, Heffron F (1991) Inhibition of macrophage phagosome-lysosome fusion by Salmonella typhimurium. Infect Immun 59(7):2232–2238
21. Desjardins M, Descoteaux A (1997) Inhibition of phagolysosomal biogenesis by the Leishmania lipophosphoglycan. J Exp Med 185(12):2061–2068
22. Mosser DM, Miles SA (2007) Avoidance of innate immune mechanisms by the protozoan parasite, Leishmania spp in protozoans in macrophages. Landes Biosci 9:118–124
23. Sibley LD, Weidner E, Krabevenhul JL (1985) Phagosome acidification blocked by intracellular Toxoplasma gondii. Nature 315(6018):416–419
24. Borlase GN, Jones HF, Keep SJ, Butle R, Brooks DA (2011) Helicobacter pylori phagosome maturation in primary human macrophages. Gut Pathog 3:3
25. Ghosh M, Bandypadhyay S (2004) Interaction of Leishmania parasites with dendritic cells and its functional consequences. Immunobiology 209(1–2):173–177
26. Swanson MS, Isberg RR (1995) Association of Legionella pneumophila with the macrophage endoplasmic reticulum. Infect Immun 63(9):3609–3620
27. Roy CR (2005) Trimming the fat: a Brucella abortus survival strategy. Nat Immunol 6(6):546
28. Roy CR, Bandyopadhyay S (2004) Interaction of Leishmania parasites with dendritic cells and its functional consequences. Immunobiology 209(1–2):173–177
29. Berger KH, Isberg RR (1993) Two distinct defects in intracellular growth complemented by a single genetic locus in Legionella pneumophila. Mol Microbiol 7(1):7–19
30. Ray K, Marteyn B, Sansonetti PJ, Tang CM (2009) Life on the inside: the intracellular lifestyle of cytosolic bacteria. Nat Rev Microbiol 7(5):333–340
31. Dermine JF, Desjardins M (1999) Survival of intracellular pathogens within macrophages. Protoplasma 210(1–2):11–24
32. Dramsi S, Cossart P (2002) Listeriolysin O: a genuine cytolysin optimized for an intracellular parasite. J Cell Biol 156(6):943–946
33. Whitworth T, Popov VL, Yu XJ, Walker DH, Bouyer DH (2005) Expression of the Rickettsia prowazekii pld or tlyC gene in Salmonella enterica serovar typhimurium mediates phagosomal escape. Infect Immun 73:6668–6673
34. Mumy KL, Bien JD, Pazos MA, Gronert K, Hurley BP, McCormick BA (2008) Distinct isoforms of phospholipase A2 mediate the ability of Salmonella enterica serotype typhimurium and Shigella flexneri to induce the transepithelial migration of neutrophils. Infect Immun 76(8):3614–3627
35. Kubica M, Guzik K, Koziel J, Zarebski M, Richter W, Gajkowska B, Golda A, Maciag-Gudowska A, Brix K, Shaw L, Foster T, Potempa J (2008) A potential new pathway for Staphylococcus aureus dissemination: the silent survival of S. aureus phagocytosed by human monocyte-derived macrophages. PLoS One 3(1):1409
37. Shin JS, Gao Z, Abraham SN (2000) Involvement of cellular caveolae in bacterial entry into mast cells. Science 289:785–788
38. Sibley LD, Charron AJ, Hakansson S, Mordue DG (2007) Invasion and intracellular survival by Toxoplasma in Protozoans in Macrophages. Landes Bioscience 16–21
39. Dabiri GA, Sanger JM, Portnoy DA, Southwick FS (1990) Listeria monocytogenes moves rapidly through the host-cell cytoplasm by inducing directional actin assembly. Proc Natl Acad Sci U S A 87(16):6068–6072
40. Van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, Pierson J, Brenner M, Peters PJ (2007) M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. Cell 129:1287–1298
41. Schroeder GN, Hilbi H (2008) Molecular pathogenesis of Shigella spp.: controlling host cell signaling, invasion, and death by type III secretion. Clin Microbiol Rev 21(1):134–156
42. Santic M, Asare R, Skrobonja I, Jones S, Abu KY (2008) Acquisition of the vacuolar ATPase proton pump and phagosome acidification are essential for escape of Francisella tularensis into the macrophage cytosol. Infect Immun 76(6):2671–2677
43. Andrews NW (1994) From lysosomes into the cytosol: the intracellular pathway of Trypanosoma cruzi. Braz J Med Biol Res 27(2):471–475
44. Winkler HH, Daugherty RM (1983) Cytoplasmic distinction of avirulent and virulent Rickettsia prowazekii: fusion of infected fibroblasts with macrophage-like cells. Infect Immun 40(3):1245–1247
45. Alexander J, Vickerman K (1975) Fusion of host cell secondary lysosomes with the parasitophorous vacuoles of Leishmania mexicana-infected macrophages. J Protozool 22:502–508
46. Clemens DL, Horwitz MA (1993) Hypoexpression of major histocompatibility complex molecules on Legionella pneumophila phagosomes and phagolysosomes. Infect Immun 61(7):2803–2812
47. Burton PR, Stueckemann J, Welsh RM, Paretsky D (1978) Some ultrastructural effects of persistent infections by the rickettsia Coxiella burnetii in mouse L cells and green monkey kidney (Vero) cells. Infect Immun 21:556–566
48. Straley SC, Harmon PA (1984) Yersinia pestis grows within phagolysosomes in mouse peritoneal macrophages. Infect Immun 45(3):655–659
49. Miller M, Dreisbach A, Otto A, Becher D, Bernhardt J, Hecker M, Peppelenbosch MP, van Dijl JM (2011) Mapping of interactions between human macrophages and Staphylococcus aureus reveals an involvement of MAP kinase signaling in the host defense. J Proteome Res 10(9):4018–4032
50. Gatfield J et al (2000) Essential role for cholesterol in entry of mycobacteria into macrophages. Science 288:1647–1650
51. Catron DM, Sylvester MD, Lange Y, Kadekoppala M, Jones BD, Monack DM, Falkow S, Haldar K (2002) The Salmonella containing vacuole is a major site of intracellular cholesterol accumulation and recruits the GPI-anchored protein CD55. Cell Microbiol 4:315–328
52. Simons K, Ehehalt R (2002) Cholesterol, lipid rafts, and disease. J Clin Invest 110(5):597–603
53. Miller BH, Fratti RA, Poschet JF, Timmins GS, Master SS, Burgos MM, Marletta MA, Deretic V (2004) Mycobacteria inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection. Infect Immun 72(5):2872–2878
54. Schlesinger LS (1996) Entry of Mycobacterium tuberculosis into mononuclear phagocytes. Curr Top Microbiol Immunol 215:71–96
55. Brown EJ (1991) Complement receptors and phagocytosis. Curr Opin Immunol 3:76–82
56. Schorey JS et al (1997) A macrophage invasion mechanism of pathogenic mycobacteria. Science 277:1091–1093
57. Schlesinger LS (1993) Macrophage phagocytosis of virulent but not attenuated strains of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. J Immunol 150(7):2920–2930
58. Armstrong JA, Hart PDA (1971) Response of cultured macrophages to Mycobacterium tuberculosis, with observations on fusion of lysosomes with phagosomes. J Exp Med 134:713–740
59. Zimmerli S et al (1996) Selective receptor blockade during phagocytosis does not alter the survival and growth of Mycobacterium tuberculosis in human macrophages. Am J Respir Cell Mol Biol 15:760–770

60. Ernst JD (1998) Macrophage receptors for Mycobacterium tuberculosis. Infect Immun 66:1277–1281

61. Romling U, Sierralta WD, Eriksson K, Normark S (1998) Multicellular and aggregative behavior of Salmonella typhimurium strains is controlled in the mutations in the agfD promoter. Mol Microbiol 28:249–264

62. Anriany YA, Weiner RM, Johnson JA, Rezende CA, Joseph SW (2001) Salmonella enterica serovar Typhimurium DT104 displays a rugose phenotype. Appl Environ Microbiol 67:4048–4056

63. Petter G, Keller J, Rahman LH, Carlson MM, Silvers S (1996) A novel relationship between O-antigen variation, matrix formation, and invasiveness of Salmonella enteritidis. Epidemiol Infect 117:219–231

64. White AP, Gibson DL, Kim W, Kay WW, Surette MG (2006) Thin aggregative fimbriae and cellulose enhance long-term survival and persistence of Salmonella. J Bacteriol 188:3219–3227

65. Collinson SK et al (1993) Thin, aggregative fimbriae mediate binding of Salmonella enteritidis to fibronectin. J Bacteriol 175:12–18

66. Zogaj XM, Nimtz M, Rohde M, Bokranz W, Romling U (2001) The multicellular morphotypes of Salmonella typhimurium and Escherichia coli produce cellulose as the second component of the extracellular matrix. Mol Microbiol 39:1452–1463

67. Ryu JH, Beuchat LR (2005) Biofilm formation by Escherichia coli O157:H7 on stainless steel: effect of exopolysaccharide and Curli production on its resistance to chlorine. Appl Environ Microbiol 71:247–254

68. Scher K, Romping U, Yaron S (2005) Effect of heat, acidification, and chlorination on Salmonella enterica serovar Typhimurium cells in a biofilm formed at the air-liquid interface. Appl Environ Microbiol 71:1163–1168

69. Solano CB, Garcia JV, Berasain C, Ghigo GM, Gamazo C, Lasa I (2002) Genetic analysis of Salmonella enteritidis biofilm formation: critical role of cellulose. Mol Microbiol 43:793–808

70. Gibson DL, White AP, Snyder SD, Martin S (2006) Salmonella produces an O-antigen capsule regulated by AgfD and important for environmental persistence. J Bacteriol 188(22):7722

71. Teixeir HD, Schumacher RI, Meneghini R (1998) Lower intracellular hydrogen peroxide levels in cells overexpressing CuZn-superoxide dismutase. Proc Natl Acad Sci U S A 95:7872–7875

72. Fries BC, Taborda CP, Serfass E, Casadevall A (2001) Phenotypic switching of Cryptococcus neoformans occurs in vivo and influences the outcome of infection. J Clin Invest 108:1639–1648

73. Sebastiaan MB et al (2011) HIV-1 and the macrophages. Future Virol 6(2):187–208

74. Marechal V et al (2001) Human immunodeficiency virus type 1 entry into macrophages mediated by macroinocytosis. J Virol 75(11):66–77

75. Gantt KR, Cherry S, Rodriguez N, Jeronimo SM, Nascimento ET et al (2003) Activation of TGF-beta by Leishmania chagasi: importance for parasite survival in macrophages. J Immunol 170:2613–2620

76. Bray RS, Heikal B, Kaye PM, Bray MA (1983) The effect of parasitization by Leishmania mexicana on macrophage function in vitro. Acta Trop 40:29–38

77. Olivier M, Gregory DJ, Forget G (2005) Subversion mechanisms by which immune response: a signaling point of leishmania parasites can escape the host. Clin Microbiol Rev 18(2):293

78. D’Orsogna MR, Mail TC (2009) Optimal cytoplasmic transport in viral infections. PLoS One 30(412):8165

79. Roop RM, Bellaire BH, Valderas MH, Cardelli JA (2004) Adaptation of the Brucellae to their intracellular niche. Mol Microbiol 52(3):621–630
80. Celli J, Chastellier CD, Franchini DM et al (2003) Brucella evades macrophage killing via virb-dependent sustained interactions with the endoplasmic reticulum. J Exp Med 198(4):545–556
81. Libraty DH et al (2002) Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. J Infect Dis 85(9):1213–1221
82. Moradpour D et al (2003) Membrane association of hepatitis C virus nonstructural proteins and identification of the membrane alteration that harbors the viral replication complex. Antivir Res 60(2):103–109
83. Heinz C, Falke D, Weise K, Bachmann M, Fonseca MC, Zaubitzer T, Müller WEG (1989) Change of processing and nucleocytoplasmic transport of mRNA in HSV-1-infected cells. Virus Res 13(1):61–78
84. Arnheiter H, Skuntz S, Noteborn M, Chang S, Meier E (1990) Transgenic mice with intracellular immunity to influenza virus. Cell 62(1):51–61
85. Tilney LG, Harb OS, Connelly PS, Robinson CG, Roy CR (2001) How the parasitic bacterium Legionella pneumophila modifies its phagosome and transforms it into rough ER: implications for conversion of plasma membrane to the ER membrane. J Cell Sci 114(24):4637–4650
86. Handman E, Greenblatt CL, Goding JW (1984) An amphipathic sulphated glycoconjugate of Leishmania: characterization with monoclonal antibodies. EMBO J 3(10):2301–2306
87. Collins B et al (2012) Assessing the contributions of the LiaS histidine kinase to the innate resistance of listeria monocytogenes to nisin, cephalosporins, and disinfectants. Appl Environ Microbiol 78(8):2923–2929
88. Moulder J (1962) The biochemistry of intracellular parasitism. p 172
89. Trebichavsky I, Spichal I, Spichalova A (2010) Innate immune response in the gut against Salmonella - review. Folia Microbiol (Praha) 55(3):295–300
90. Skvortsov TA, Azhikina TL (2012) Adaptive changes in gene expression of Mycobacterium tuberculosis during the development of the infection. Russ J Bioorg Chem 38(4):341–353
91. Khodor S, Kwaik YA (2010) Triggering Ras signalling by intracellular Francisella tularensis through recruitment of PKCa and bI to the SOS2/GrB2 complex is essential for bacterial proliferation in the cytosol. Cell Microbiol 12(11):1604–1621
92. Herberman RB, Oraldo JR (1981) Natural killer cells: their role in defenses against disease. Science 214(2):24
93. Ojo E, Wigzell H (1978) Natural killer cells may be the only cells in normal mouse lymphoid cell populations endowed with cytolytic ability for antibody-coated tumour target cells. Scand J Immunol 7:297
94. Lopez C (1980) Genetic control of natural resistance to infection and malignancy. p 253
95. Bancroft GJ, Shellam GR, Chalmer JE (1981) Genetic influences on the augmentation of natural killer (NK) cells during murine cytomegalovirus infection: correlation with patterns of resistance. J Immunol 126:988
96. Welsh RM (1981) Natural cell-mediated immunity during viral infections. Curr Top Microbiol Immunol 92:83–106
97. Golub ES (1980) The cellular basis of the immune response. an approach to immunobiology. Sinauer, Sunderland, MA
98. Powrie F, Maloy KJ (2003) Regulating the regulators. Immunology 299(5609):1030–1031
99. Mills KH (2004) Regulatory T cells: friend or foe in immunity to infection? Nat Rev Immunol 4:841–855
100. Belkaid Y, Rouse BT (2005) Natural regulatory T cells in infectious disease. Nat Immunol 6:353–360
101. N'Diaye EN, Darzacq X, Astarie-Dequeker C, Daffe M, Calafat J, Maridonneau-Parini I (1998) Fusion of azurophil granules with phagosomes and activation of the tyrosin ekinase Hck are specifically inhibited during phagocytosis of mycobacteria by human neutrophils. J Immunol 161:4983–4991
102. Roy CR, Berger KH, Isberg RR (1998) Legionella pneumophila DotA protein is required for early phagosome trafficking decisions that occur within minutes of bacterial uptake. Mol Microbiol 28:663–674
103. Gao LY, Abu KY (1999) Apoptosis in macrophages and alveolar epithelial cells during early stages of infection by Legionella pneumophila and its role in cytopathogenicity. Infect Immun 67:862–870
104. Segal G, Shuman HA (1999) Legionella pneumophila utilizes the same genes to multiply within Acanthamoeba castellani and human macrophages. Infect Immun 67:2117–2124
105. Gomes MS, Paul S, Moreira AL, Appelberg R, Rabinovitch M, Kaplan G (1999) Survival of Mycobacterium avium and Mycobacterium tuberculosis in acidified vacuoles of murine macrophages. Infect Immun 67:3199–3206
106. Liautard JP, Gross A, Dornand J, Kohler S (1996) Interactions between professional phagocytes and Brucella spp. Microbiology 12:197–206
107. Cole SP, Eckmann L, Guiney DG (1998) Nitric oxide-mediated crosstalk between Helicobacter and gastric mucosa. In: 38th interscience conference of antimicrobial agents and chemotherapy. 24–27 Sept, San Diego, CA. Abstract B15
108. Costerton JW, Stewart PS, Greenberg ES (1999) Bacterial biofilms: a common cause of persistent infections. Science 284(5418):1318–1322
109. Brown MR, Allison DG, Gilbert P (1988) Growth rate control of adherent bacterial populations. J Antimicrob Chemother 22:777
110. Cheema MS, Rassing JE, Marriott C (1986) The diffusion characteristics of antibiotics in mucus glycoprotein gels. J Pharm Pharmacol 38(S12):53
111. Stewart PS (1998) A review of experimental measurements of effective diffusive permeabilities and effective diffusion coefficients in biofilms. Biotechnol Bioeng 59:261
112. Piddock LJV (2006) Multidrug-resistance efflux pumps not just for resistance. Nat Rev Immunol 4(8):629–639
113. Paulsen IT (2003) Multidrug efflux pumps and resistance: regulation and evolution. Curr Opin Microbiol 6:446–451
114. Nikaido H, Zgurskaya HI (1999) Antibiotic efflux mechanisms. Curr Opin Infect Dis 12(6):529–536
115. Neyfakh AA (1992) The multidrug efflux transporter of Bacillus subtilis is a structural and functional homolog of the Staphylococcus NorA protein. Antimicrob Agents Chemother 36:484–485
116. Yoshida H, Bogaki M, Nakamura S, Ubakata K, Konno M (1990) Nucleotide sequence and characterization of the Staphylococcus aureus norA gene, which confers resistance to quinolones. J Bacteriol 172:6942–6949
117. Kaatz GW, Seo SM, Ruble CA (1993) Efflux-mediated fluoroquinolone resistance in Staphylococcus aureus. Antimicrob Agents Chemother 37:1086–1094
118. Wright GD (2005) Bacterial resistance to antibiotics: enzymatic degradation and modification. Adv Drug Deliv Rev 57:1451–1470
119. Torchillin VP (2000) Drug targeting. Eur J Pharmaceut Sci 11(2):S81–S91
120. Garnett MC (2001) Targeted drug conjugates: principles and progress. Adv Drug Deliv Rev 53(2):171–216
121. Vasir Jaspreet K, Reddy MK, Labhasetwar VD (2005) Nanosystems in drug targeting, opportunities and challenges. Curr Nanosci 1:47–64
122. Schiffelers RM et al. (2001) Targeted drug delivery to enhance efficacy and shorten treatment duration in disseminated Mycobacterium avium infection in mi host factors influencing the preferential localization of sterically stabilized liposomes in klebsiella pneumoniae-infected rat lung tissue. IAJM Pharm Res 18:780–787
123. Edens HA, Levi BP, Jaye DL, Walsh S, Reaves TA, Turner JR, Nusrat A, Parkos CA (2002) Neutrophil transepithelial migration: evidence for sequential, contact-dependent signaling events and enhanced paracellular permeability independent of transjunctional migration. J Immunol 169:476–486
124. Maeda H et al (2000) Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 65(1–2):271–284
125. Powers KW, Palazuelos M, Moudgil BM, Roberts SM (2007) Characterization of the size, shape, and state of dispersion of nanoparticles for toxicological studies. Nanotoxicology 1:42
126. Duan X, Li Y (2013) Physicochemical characteristics of nanoparticles affect. Small 9(9–10):1521–1532
127. Gratton SEA, Ropp PA, Pohlhaus PD et al (2008) The effect of particle design on cellular internalization pathways. Proc Natl Acad Sci U S A 105:11613
128. Hillaireau HP, Couvreur C (2009) Nanocarriers entry into the cell: relevance to drug delivery. Cell Mol Life Sci 66:2873
129. Alipoura M, Halwania M, Omria A et al (2008) Antimicrobial effectiveness of liposomal polymyxin B against resistant Gram-negative bacterial strains. Int J Pharm 355(1–2):293–298
130. Mehta RT et al (1993) In vitro activities of free and liposomal drugs against Mycobacterium avium-M. intracellular complex and M. tuberculosis. Antimicrob Agents Chemother 37(12):2584–2587
131. El-Ridy MS, Mostafa DM, Shehab BA, Nasr ES, Abd El-Alim S (2007) Biological evaluation of pyrazinamide liposomes for treatment of Mycobacterium tuberculosis. Int J Pharm 330(1–2):82–88
132. Gaspara MM, Cruz A et al (2008) Rifabutin encapsulated in liposomes exhibits increased therapeutic activity in a model of disseminated tuberculosis. Int J Antimicrob Agents 31(1):37–45
133. Fattal E et al (1991) Liposome-entrapped ampicillin in the treatment of experimental murine listeriosis and salmonellosis. Antimicrob Agents Chemother 35(4):770
134. Fountain MW, Weiss SJ, Fountain AG (1985) Treatment of Brucella canis and Brucella abortus in vitro and in vivo by stable plurilamellar vesicle-encapsulated aminoglycosides. J Infect Dis 152(3):529–535
135. Magallanes M, Dijkstra J, Fierer J (1993) Liposome-incorporated ciprofloxacin in treatment of murine salmonellosis. Antimicrob Agents Chemother 37(11):2293–2297
136. Date AA, Joshi MD, Patravale VB (2007) Parasitic diseases: liposomes and polymeric nanoparticles versus lipid nanoparticles. Adv Drug Deliv Rev 59:505–521
137. Oussoren C et al (1999) Liposomes as carriers of the antiretroviral agent dideoxycytidine-5 %-triphosphate. Int J Pharm 180:261–270
138. Saraogia GK, Gupta P, Gupta UD, Jain NK et al (2010) Gelatin nanocarriers as potential vectors for effective management of tuberculosis. Int J Pharm 385:143–149
139. Kaur M, Malik B., Garg T, Rath G, Goyal AK (2014) Development and characterization of guar gum nanoparticles for oral immunization against tuberculosis. Drug Deliv. (Ahead of Print): 1–7
140. Booyse F et al (2013) In vivo/in vitro pharmacokinetic and pharmacodynamic study of spray-dried poly-(dl-lactic-co-glycolic) acid nanoparticles encapsulating rifampicin and isoniazid. Int J Pharm 444(28):10–17
141. Esmaeili F et al (2007) Preparation and antibacterial activity evaluation of rifampicin-loaded poly lactide-co-glycolide nanoparticles. Nanomedicine 3(2):161–167
142. Dou H et al (2009) Macrophage delivery of nanoformulated antiretroviral drug to the brain in a murine model of NeuroAIDS. J Immunol 183(1):661–669
143. Toti US et al (2011) Targeted delivery of antibiotics to intracellular chlamydial infections using PLGA nanoparticles. Biomaterials 32:6606–6613
144. Tyagi R et al (2005) Targeted delivery of arjunglucoside I using surface hydrophilic and hydrophobic nanocarriers to combat experimental leishmaniasis. J Drug Target 13(3):161–171
145. Zhang Q, Liao G, Wei D, Nagai T (1998) Increase in gentamicin uptake by cultured mouse peritoneal macrophages and rat hepatocytes by its binding to polybutylcyanoacrylate nanoparticles. Int J Pharm 164:21–27
146. Azarmi S, Rao WH, Löbenberg R (2008) Targeted delivery of nanoparticles for the treatment of lung diseases. Adv Drug Deliv Rev 60(8):863–875
147. Kisich KO et al (2007) Encapsulation of moxifloxacin within poly(butyl cyanoacrylate) nanoparticles enhances efficacy against intracellular Mycobacterium tuberculosis. Int J Pharm 345(1–2):154–162

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148. Haas SE et al (2009) Nanoencapsulation increases quinine antimalarial efficacy against Plasmodium berghei in vivo. Int J Antimicrob Agents 34:156–161
149. Espuelas MS, Legrand P, Loiseau PM et al (2002) In vitro antileishmanial activity of amphoterin C loaded in poly(e-caprolactone) nanospheres. J Drug Target 10(8):593–599
150. Yadav AB et al (2009) Inhalable microparticles containing isoniazid and rifabutin target macrophages and “stimulate the phagocyte” to achieve high efficacy. Indian J Exp Biol 47(6):469–474
151. Zhoua H, Zhang Y, Biggs DL, Mark C et al (2005) Microparticle-based lung delivery of INH decreases INH metabolism and targets alveolar macrophages. J Control Release 107(2):288–299
152. Alex MR, Chacko AJ, Jose S, Souto EB (2011) Lopinavir loaded solid lipid nanoparticles (SLN) for intestinal lymphatic targeting. Eur J Pharm Sci 42:11–18
153. Bargoni A et al (2001) Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles after duodenal administration to rats. Part ii tissue distribution. Pharmacol Res 43(5):497
154. Heiatia H, Rashad T, Richard R, Phillips NC (1997) Solid lipid nanoparticles as drug carriers. Incorporation and retention of the lipophilic prodrug 3´-azido-3´-deoxythymidine palmitate. Int J Pharm 146(1):123–131
155. Chattopadhyay N, Jason ZW et al (2008) Solid lipid nanoparticles enhance the delivery of the HIV protease inhibitor, atazanavir, by a human brain endothelial cell line. Pharm Res 25:10
156. Bhandari R, Kaur IP (2011) Pharmacokinetics, tissue distribution and relative bioavailability of isoniazid-solid lipid nanoparticles. Int J Pharm 441(1–2):202–212
157. Clemens DL et al (2012) Targeted intracellular delivery of antituberculosis drugs to mycobacterium tuberculosis-infected macrophages via functionalized mesoporous silica nanoparticles. Antimicrob Agents Chemother 56(5):2535–2545
158. Singh G, Dwivedi H, Saraf SK, Saraf SA (2011) Niosomal delivery of isoniazid development and characterization. Trop J Pharm Res 10(2):203–210
159. Singh KK, Vingkar SK (2008) Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine. Int J Pharm 347(1–2):136–143
160. Falk R, Domb AJ, Polacheck I (1999) A novel injectable water-soluble amphotericin B-arabinogalactan conjugate. Antimicrob Agents Chemother 43(8):1975–1981
161. Dutta T, Jain NK (2007) Targeting potential and anti-HIV activity of lamivudine loaded mannosylated poly (propyleneimine) dendrimer. Biochim Biophys Acta 1770(4):681–686
162. Chatterjee S, Bandyopadhyay A, Keka S (2011) Effect of iron oxide and gold nanoparticles on bacterial growth leading towards biological application. J Nanobiotechnology 9(34):1
163. Champion JA, Mitragotri S (2006) Role of target geometry in phagocytosis. PNAS 103(13):4930–4934
164. Sharma G et al (2010) Polymer particle shape independently influences binding and internalization by macrophages. J Control Release 147:408
165. Patil RR, Gaikwad RV, Samad A, Devarajan PV (2008) Role of lipids in enhancing uptake of polymer-lipid (LIPOMER) nanoparticles. J Biomed Nanotechnol 4(3):359–366
172. Devarajan PV, Jindal AB, Patil R et al (2010) Particle shape: a new design parameter for passive targeting in splenotropic drug delivery. J Pharm Sci 99(6):2576
173. Schipper ML et al (2009) Particle size, surface coating, and PEGylation influence the biodistribution of quantum dots in living mice. Small 5:126
174. Esmaeili F, Gahremanei F, Esmaeili B, Khoshayand MR, Atyabi F, Dinarvand R (2008) PLGA nanoparticles of different surface properties: preparation and evaluation of their body distribution. Int J Pharm 349:249
175. Verma A, Stellacci F (2010) Effect of surface properties on nanoparticle–cell interactions. Small 6(1):12–21
176. Roser M, Fischer D, Kissel T (1998) Surface-modified biodegradable albumin nano- and microspheres. II: effect of surface charges on in vitro phagocytosis and biodistribution in rats. Eur J Pharm Biopharm 46:255
177. Blau S, Jubeh TT, Haupt SM, Rubinstein A (2000) Drug targeting by surface cationization. Crit Rev Ther Drug Carrier Syst 17(5):425
178. Xiao K, Li Y, Luo J, Lee JS, Xiao W, Goni AM, Agarwal RG, Lam KS (2011) The effect of surface charge on in vivo biodistribution of PEG-oligocholic acid based micellar nanoparticles. Biomaterials 32:3435
179. Iversena T, Sandvig K (2011) Endocytosis and intracellular transport of nanoparticles: present knowledge and need for future studies. Nano Today 6(2):176–185
180. Ford MG et al (2001) Simultaneous binding of PtdIns P2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes. Science 291(105):1–55
181. Roth TF, Porter KR (1964) Yolk protein uptake in the oocyte of the mosquito Aedes aegypti. L. J Cell Biol 20:313–332
182. Parton RG, Simons K (2007) The multiple faces of caveolae. Nat Rev Mol Cell Biol 8:185–194
197. Marbet P, Rahner C, Stieger B, Landmann L (2006) Quantitative microscopy reveals 3D organization and kinetics of endocytosis in rat hepatocytes. Microsc Res Tech 69:693–707
198. Krueger EW, Orth JD, Cao H, McNiven MA (2003) A dynamin-cortactin-Arp2/3 complex mediates actin reorganization in growth factor-stimulated cells. Mol Biol Cell 14:1085–1096
199. Grassart A, Dujeancourt A, Lazarow PB, Dautry-Varsat A, Sauvonnet N (2008) Clathrin-independent endocytosis used by the IL-2 receptor is regulated by Rac1, Pak1 and Pak2. EMBO Rep 9:356–362
200. Lamaze C, Dujeancourt A, Baba T, Lo CG, Benmerah A, Dautry-Varsat A (2001) Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway. Mol Cell 7:661–671
201. Ezekovitz RA, Williamsi DJ, Kozieltil H et al (1991) Uptake of Pneumocystis carinii mediated by the macrophage mannose receptor. Nature 351(6322):155–158
202. Agrawal AK, Gupta CM (2000) Tuftsin-bearing liposomes in treatment of macrophage-based infections. Adv Drug Deliv Rev 41(2):135–146
203. Tzehoval E et al (1978) Tuftsin (an Ig-associated tetrapeptide) triggers the immunogenic function of macrophages: implications for activation of programmed cells. Proc Natl Acad Sci 75(7):3400
204. Wilkinson K, Khoury JEI (2012) Microglial scavenger receptors and their roles in the pathogenesis of Alzheimer’s disease. Int J Alzheimers Dis 489456:1
205. Graversen JH, Svendsen P, Dagnæs-Hansen F, Dal J, Anton G, Etzerodt A, Petersen MD, Christensen PA, Møller HJ, Moestrup SK (2012) Targeting the hemoglobin scavenger receptor CD163 in macrophages highly increases the anti-inflammatory potency of dexamethasone. Mol Ther 20(8):1550–1558
206. Guilliams M, Pierre B, Van SY, Hamida H, Lambrecht BN (2014) The function of Fcγ receptors in dendritic cells and macrophages. Nat Rev Immunol 14:94–108
207. Taylor ME, Conary JT, Lennartz MR, Drickamer K (1990) Primary structure of the mannose receptor contains multiple motifs resembling carbohydrate-recognition domains. J Biol Chem 265:12156–12162
208. Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Rev Immunol 10:373–384
209. Campagne S, Wiesmann B (2007) Macrophage complement receptors and pathogen clearance. Cell Microbiol 9(9):2095–2102
210. Kotenko SV, Gallagher G, Baurin VV, Antes AL, Sheikf F, Dickensheets H, Donnelly RP (2002) IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 4:69–77
211. Dobrovolskaia MA, Vogel S (2002) Toll receptors, CD14, and macrophage activation and deactivation by LPS. Microbes Infect 4(9):903–914
212. Banerjee G et al (1996) Drug delivery system: targeting of pentamidines to specific sites using sugar grafted liposomes. J Antimicrob Chemother 38(1):1145–1150
213. Chono S, Tanino T, Seki T, Morimoto K (2008) Efficient drug delivery to alveolar macrophages and lung epithelial lining fluid following pulmonary administration of liposomal ciprofloxacin in rats with pneumonia and estimation of its antibacterial effects. Drug Dev Ind Pharm 34(10):1090–1096
214. Galnøy YG, Zor T, Margalit R (2012) Hyaluronan-modified and regular multilamellar liposomes provide sub-cellular targeting to macrophages, without eliciting a pro-inflammatory response. J Control Release 1609(2):388–393
218. Kim J, Basak JM, Holtzman DM (2009) The role of apolipoprotein E in Alzheimer’s disease. Neuron 63(3):287–303
219. Tempone AG et al (2004) Targeting Leishmania (L.) chagasi amastigotes through macrophage scavenger receptors: the use of drugs entrapped in liposomes containing phosphatidylserine. J Antimicrob Chemother 54(1):60–68
220. Deol P, Khuller GK, Joshi K (1997) Therapeutic efficacies of isoniazid and rifampin encapsulated in lung-specific stealth liposomes against Mycobacterium tuberculosis infection induced in mice. Antimicrob Agents Chemother 41(6):1211–1214
221. Vyas SP, Quraishi S, Gupta S, Jaganathan KS (2005) Aerosolized liposome-based delivery of amphotericin B to alveolar macrophages. Int J Pharm 296(1–2):12–25
222. Derksen JTP, Morselt HWM, Scherphof GL (1988) Uptake and processing of immunoglobulin-coated liposomes by subpopulations of rat liver macrophages. Biochim Biophys Acta 971(2):127–136
223. Bestman-Smith J, Gourde P, Désormeaux A, Tremblay MJ, Bergeron MJ (2000) Sterically stabilized liposomes bearing anti-HLA-DR antibodies for targeting the primary cellular reservoirs of HIV-1. Biochim Biophys Acta 1468(1–2):161–174
224. Gagne JD et al (2002) Targeted delivery of indinavir to HIV-1 primary reservoirs with immunoliposomes. Biochim Biophys Acta 1558:198–210
225. Chaubey P et al (2014) Development and optimization of curcumin-loaded mannosylated chitosan nanoparticles using response surface methodology in the treatment of visceral leishmaniasis. Carbohydr Polym 101:1101–1108
226. Date PV, Patel MD, Majee SB, Abdul S, Devarajan PV (2013) Ionic complexation as a non-covalent approach for the design of folate anchored rifampicin gantrez nanoparticles. J Biomed Nanotechnol 9(5):765–775
227. Chattopadhyay S, Chakraborty SP, Laha D, Baral R, Pramanik P, Roy S (2012) Surface-modified cobalt oxide nanoparticles: new opportunities for anti-cancer drug development. Cancer Nanotechnol 3(1–6):13–23
228. Rao KS, Reddy S, Horning JL, Labhasetwar V (2008) TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs. Biomaterials 29(33):4429–4438
229. Mishra V et al (2006) Targeted brain delivery of AZT via transferrin anchored pegylated albumin nanoparticles. J Drug Target 14(1):45–53
230. Ulbrich K et al (2009) Transferrin- and transferrin-receptor-antibody-modified nanoparticles enable drug delivery across the blood–brain barrier (BBB). Eur J Pharm Biopharm 71(2):251–256
231. Kaur A, Jain S, Tiwary A (2008) Mannan-coated gelatin nanoparticles for sustained and targeted delivery of didanosine: in vitro and in vivo evaluation. Acta Pharma 58(1):61–74
232. Yu W, Liu C, Liu Y, Zhang N, Xu W (2010) Mannan-modified solid lipid nanoparticles for targeted gene delivery to alveolar macrophages. Pharm Res 27(8):1584–1596
233. Nimje N et al (2009) Mannosylated nanoparticulate carriers of rifabutin for alveolar targeting. J Drug Target 17(10):777–787
234. Gupta Y, Jain A, Jain SK (2007) Transferrin-conjugated solid lipid nanoparticles for enhanced delivery of quinine dihydrochloride to the brain. J Pharm Pharmacol 59:935–940
235. Gao Q, Han J, Ma Z (2013) Polyamidoamine dendrimers-capped carbon dots/Au nanocrystal nanocomposites and its application for electrochemical immunosensor. Biosens Bioelectron 49:323–328
236. Dutta T et al (2008) Toxicological investigation of surface engineered fifth generation poly(propylenimine) dendrimers in vivo. Nanotoxicology 2(2):62
237. Dutta T, Garg M, Jain NK (2008) Targeting of efavirenz loaded tuftsin conjugated poly(propylenimine) dendrimers to HIV infected macrophages in vitro. Eur J Pharm Sci 34(4):181–189
238. Kumar VP, Asthana A, Dutta T, Jain NK (2006) Intracellular macrophage uptake of rifampicin-loaded mannosylated dendrimers. J Drug Target 14(8):546–556
239. Pruthi J, Mehra NK, Jain NK (2012) Macrophages targeting of amphotericin B through mannosylated multiwalled carbon nanotubes. J Drug Target 20(7):593–604
240. Verma RK et al (2012) Inhalable microparticles containing nitric oxide donors: saying NO to intracellular mycobacterium tuberculosis. Mol Pharm 9:3183–3189
241. Charpian S, Przyborski JM (2008) Protein transport across the parasitophorous vacuole of Plasmodium falciparum: into the great wide open. Traffic 9:157–165
242. Vauthier C, Labarre D, Ponchel G (2007) Design aspects of poly(alkylcyanoacrylate) nanoparticles for drug delivery. J Drug Target 15(10):641–663
243. Agrawal AK, Singhal A, Gupta CM (1987) Functional drug targeting to erythrocytes in vivo using antibody bearing liposomes as drug vehicles. Biochem Biophys Res Commun 148:357–361
244. Joshi VM, Devarajan PV (2014) Receptor-mediated hepatocyte-targeted delivery of primaquine phosphate nanocarboxoplex using a carbohydrate ligand. Drug Deliv Transl Res 4:353–364
245. Blankson J, Deborah P, Siliciano RF (2002) The challenge of viral reservoirs in hiv-1 infection. Annu Rev Med 53:557–593
246. Ramana KV (2012) HIV disease management in the highly active antiretroviral therapy (HAART) era. J Med Microbiol 1(1):101
247. Fraternale A, Casabianca A, Tonelli A, Chiariantini L, Brandi G, Magnani M (2001) New drug combinations for the treatment of murine AIDS and macrophage protection. Eur J Invest 31(3):248–252
248. Kim SS et al (2010) RNAi-mediated CCR5 silencing by LFA-1-targeted nanoparticles prevents HIV infection in BLT mice. Mol Ther 18(2):370–376
249. Gerson T, Makarov E, Senanayake TH, Santhi G, Larisa Y (2014) Nano-NRTIs demonstrate low neurotoxicity and high antiviral activity against HIV infection in the brain. Nanomedicine 10(1):177–185
250. Marques C, Carvalheiro M, Pereira MA, Jorge J, Cruz ME, Santos-Gomes GM (2008) Efficacy of the liposome trifluralin in the treatment of experimental canine leishmaniasis. Vet J 178:133–137
251. Ribeiro RR, Moura EP, Pimentel VM, Sampaio WM, Silva SM, Schettini DA (2008) Reduced tissue parasitic load and infectivity to sand flies in dogs naturally infected by Leishmania (Leishmania) chagasi following treatment with a liposome formulation of meglumine antimoniate. Antimicrob Agents Chemother 52:2564–2572
252. Nicoletti P, Lenk RP, Popescu MC, Swenson CE (1989) Efficacy of various treatment regimens, using liposomal streptomycin in cows with brucellosis. Am J Vet Res 50:1004–1007
253. Krawiec DR, McKiernan BC, Twardock AR, Swenson CE, Itkin RJ, Johnson LR, Kurowsky LK, Marks CA (1996) Use of an amphotericin B lipid complex for treatment of blastomyces in dogs. J Am Vet Med Assoc 209:2073–2075
254. Timofeev BA, Bolotin IM, Stepanova LP, Bogdanov AA, Georgiu K, Malysh SN, Petrovsky VV, Klibanov AL, Torchilin VP (1994) Liposomal diamidine (imidocarb), preparation and animal studies. J Microencapsul 11:627–632
255. Cubillos C et al (2008) Enhanced mucosal immunoglobulin A response and solid protection against foot-and-mouth disease virus challenge induced by a novel dendrimeric peptide. J Virol 82:7223–7230
256. Hiszczynska SE, Oledzka G, Gasior H, Li H, Xu JB, Sedcole R, Kur J, Bickerstaffe R, Stankiewicz M (2011) Evaluation of immune responses in sheep induced by DNA immunization with genes encoding GRA1, GRA4, GRA6 and GRA7 antigens of Toxoplasma gondii. Vet Parasitol 177:281–289
257. Klei TR, Torbert BJ, Chapman MR, Turk MA (1984) Efficacy of ivermectin in injectable and oral paste formulations against eight-week-old Strongylus vulgaris larvae in ponies. Am J Vet Res 45:183–185
258. Soni MK et al (2014) Buparvaquone loaded solid lipid nanoparticles for targeted delivery in theileriosis. J Pharm Bioall Sciences 6(1):22–30
259. Devarajan PV, Soni MKP (2014) Targeted nanomedicine strategies for livestock infections. Nanotechnology for Animal Health and Production. 61