Chapter 10
Macrophage-Targeted Nanomedicines

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Abstract Macrophages are versatile cells of the innate immune system responsible for the control and progressions of a variety of autoimmune inflammatory, infectious, and metabolic diseases and cancer. Macrophage polarization (pro-inflammatory and tissue injury M1 or anti-inflammatory, tissue repair, and proangiogenic M2) occurs in health tissues and in diseases, being a vital element of disease development or reversion. Macrophages play important roles in diverse diseases that affect millions of people and have significant health and economic costs since generally they are chronic, relapsing, and disabling. Therapies focus on elimination, repolarization, reduction of pro-inflammatory mediators, activation of antimicrobial activity, or induction of immune response by macrophages is being considered of increasing interest. However, issues associated with inappropriate pharmacokinetics, lack of tissue selectivity, and poor intracellular delivery make such pharmacological approaches poorly efficient and/or toxic. Macrophage-targeted nanomedicines may increase intracellular drug concentration on activated macrophages, reduce toxicity, and improve activity. In this chapter, we will describe strategies for macrophage targeting employing nanoparticles for treatment of inflammatory diseases such as cardiovascular diseases, lung inflammatory diseases, inflammatory bowel diseases and rheumatoid arthritis, and infectious diseases such as leishmaniasis, tuberculosis, and nontuberculous mycobacterial disease developed in the last 5 years.

Keywords Inflammatory diseases · Infectious diseases · Liposomes · Nanoparticles · Active targeting

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10.1 Introduction

Macrophages (Mϕ) are cells of the innate immune system with diverse functions. Mϕ sense their environment, kill pathogens, take up apoptotic and necrotic cells, heal tissue damage, present antigens to T cells and produce cytokines and chemokines that serve many important roles in innate and adaptive immune responses (Hume et al. 2019). Mϕ are found as resident self-maintaining populations distributed as sinus-lining and interstitial resident Mϕ in lymphohematopoietic and other tissues, such as Langerhans cells of the skin, alveolar Mϕ (AMϕ), Kupffer cells (KC) of the liver, intestinal Mϕ, microglia cells, and osteoclasts, with specialized functions and phenotypes (Ginhoux and Guilliams 2016). Bone marrow-derived blood monocytes restock resident Mϕ with high turnover and are recruited to places of injury, infection, sterile inflammation, and in response to metabolic, atherogenic, and neoplastic stimuli, generating infiltrating activated tissue Mϕ.

Mϕ are dynamic plastic cells, they can alter their functional phenotype depending on the microenvironment. Mϕ sense multiple signals from pathogens (though pathogen-associated molecular patterns, PAMPs, such as toll-like receptors-TLRs), from damage tissue (though damage-associated molecular patterns, DAMPs), and from normal tissue environment (lineage-determining growth factors and cytokines) (Shapouri-Moghaddam et al. 2018). According to the combination of these stimuli, Mϕ polarized into two subsets, classically activated (M1) or alternatively activated (M2) (Murray 2017). Briefly, interferon-gamma (INF-γ) produced by Th1 lymphocyte and PAMPs or DAMPs induce the M1 phenotype with antimicrobial, inflammatory, and antigen-presenting activities, whereas cytokines produced mainly by Th2 lymphocytes promote the M2 phenotype characterized by anti-inflammatory actions and antiparasitic actions (Fig. 10.1).

Generally, M1 Mϕ are involved in microorganism and cell matrix debris phagocytosis, in the early phase of tissue healing and antigen presentation. M1 Mϕ produced pro-inflammatory cytokines (TNF, IL-1β, IL-6, IL-12, IL-23), low levels of anti-inflammatory cytokines (IL-10), and several chemokines. M1 Mϕ highly express cyclo-oxygenase 2 (COX 2) and inducible nitric oxide synthase (iNOS) for nitric oxide (NO) synthesis, secrete high levels of reactive nitrogen intermediates (RNIs), reactive oxygen species (ROS), and produce collagenase, that leads tissue damage.

M2 Mϕ, on the other hand, are associated with allergy, extracellular parasitic infection, tissue remodeling and healing, acetogenesis, and tumor progression (Mantovani et al. 2013). M2 Mϕ are highly endocytic and partially phagocytic, secrete high levels of IL-10 and low levels of IL-12 and IL-23 (Arora et al. 2018), produce angiogenesis mediators such as transforming growth factor (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and express scavenger, mannose, and galactose receptors and COX 1. M2 Mϕ can be further divided into M2a, M2b, M2c, and M2d subsets depending on the activation stimulus (Martinez and Gordon 2014). M2a and M2c Mϕ express high levels of Arg-1 that plays a role in catalysis polyamines, which is necessary for collagen synthesis,

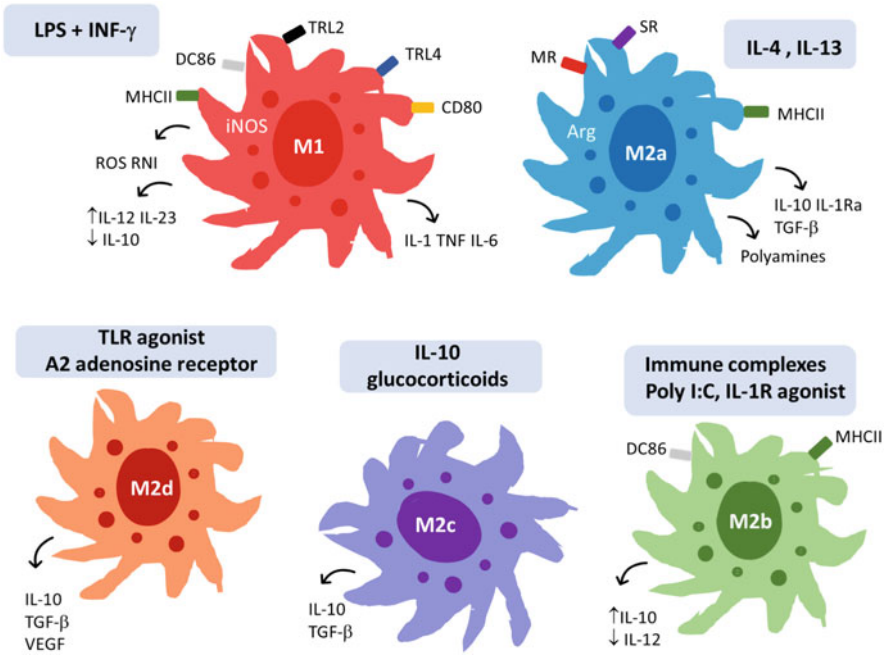

Fig. 10.1 M1 Mϕ are induced when naïve or M0 Mϕ are exposed to bacterial moieties including LPS and Th1 cytokines including IFN-γ, IL-2, IL-12, IL-18, and TNF-β [lymphotoxin β (LT-β)]. M1 Mϕ express pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and type I IFN, and promote cytotoxic adaptive immunity by upregulating MHC class II molecules in conjunction with co-stimulatory molecules CD40, CD80, CD86, TLR2, and TLR4. Also, M1 macrophages express Th1- and Th17-polarizing cytokines IL-12, IL-23, IL-27, and Th1-recruiting chemokines CXCL9, CXCL10, CXCL11. M1 Mϕ are finally characterized by microorganism and matrix debris phagocytosis in the early phases of healing and high antigen presentation capacity

fibroblast proliferation, fibrosis, and other tissue remodeling functions. Healthy tissues associated with immune-suppressed states such as placenta and lung are rich in M2 Mϕ. In this chapter, we will generally refer to M2a as M2 Mϕ.

Classification into two subsets, M1 and M2, is a simplified description of Mϕ heterogeneity and plasticity, being a continuum of functional states more realistic. Repolarization or switching Mϕ phenotype in response to new environmental influences is possible (Martinez and Gordon 2014).

M2 Mϕ are developed upon exposure to Th2 cytokines including IL-4, IL-5, IL-6, and IL-10. The M2 Mϕ can further be divided into M2a, M2b, M2c, and M2d depending on their stimulus for the activation.

Upon stimulation with IL-4 or IL-13, M2a (a stands for alternative) Mϕ express high levels of MR (CD206), IL-1 receptor (IL-1R) and CCL17, and secrete profibrotic factors, such as TGF-β, IGF, and fibronectin, essential for tissue repair. M2 phenotype expressed MMP2, MMP7, MMP9, and arginase receptors.

M2b Mϕ or "regulatory" Mϕ are induced by poly I:C or TLR or IL-1R agonists, which leads to activation of multiple transcription factors such as NF-κB, MAPK, and interferon regulatory factor 3, as well as PI3K–AKT signaling. Besides producing several pro-inflammatory cytokines (IL-1β, IL-6, TNF, CCL1, and TNF SF14), M2b cells also secrete anti-inflammatory cytokines such as IL-10 and low levels of IL-12 thereby opposing M1 Mϕ.

M2c or "deactivation" Mϕ are induced by IL-10 and glucocorticoids, which leads to high levels of IL-10 and TGF-β. This anti-inflammatory phenotype of M2c Mϕ is further driven by their efficient capability to phagocytose apoptotic cells by high expression of Mer receptor tyrosine kinase.

M2d or tumor-associated Mϕ are induced by TLR agonists through the adenosine receptor, which leads to production of high levels of IL-10, TGF-β, and VEGF and low levels of IL-12, TNF, and IL-1β thereby providing proangiogenic properties with the features of tumor-associated Mϕ (TAMs).

Mϕ polarization occurs both in physiological and pathological conditions and is a key element of disease development and progression or resolution (Sica et al. 2015). For instance, Mϕ with sustained M1 phenotype are implicated in the development and maintenance of diseases-sepsis, infections, chronic inflammatory diseases [atherosclerosis, chronic obstructive pulmonary disease (COPD), asthma, rheumatoid arthritis (RA), inflammatory bowel disease (IBD), diabetes, lupus, muscle injury, psoriasis, etc.], and neurodegenerative disease. M1 Mϕ release pro-inflammatory cytokines, chemokines, extracellular matrix (ECM) digestive enzymes, prostaglandins, and ROS that aggravate and accelerate damage to the tissues during diseases. On the other hand, M2 Mϕ contributes to lung remodeling and fibrosis leading to lung dysfunction in asthma and COPD (Arora et al. 2018). M2 Mϕ also suppress anti-tumour T cell responses and stimulate tumor angiogenesis (Lee et al. 2019). Additionally, Mϕ are host cells of numerous intracellular pathogens such as *Mycobacterium tuberculosis and Mycobacterium avian complex, Leishmania* parasites, *Salmonella enterica*, *Legionella pneumophila, and Listeria monocytogenes*. These microorganisms have developed different strategies to survive inside Mϕ including modulation of Mϕ phenotypes and reduction of their immune response.

Mϕ targeting aiming at their elimination, reduction of pro-inflammatory cytokines production, phenotype switching or repolarization, enhancement of antimicrobial activity and immune response could improve the current therapy of diverse diseases. However, issues associated with pharmacokinetics (PK, plasma transient peaks), biodistribution (BD, lack of tissue selectivity), and pharmacodynamics (PD, poor intracellular delivery) make such pharmacological approaches poorly efficient and/or toxic. Mϕ targeting nanomedicines may help to solve these limitations suffered by free drug-based therapies.

Therapeutic nanomedicines are composed of nano-object [generally nanoparticles (Nps), nano-objects with three dimensions in the nanoscale, such as liposomes, micelles, lipidic, and polymeric Nps] associated with an active pharmaceutical ingredient (API, low-molecular-weight molecules, or macromolecules such as nucleic acids, polysaccharides, or proteins). Due to their small size and high surface area, nanomedicines (1–1000 nm) exhibit related dimension-dependent

properties or phenomena, that are different from bulk materials, such as endocytic uptake by cells. However, the most important aspect of therapeutic nanomedicines is that upon incorporation into its structure, the API's PK, BD and intracellular traffic no longer depend on the chemical structure of the API but on the structural features of the Nps, such as size, shape, charge, and surface properties. Hence, incorporation into Nps could avoid unspecific distribution in healthy tissues and reduce the adverse effects of API's. Besides, increased solubility and protection from degradation could be achieved by API's incorporation into Nps.

Plain, pegylated, or surface ligand-modified Nps are used for passive and active Mϕ-targeting. The use of plain Nps is based on the natural capacity of Mϕ to engulf micro and nano materials that are anatomically accessible. For instance, KC in the hepatic sinusoids, spleen, and bone-marrow Mϕ (Mϕ of the mononuclear phagocyte system, MPS) rapidly eliminate intravenously (iv) administered plain Np, while AMϕ eliminate inhaled plain Np. On the other hand, pegylated Np [Nps covered by poly(ethylene glycol) (Peg) shield] should be used to access Mϕ others than those of the MPS. Pegylated Np could partially, evade or delay such uptake, circulate for a longer time, and extravasate by convection in zones where vascular endothelium barrier dysfunction and increased permeability occurs for example in tumors or inflamed tissues [enhanced permeation and retention (EPR) effect]. Though the lymph drainage in inflamed zones is not impaired, as in tumors, accumulation in inflamed zones of pegylated Np is anticipated (Maeda 2012; Chen et al. 2017). After site-specific accumulation, either API release or phagocytosis of Nps by accessible Mϕ may occur. However, this last option is less possible since pegylation strongly inhibits cellular uptake (Hatakeyama et al. 2011). Then, active targeting, where ligands (i.e., antibodies, peptides, proteins, sugars) of endocytic receptors are superficially exposed, could increase selective intracellular delivery of nanomedicines to Mϕ. Mannose receptors (MR), scavenger receptors (SR), macrophage galactose lectin (MGL), folate receptor (FR-β), and CD44 are endocytic receptors overexpress on inflammatory Mϕ generally used for active Mϕ targeting nanomedicines (Table 10.1).

Once a nanomedicine is up taken by Mϕ it follows, such as a pathogen, the endo-lysosomal pathway where Np and the loaded API are degraded. Thus, unless the molecular target is localized in the endo-lysosomal system, the loaded API should scape such system to access the cytoplasm and other organelles like the nucleus, mostly relevant for the delivery of DNA, RNA, and proteins. Numerous mechanisms including membrane fusion, membrane destabilization, particle swelling, and osmotic rupture could be used as strategies for Nps and API endosomal escape (Smith et al. 2018).

In this chapter, we will describe the strategies for Mϕ targeting nanomedicine for inflammatory and infectious diseases developed and tested in in vivo models in the last 5 years.

Table 10.1 Surface phagocytic receptors used for active macrophage targeting nanomedicines

Name	Type	Ligands	Cell expression	Overexpression
Mannose receptor (CD206) (MR)	Type I membrane glycoprotein composed of short cytoplasmatic domain, a transmembrane domain, and an extracellular region comprising eight C-type lectin-like domains, a fibronectin type II domain, and an N-terminal CRD	Mannose, fucose, sulfated sugars (sLex), collagen, CD45, tumoral mucins, and neutrophil-derived myeloperoxidases	MØ, endothelial cells: hepatic sinusoidal endothelial cells, dermal endothelial cells lymphatic endothelial cells	Inflammatory conditions M2 MØ Upregulated by IL-4, IL-13, IL-10 Downregulated by IFN-γ
MGL (CD301)	Type II C-type lectin composed of N-terminal cytoplasmic domains, transmembrane domains, extracellular stalk domains, and C-terminal CRD	Galactose and *N*-acetylgalactosamine		Inflammatory conditions M2 MØ
Folate receptor (FR-β)	Glycosylphosphatidylinositol-linked protein receptor	Folic acid	Rapid dividing cells	Inflammatory conditions (IBD, RA, atherosclerotic lesions, TAM) Cannot be detected on resting MØ or any other normal cells M1 and M2 MØ
Scavenger receptors SR-A1 (CD240)	Type II membrane protein, composed of short N-cytoplasmic tail, a transmembrane domain, a spacer region, an α-helical coiled-coil domain, a collagen-like domain, and a C-terminal scavenger receptor CRD	Polyionic ligands including gram-positive LTA, gram-negative bacteria lipid-A moiety of LPS, AcLDL, OxLDL, malondialdehyde-LDL, maleylated-LDL	MØ monocytes	M2 MØ
CD44	Type I transmembrane protein	Hyaluronic acid Chondroitin sulfate	Present in almost all cells: endothelial cells, epithelial cells, fibroblasts, keratinocytes, and leukocytes	Cancer cells, inflammatory epithelial cells, and MØ

AcLDL acetylated LDL, *CRD* cysteine-rich domain, *CD* cluster of differentiation, *LDL* low-density lipoprotein, *LPS* lipopolysaccharide, *LTA* lipoteichoic acid, *MGL* macrophage galactose lectin, *OxLDL* oxidized LDL, *RA* rheumatoid arthritis, *TAM* tumor-associated macrophages

10.2 Macrophage-Targeted Nanomedicines for Inflammatory Diseases

10.2.1 Cardiovascular Diseases and the Role of Macrophages in Atherosclerosis

Cardiovascular diseases (CVD) are the foremost cause of morbidity and mortality worldwide, representing 31% of all global deaths in 2016 (World Health Organization 2017). Atherosclerosis is the main underlying factor of the most common forms of life-threatening CVD such as coronary artery disease and cerebrovascular disease (Frostegård 2013). Atherosclerosis is a chronic arterial disease of inflammatory and metabolic origin. It takes place upon binding of aberrantly elevated levels of low-density lipoproteins (LDL), to the rich proteoglycans extracellular matrix of the endothelium. In there, LDL are chemically modified, mostly oxidated, and also suffering aggregation (Hansson and Hermansson 2011). Oxidized LDL are anomalously deposited in the intima, which inhibit the production of the anti-atherosclerotic labile liposoluble radical NO. NO prevents smooth muscle cell proliferation, leukocyte adhesion, and regulates the vascular tone. Decreased levels of NO prompt coronary vasospasm and cardiac ischemia producing the characteristic anginal pain (Steinberg 2002; Witztum and Steinberg 2001). Oxidized LDL are potent chemoattractant provoking the secretion of monocyte-chemotactic protein 1 (MCP-1) by the inflamed vascular endothelium, which also express cell adhesion molecules, including selectins, cell adhesion protein 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) that attach and collect circulatory monocytes (Libby 2012). The inflammatory process initiated in the endothelium thus is magnified by the arrival of attracted circulatory monocytes that accumulate in the intima and differentiate into tissue Mϕ. Such Mϕ display multiple phenotypes, being the prevailing M1 phenotype releasing pro-inflammatory cytokines and chemokines such as MCP-1, that recruit C–C chemokine receptor-2 (CCR2)-expressing monocytes and promotes the transfer of vascular smooth muscle cell (VSMC) from the media to the intimal layers (Adamson and Leitinger 2011).

The asymptomatic thickening of the intima could start as early as the first months of life. Later, the continuous cell collection and production of inflammatory mediators may induce progressive vascular alterations. A protagonist role in this process is played by Mϕ that express receptors relevant to perpetuate the local inflammation. TLRs for instance, recognize molecular patterns foreign to the body like bacterial pathogens (Krieger 1997), mostly TLR2 and 4, and produce cytokines such as TNF-α that augment local inflammation and VSMC proliferation (Ionita et al. 2010). Mϕ also express SR such as CD36 and SR-AI/II, which account for 75–90% of oxidized or acetylated LDL uptake and degradation; SR-B1 and lectin-like oxidized LDL receptor-1 (LOX-1) participate also in the uptake of oxidized LDL. The phagocytosis of oxidized aggregated and ECM-bound LDL causes intracellular cholesteryl ester accumulation in cytoplasmic lipid droplets. The resulting lipid-laden Mϕ or foam cells are the typical cells present in atherosclerotic

plaque. These foam cells result from cholesterol (chol) imbalance caused by increased chol influx or its esterification [regulated by Acyl coenzyme A: chol acyltransferase-1 (ACAT1) and neutral cholesteryl ester hydrolase (nCEH)] or decreased chol efflux [mediated by ATP-binding cassette transporters A1 (ABCA1), ABCG1 and SR-B1] (Chistiakov et al. 2017; Kzhyshkowska et al. 2012; Li and Glass 2002). The pathological thickening of the intima progress through the accumulation of VSMC, Mϕ, and foam cells, and end up in an anomalous structure known as fibroatheroma, which occludes in variable degree the vascular light. Common features of important lesions such as late core fibroatheromas are the acellular necrotic, hypoxic, acidic cores (made of chol released from senescent cells), absence of ECM (containing hyaluronan, proteoglycans, collagen) immersed in a fibrous cap produced by VSMC, irrigated by variable neovascularization. VSMC and endothelia also capture oxidized LDL employing SR-B1, contributing to the fibroatheroma structure.

Fibroatheromas can be structurally stable for a long time, or the fibrous cap may tend to erode or experience sudden rupture. Stable plaques are clinically silent while unstable plaques can rupture and produce vessel-occluding thrombosis and end-organ damage. Stable plaques have a thick protective fibrous cap, which largely consists of VSMCs that express CCR2, synthesize fibrin and collagen (Sakakura et al. 2013), and produce mostly MMP-2 (Sluijter et al. 2006). Different from stable fibroatheromas, thin (below 65 μm thick) cap fibroatheromas (TCFA) are characterized by immune cell infiltration, inflammatory cytokines production, decreased apoptosis of Mϕ, and necrotic processes. TCFA contain few VSMC, and are rich in inflammatory Mϕ producing proteases that degrade ECM, such as MMP-1, MMP-8 and 9, gelatinases, and stromelysin, which break down collagen and lead to fibrous cap thinning, plaque destabilization, and rupture (Libby 2013). Overall, the presence of senescent cells and prolonged inflammation promotes plaque instability, including elastic fiber degradation and fibrous cap thinning (Goetzl et al. 1996; Childs et al. 2016).

Importantly, plaque Mϕ show *reduced* migratory properties, which hinder the inflammation resolution, attract lymphocytes, and stimulate the progression of lesions into TCFA. The persistent inflammation induces Mϕ apoptosis, which in the absence of efficient phagocytic clearance of apoptotic cells (efferocytosis) and accumulation of debris, enables the plaque necrotic core development (Tabas 2000, 2010; Bäck et al. 2019). The rupture or erosion of TCFA exposes necrotic core contents to the circulating blood and induces platelet activation, which generates rapid thrombotic vascular occlusion and can induce myocardial infarction, stroke, acute limb ischemia, and cardiovascular death (Lusis 2000). Mϕ and smooth muscle cells within atherosclerotic plaques also overexpress the CD40 ligand (CD154), a potent procoagulant tissue factor. Inflammation and thrombotic complications of atherosclerosis are linked since both integrity of the protective fibrous cap and the thrombogenicity of the plaque are controlled by inflammation.

10.2.2 Current Therapeutics for Atherosclerosis

Most of the currently available therapies for atherosclerosis do not focus on the disease-causing pathways active in the vessel walls, but target risk factors such as hypertension and hyperlipidaemia. Current pharmacological treatments aimed to diminish the chol/LDL levels by inhibiting chol synthesis employing statins (atorvastatin, simvastatin, rosuvastatin, and pravastatin). Statins decrease plasma levels of LDL chol and triglycerides and increase plasma levels of high-density lipoprotein (HDL) chol reducing the risk of atherosclerotic CDV and suppressing the progression of atherosclerosis. However, statins induce myopathy, hepatotoxicity, and increase the risk of diabetes mellitus. Other drugs are also used to decrease morbidity and mortality of CVD such as antiplatelet agents, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, beta-adrenergic antagonists, calcium channel blockers, and diuretics (Arnett et al. 2019). Advanced cases require stent-assisted therapies, associated with complications, such as restenosis, inflammation, and thrombosis or coronary artery bypass surgery, the last needing of a longer time for recovery (d'Souza et al. 2017).

Remarkably however even if chol levels are strongly reduced, only a limited regression of the fibroatheroma is achieved (Nicholls et al. 2016) and is not sufficient for many patients to avoid major adverse cardiac event (Sabatine et al. 2017). The underlying reason is that atherosclerotic CVD is the consequence of the unresolve inflammatory response (Moore et al. 2013; Ross 1999; Witztum and Lichtman 2014). Besides the poor outcome of pharmacological treatments, current analytical techniques do not allow satisfactorily detect dangerous TCFA. Altogether these facts underscore the urgent need for better therapeutic and diagnosis strategies to treat and detect TCFA.

Vascular inflammation enhances the risk of recurrent atherothrombotic events, while Mϕ contribute to inflammatory risk (Barrett 2020). Therefore, therapies aimed to induce Mϕ efferocytosis, Mϕ emigration, or Mϕ polarization to a pro-resolving phenotype, should have clinical benefits. Counting on agents capable of selectively localizing within TCFA and performing specific targeting on enriched Mϕ and foam cells, would be key tools to meet such aims.

10.2.3 Macrophage-Targeted Nanomedicines for Atherosclerosis

Nanomedicines may provide the tools to overcome the challenges posed by the treatment and diagnostic of TCFA. Nanomedicines-mediated TCFA treatments and diagnostics should pursue two goals: the first is deceptively simple and consists of accumulating nanomedicines into the plaque; the second is detecting features of plaque lability, such as inflammatory Mϕ or any inflammatory Mϕ related activity. Intravenously injected nanomedicines are optimal to accomplish the first objective,

since different from conventional low-molecular-weight drugs, Nps below 300–400 nm tend to extravasate by convection at inflamed sites. Plaques progress at the walls of the vasculature of blood flow disturbance and low shear stresses. Upon convective extravasation, nanomedicines may passively accumulate at places of high vascular permeability present at inflamed vessels from fibroatheromas by passive targeting (Hu et al. 2018). The extent of nanomedicines accumulation into the plaque is dependent on their size, shape, and surface characteristics (tailored to minimize their liver uptake), and also on the site permeability. Plaque-specific extravasation brings nanomedicines close to plaque Mϕ, but alone is insufficient: to specifically deliver carried therapeutics or execute diagnosis, nanomedicines must be captured by inflammatory Mϕ. To do so, nanomedicines must display ligands that will be recognized and internalized by plaque Mϕ overexpressing specific receptors. Many of these candidate receptors for specific delivery however are ubiquitous. Such is the case of SR-B1 (also expressed in tumors); p-selectin, endothelial cell junctional molecules, such as PECAM1, expressed on most leukocyte sub-types, platelets, and at junctions between endothelial cells (overexpressed not only in infarcted myocardium, but also in arthritis, renal and hepatic diseases, acute lung injury, and graft rejection). Most of the preclinical active targeted nanomedicines for atherosclerosis rely on targeting either the transferrin receptor 1 (TfR1) (overexpressed not only in foam cells, Mϕ, and VSMC, but also on tumor cells) or the FR-β (overexpressed on activated, but not on resting Mϕ, and implicated in a diversity of inflammatory and autoimmune diseases) (Chen et al. 2020). The use of targeted delivery to a population with prevalent comorbidities thus may hamper its effective translation (Wang et al. 2021a). Nonetheless, even the newest strategies target Mϕ receptors which are not selectively expressed on plaque Mϕ, such as MR CD-206 (expressed only on immature monocyte-derived DCs); CD9 (ubiquitously expressed tetraspanin protein); hyaluronan acid receptors; CD36 SR (upregulated during monocyte-to-Mϕ differentiation and stimulated by hypertension, high glucose and oxidative stress, also expressed on DC, erythrocytes, adipocytes, and platelets), SR-AI/II (not expressed on monocytes, upregulated during monocyte-to-Mϕ differentiation), and chemokine receptor CCR2-binding motif of MCP-1.

Active targeting strategies for plaque Mϕ may be aimed at diagnostic or therapeutic purposes. The current diagnostic methods cannot detect nascent lesions. Targeting specific molecules or cells such as inflammatory Mϕ as a sign of plaque vulnerability are an emerging field for molecular imaging. Therapy instead pursues to reduce the inflammation within the plaque, on the basis of an array of strategies, such as (1) shifting Mϕ phenotype from M1 to M2 (to reduce the inflammatory component), (2) minimizing Mϕ chol content, by reducing its uptake and/or increasing its efflux (to reduce the formation of foam cells), (3) avoiding foam cells necrosis by delivering anti-senescent agents (to reduce their input to inflammation), and (4) increasing their ability to make efferocytosis (to avoid the senescence of foam cells).

In the last 5 years, a growing number of approaches showing the response of ex vivo and in vivo fibroatheroma/TCFA models to pharmacological treatments, as well as improvements in image diagnosis techniques performed with

macrophage-targeted nanomaterials, has been published. A detailed description of such voluminous information is out of the scope of this section, but the emerging landscape shows that most of the preclinical approaches developed between 2017 and 2020 employed polymeric [mostly *poly*(*lactic-co-glycolic acid*), PLGA, and hyaluronic acid, HA, based] and lipid-based (liposomes, HDL, other) nanomedicines, followed by micelles, dendrimers, cyclodextrins, and carbon-based, inorganic, biomimetic NPs. Most therapeutic nanomedicines combined macrophage targeting plus increased cholesterol efflux. Along 19 years (2001–2020), 27 clinical trials assessed the performance of mainly lipid-based therapeutics nanomedicines (Chen et al. 2021).

A selection of relevant strategies illustrating the latest experimental approaches will be discussed in the following sections. Several targeted nanomedicines do not show therapeutic improvements but are proof of concepts for effective macrophage targeting. Included are examples of macrophage targeting achieved only by passive targeting to the plaque, by tailoring nanomedicines size, shape, and surface features to control PK and extent of extravasation.

10.2.3.1 mAbCD9-Targeted Nanomedicines for Anti-senescence Drug Delivery

Statins are used to reduce blood chol level by inhibiting HMG-CoA reductase and are also being experimentally used as anti-senescent agents, because of their telomerase shortening inhibition, anti-inflammatory, and antioxidant activities (Boccardi and Paolisso 2014; Sørensen et al. 2019). Since dying Mϕ and foam cells are deleterious for fibroatheroma stability, a recent work has developed Mϕ-targeted NPs for delivery of statins as anti-senescent agents (Pham et al. 2021). To that aim, NPs were designed that combined monoclonal antibody (mAb) anti-CD9-mediated senescent Mϕ targeting, with delivery of rosuvastatin (RSV). CD-9 is a cell surface glycoprotein highly expressed in some smooth muscle cells and in Mϕ-rich plaques in atherosclerotic lesions that controls cell migration, proliferation, and adhesion (Nishida et al. 2000). CD9 expression is considered a marker of inflammatory cells; it induces cellular senescence through the phosphatidylinositide 3 kinase-AKT-mTOR-p53 signal pathway and aggravates atherosclerotic plaque formation in apolipoprotein E knockout apo E (−/−) mice (Cho et al. 2020). RSV was loaded in mesoporous silica Nps (MSN) (137 nm, $-\zeta$ potential 16.3 mV, 2.8 nm pore size, 0.1517 cm^3/g pore density) and covered by 3 layers of polymers: poly(ethylene glycol)-*block*-poly-glutamic acid) [PGA], poly-lysine (PLL), and hyaluronic acid (PHA). Then Nps were surface decorated with ~55 anti-CD9 mAbs (CD9-HMSN@RSV) per Np. The inner PGA and PLL layers avoided fast RSV leakage, inhibited plasma protein opsonization, decreased MPS uptake, and prolonged MSN circulation. In an in vivo model of atherosclerosis, the anti-CD9 mAb was observed to efficiently target the MSN, delivering RSV to inflammatory Mϕ; an additional release of free anti-CD9 mAb, that restrained the progression of

cell senescence, occurred upon dissociation of the external PHA layer by plaque hyaluronidase.

10.2.3.2 MCP-1-Targeted Nanomedicines for Competitive Inhibition of MMP1

To recognized and treat rupture-prone plaques possessing thin fibrous caps monocyte-binding, collagenase-inhibiting, and gadolinium-containing peptide amphiphile micelles (MCG PAMs) were recently developed (Chin et al. 2020). The micellar structure was made of three components: MCG (a peptide binding motif of MCP-1 to target monocytes and VSMC); Col-1 peptide (peptides having collagen cleavage recognition site sequence, [VPMS-MRGG] which are recognized by MMP-1 undergoing rapid degradation) to inhibit MMP collagenases and preserve the integrity of plaques; and diethylenetriamine pentaacetic acid (DTPA)-chelated Gd^{3+} to allow simultaneous magnetic resonance imaging of plaques. These micelles bonded to monocytes and Mϕ and were small enough ~15 nm since leaky endothelial tight junctions are 20–1330 nm range (Chin et al. 2019) to extravasate and labeled atherosclerotic aortas in apo E (−/−) mice in proportion to the severity of the lesions. Furthermore, micelles successfully detected plaques in diseased mice and acted as contrast agents for molecular imaging. Micelles competed with collagenases, treated mice showed 61% and 113% increase in fibrous cap thickness compared to non-targeting micelle- and PBS-treated mice, respectively. Overall, this multimodal Nps offers new opportunities for noninvasive diagnosis and treatment of atherosclerotic plaques.

The efficacy of the (a) and (b) approaches may be counterbalanced however by their huge structural complexity, which would make difficult its industrial scaling up.

On the other side, the following approaches employed nanomedicines of higher structural simplicity:

10.2.3.3 Hyaluronan-Targeted Nanomedicines

Hyaluronan (HA), a key component of the extracellular matrix, is a linear polymer of *N*-acetylglucosamine and a β-glucuronic acid. HA regulates cell adhesion, migration, and proliferation. The HA lining on vascular endothelium mediates immune cell rolling and extravasation during inflammation. Mϕ express several HA-binding receptors, including CD44, ICAM-1, LYVE-1, RHAMM, and TLR-4. The biological activity of HA depends on its degree of polymerization: low-molecular-weight (MW) HA stimulate inflammation and angiogenesis, whereas high MW (megadalton) HA inhibit these processes. The lack of immunogenicity and the low cost of HA have driven its application in biomedicine; however, its systemic administration is hampered by its rapid blood clearance and susceptibility to hydrolysis. Nanoparticulate HA can be prepared by deposition on the surface of either

lipid or polymeric Nps, or through transformation of its polymeric backbone in Nps by chemical modification of their carboxyl groups. A recent report showed that HA-Nps (90 nm, ζ potential −31.3 mV) are structurally stable under hydrolysis and efficiently target fibroatheroma-associated pro-inflammatory Mϕ in an apo E (−/−) atherosclerotic mice (Beldman et al. 2017).

10.2.3.4 Oxidized Phosphatidylcholines-Targeted Nanomedicines

Since oxidized phosphatidylcholines (oxPCs) of oxidized LDL bind to the CD36 receptor of intimal Mϕ in atherosclerotic lesions, Mϕ-targeted liposomes were designed by including a type of oxPCs (1-palmitoyl-2-(4-keto-dodec-3-enedioyl) PC) in the liposomal bilayer (Dhanasekara et al. 2021). Targeted liposomes (90 nm) co-localize with intimal Mϕ and CD36 receptors and show 1.4-fold higher accumulation in aortic lesion areas than non-targeted liposomes. This strategy could be useful to detect early stages of lesions and identify Mϕ amounts and distribution in the lesion, providing evidence of lesion vulnerability.

Biomimetic nano-carrier platforms are nanomedicines inspired by the way cells (erythrocytes, leukocytes, thrombocytes) or lipoproteins that interact with the vascular system behave and represent an alternative to drug synthetic Nps because of their longer circulation times, MPS evasion, and favorable interactions with target cells (Zinger et al. 2021). The following examples illustrate recent biomimetic approaches used to target plaque Mϕ.

10.2.3.5 Synthetic HDL Biomimetic-Based Passively Targeted Nanomedicines for LXR Delivery

Synthetic HDL (sHDL) Nps mimic the structure and function of pre-β HDL a specific small fraction (2–5%) of endogenous HDL (Fielding and Fielding 1995). sHDL are nanodisc structures of 8–12 nm of lipid bilayers wrapped around apolipoprotein A-I (apoA-I) or apoA-I synthetic peptide (ETC-642) at 1:2 w/w peptide to lipid ratio. Pre-β HDL accumulate in the atheroma area where they efflux the excess of chol from foam cells and deliver it to the liver for elimination (Kingwell et al. 2014). Acute treatment with sHDL, 4–6 times a week, reduces plaque burden in coronary artery disease patients (Tardif et al. 2007, 2014).

Liver X nuclear receptor (LXR) agonists induce the expression of chol transporters (ATP-binding cassette transporters ABCA1 and ABCG1), which stimulates excess chol efflux from plaque Mϕ to endogenous HDL acceptors. LXR agonists reduced plaque burden in apo E (−/−) murine models of atherosclerosis (Kratzer et al. 2009). However, LXR agonist induce liver toxicity. LXR agonists activate lipogenesis in liver, which induce hepatic steatosis and excretion of excess triglycerides into systemic circulation, increasing the levels of very low-density lipoproteins (VLDL) and pro-atherogenic LDL and intermediate-density lipoprotein. Besides, the high doses of LXR agonists needed to get anti-atherosclerotic effects,

the poor aqueous solubility, and the low levels of endogenous HDL acceptors in patients, limit the clinical translation of LXR agonists.

sHDL has been used as a carrier for the LXR agonist T0901317 (T1317), where sHDL acted also as an acceptor of chol efflux from Mϕ. The formulation induces atheroma regression in a severe model of atherosclerosis (Guo et al. 2018; Aye et al. 2010; Costet et al. 2000). Then HDL delivers efflux chol to the liver resulting in reduction of fibroatheromas (Kingwell et al. 2014).

In a recent work, the original formulation of sHDL-LXR agonist was adjusted to maximize encapsulation efficiency, drug retention, Np purity, upregulation of ABCA1/ABCG1 gene expression, and chol efflux. The improved formulation was observed to halt the development of atherosclerotic lesions on an early onset plaque formation apo E −/− murine atherogenesis model, with an average atheroma area of 4.9% compared to 12.2% and 10.8% after treatment with T1317 or sHDL alone, respectively (Yuan et al. 2021). sHDL-mediated delivery of LXR agonists would avoid hepatic toxicity by achieving effects with doses between 15 and 78-fold lower than those administered by oral route. In this approach however the sHDL were administered by the intraperitoneal (ip) route, which is not suitable for clinical use. Besides, despite two decades of preclinical studies, the industrial production of HDL proteins is still dealing with unsolved challenges (Brusinia et al. 2020). An interesting alternative to the industrial synthesis of lipoproteins is the therapeutic platform based on squalene (SQ), a cholesterol precursor, that can be bonded to drugs and upon iv injection, it is spontaneously inserted within lipoproteins, such as LDL, to be used for targeted delivery. SQ-based Nps (SQ Nps) were recently shown to target atherosclerotic plaque (Sobot et al. 2017). Indeed, significant accumulation of SQ Nps in both early and advanced atherosclerotic plaque, in apo E (−/−) mice, and interaction with plaque resident Mϕ was found (Brusinia et al. 2020).

10.2.3.6 Plaque Targeting Via Biomimetic Liposomes

Platelets, that interact with multiple substrates and release active factors, are fundamentals for atherosclerosis initiation and progression (Gawaz et al. 2008; Wu et al. 2017; Huo et al. 2003). In the early-stage platelets adhere to the injured endothelium, then platelets induce release of chemo-attractants, upregulation of endothelial adhesion molecules, and secretion of MMPs (Langer and Gawaz 2008). Activated platelets attract leukocytes, promote smooth muscle cell and fibroblast proliferation, and stimulate collagen synthesis, contributing to atherosclerotic lesion progression and maturation (Ross 1985). P-Selectins expressed on platelets directly interact with inflammatory cells (Totani and Evangelista 2010).

Another example of biomimetic nanomedicine made of liposomes covered with platelet proteins for targeted delivery of rapamycin (RAP) to the inflamed endothelium was recently published (Song et al. 2021). To that aim, hybrid vesicles made of platelet membranes and artificial lipid membranes (P-lipo) of (90 nm, ζ potential −20 mV) were prepared. RAP P-lipo reduced the average plaque area from ~53 to 14% and stabilized the atherosclerotic plaques in an apo E (−/−) mice. P-Lipo

showed a 5.91-fold increase in accumulation into the atherosclerotic lesion compared to conventional liposomes.

The last is an example of preclinical succeeding nanomedicines that because of their chemical nature and poorly explored biocompatibility and biodistribution, regulatory organisms may find difficult to accept.

10.2.3.7 Increased Efferocytosis with Passively Targeted Nanomedicines to Monocytes

Accumulation of apoptotic cells in the necrotic core is characteristic of atherosclerotic plaque. These cells are removed by efferocytosis (Latin: "to take to the grave"), a highly conserved process triggered by "eat me" ligands that signal phagocytes to induce uptake (Arandjelovic and Ravichandran 2015; Yurdagul Jr. et al. 2017). Conversely, cells may overexpress "don't eat me" ligands to avoid removal. Such is the case of the CD47 molecule, a major mechanism by which red blood cells enable immune evasion, and cancers establish and propagate disease. The anti-phagocytic CD47 signaling has a critical role in atherosclerosis, being upregulated in the atherosclerotic plaque (Kojima et al. 2014, 2016). CD47 binds with the signal regulatory protein-α (SIRPα), a transmembrane protein expressed in Mϕ and activates the SH2 domain-containing phosphatase-1 (SHP-1) initializing the intracellular signaling that inhibits phagocytosis. In this way, disease vascular cells resist to be removed and plaque expansion is promoted. Antibody-mediated blockade of CD47 accelerates the off-target removal of healthy tissue, such as the elimination of red blood cells in the spleen (Kojima et al. 2016), causing anemia and reduced oxygen-carrying capacity, which limits the translational potential of systemic pro-efferocytic therapies.

In a recent approach, nanomedicines to efficiently reduced plaque inflammation by interrupting CD47-SIRPα signaling in monocytes and Mϕ were developed (Flores et al. 2020). The system involves Peg-functionalized single-walled carbon nanotubes (SWNTs) loaded with a small-molecule inhibitor of SHP-1. Peg-SWNTs showed ability to accumulate within Ly-6Chi inflammatory monocytes (Smith et al. 2014), the primary circulating cells recruited to the diseased artery, where they differentiate into lesion Mϕ (Swirski et al. 2007). Peg-SWNTs were shown to accumulate within the atherosclerotic plaque, reactivate phagocytosis, and reduce plaque burden in atheroprone apo E (−/−) mice without compromising safety.

10.2.4 Inflammatory Lung Diseases and the Role of Macrophages

Pulmonary inflammation is generated by the innate immune system in response to harmful foreign stimuli such as invading pathogens, allergens, air pollutants, toxic chemicals, cigarette smoke, or by endogenous signals such as damaged cells.

Inflammation is an underlying pathology of several common respiratory diseases, such as COPD, asthma, acute lung injury (ALI), pulmonary fibrosis, and infectious diseases such as bacterial pneumonia or respiratory viruses. Although each disease expresses a unique inflammatory response, there are some shared characteristics: persistent inflammation, impaired repair process, and lung remodeling. The environmental adaptation of pulmonary macrophages plays a central role in pulmonary immunity response and is a determining factor in the establishment of chronic inflammatory pulmonary pathologies (Ogger and Byrne 2021). There are two distinct populations of pulmonary Mϕ: AMϕ, which are in the lung lumen in contact with the alveolar epithelial cells; and interstitial Mϕ, which reside in the parenchyma between the respiratory epithelium and the blood vessels. In healthy conditions, the main function of AM is to clear apoptotic cells and cell debris, express CD206, CD169, CD11c, CD163, and MARCO. In an inflammatory context, monocytes are recruited in the lung where they differentiate and add to the pool of AMϕ. M1 activation occurs by the classical stimulus or by losing their exposure to regulatory ligands like IL-10 or CD200 after epithelial cell injury during inflammation. The outcome of AMϕ activation is determined by pathogen-specific properties and by the host immune response to them. For example, during COVID-19-associated pneumonia Mϕ can produce a hyper-inflammation known as Mϕ activation syndrome or cytokine storm which is associated with constant production of pro-inflammatory cytokines (e.g., IL-6, IL-8, TNF-α, IL-1β) leading to acute respiratory distress syndrome (ARDS) (Ogger and Byrne 2021). Resolution of inflammation is achieved after clearance of foreign agents, elimination of recruited immune cells such as neutrophils by efferocytosis and IL-4/IL-13-mediated M2 Mϕ switching to initiate lung tissue repair (Schett and Neurath 2018). The switch to M2 phenotype has a central role in the resolution of pulmonary inflammatory conditions but could also contribute to fibrotic pathology through increased production of TGF-β as in idiopathic pulmonary fibrosis, or when leading to allergic airway chronicity as in asthma (Hussell and Bell 2014; Schett and Neurath 2018). An excess of Mϕ MMPs release produces structural changes in the lungs that can lead to pulmonary emphysema. In addition, during lung emphysema, AMϕ upregulates TLR2 and TLR4 expression and increases inflammation in response to infections. Conversely, cigarette smoke and COPD lead to decreased TLR2 and their phagocytic capacity, impaired bacterial killing, and neutrophil efferocytosis, but increase pro-inflammatory cytokines, chemokines, MMPs, and ROS production.

10.2.5 Macrophages-Targeted Nanomedicines for Pulmonary Inflammatory Diseases

Mϕ targeting nanomedicines for lung inflammatory diseases could decrease production of pro-inflammatory cytokines, induce M2 phenotype polarization, or reduce profibrotic activity of M2 Mϕ (Table 10.2). Local administration of Nps via intratracheal instillation or inhalation is a direct and more straightforward alternative

Table 10.2 Summary of macrophage-targeted nanomedicines for the treatment of inflammatory lung diseases

Disease	Receptor	Ligand	Nanomedicine	Drug	Aim	References
ALI	ud	Hexapeptide CLPFFD	Au Nps	–	M1–M2 switching	Wang et al. (2020, 2021a)
Lung fibrosis	CD44	Anti-CD44 antibody	Au Nps	Imatinib	Reduced M2 macrophages	Codullo et al. (2019)
COPD/interstitial lung disease	ud	PEI	Calcium phosphate PLGA Np	CCL-2, IP-10, and IFN-γ siRNA	Anti-inflammatory	Frede et al. (2017)
Staphylococcal pneumonia	ud	Peptide CRVLRSGSC	Porous silicon Nps coated with fusogenic liposomes	IRF5 siRNA	M1–M2 switching	Kim et al. (2018)
VILI	–	–	Lipid Np	pre-miR-146a	Mitigation lung injury	Bobba et al. (2021)
Pulmonary fibrosis	–	–	Cationic liposomes	Mdb2 siRNA	M1–M2 switching	Wang et al. (2021c)
Toxic industrial chemicals	CD44	HA	Multilamellar liposomes	DEX and *N*-acetyl cysteine	Anti-inflammatory	Rivkin et al. (2017)
ALI	–	–	Liposomes	DEX and TPGS	Anti-inflammatory	Shah and Banerjee (2019)
Asthma	SR-A1	PGP-Me	pH-sensitive archaeosomes	DEX-P	Anti-inflammatory	Altube et al. (2016, 2017)

ALI acute lung injury, *AM* alveolar macrophages, *DEX* dexamethasone, *DEX-P* dexamethasone phosphate, *PEI* polyethylenimine, *PLGA* poly lactide-*co*-glycolic acid, *TPGS* tocopheryl polyethylene glycol succinate, *ud* undetermined

than iv administration (that leads to MPS accumulation) for AMϕ targeting. Local pulmonary administration avoids the first-pass metabolism, has low enzymatic activity (compared to the gastrointestinal tract, GIT), and allows the use of lower doses than those necessary in a systemic administration, avoiding unwanted systemic effects (Loira-Pastoriza et al. 2014). For instance, intratracheal administration of AuNps coated with the hexapeptide CLPFFD for macrophage targeting (~13 nm, ζ potential −36 mV), in contrast to iv and intraperitoneal (ip) administration, led to more accumulation of AuNps in the lungs but less in the liver and other organs, in lipopolysaccharide (LPS)-induced ALI mouse model (Wang et al. 2021b). After being uptaken, CLPFFD-AuNp blocked the acidification process of the endosomes, inhibiting TLR4 signaling and the activation of NF-κB and IRF3 and the further pro-inflammatory response (Yang et al. 2016). Treatment with CLPFFD-coated AuNps increased granulocyte colony-stimulating factor (G-CSF) levels in the lung, IL-4 and IL-13 in serum, and M2 Mϕ in lungs (Wang et al. 2020).

Pulmonary fibrosis is a pathological consequence resulting from altered wound healing in response to persistent lung injury. CD44 is overexpressed by lung fibroblasts and Mϕ isolated from bronchoalveolar lavage fluid (BALF) of systemic sclerosis patients with interstitial lung disease. Anti-CD44-AuNps loaded with Imatinib (Imb), a tyrosine kinase inhibitor able to interfere with the downstream activation of profibrotic pathways (21 nm, ζ potential −46.3 mV) reduced the percentage of M2 Mϕ and IL-8 release in BALF of interstitial lung disease patients. Intratracheal administration of anti-CD44-AuNps-Imb resulted in AMϕ accumulation and reduction of pathological changes (collagen deposition and fibrotic tissue) as effective as ip administration of Imb however avoiding Imb systemic side effects, on bleomycin lung fibrosis murine model (Codullo et al. 2019). Possible translocation of AuNps to blood and local and systemic toxicity should be studied. Although intratracheal instillation is a useful tool to test nanomedicines in preclinical settings because it deposits a large number of Nps in the lungs, this administration route is not currently used in clinics.

In spite that large amount of dose could be ingested, a major part of intranasal administration of polyethyleneimine (PEI) coated calcium phosphate/PLGA Nps loaded with a mixture of pro-inflammatory cytokines siRNAs (~145 nm, ζ potential +23 mV) was observed in lung Mϕ and DC 1 h after administration to a sterile inflammation mice model. The expression of CCL-2, INF-γ inducible protein-10 (IP-10), and IFN-γ were downregulated in lung, and less loss of weight and a reduced number of cells within the BALF were observed, indicating a less severe inflammation (Frede et al. 2017). However, cationic Nps are known to be more toxic than negative or neutral counterparts and could lead to inflammation.

Silencing the Irf5 gene, that upregulates TNF, IL-1, IL-6, IL-15, IL-18, IL-23, and downregulates IL-10, induce repolarization of Mϕ toward the M2 phenotype. An interesting approach to decrease acute lung inflammation, promote bacterial phagocytosis and tissue repair during *Staphylococcal* pneumonia used porous silicon Nps with a fusogenic pegylated liposomal coating to Mϕ targeting and intracytoplasmic release Irf5 siRNA after iv administration. Nps (~190 nm, ζ potential −10 mV) targeted monocyte-derived Mϕ recruited to infected lungs and

significantly lowered expression of Irf5 in Mϕ collected from BALF, but not in pulmonary homogenates (composed of epithelial, endothelial, and interstitial cells) from *S. aureus* infected mice. This strategy allowed all the mice to be rescued from a lethal dose of *S. aureus* (Kim et al. 2018).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible and pathogenic coronavirus that emerged in late 2019. SARS-CoV-2 infection in humans manifests as mild symptoms to severe life-threatening pneumonia. SARS-CoV-2 begins replicating in the epithelial cells of the respiratory tract, then migrates down into the airways and enters alveolar epithelial cells in the lungs (Hu et al. 2020). This rapid replication and the destruction of lung cells triggers a local immune response, recruiting Mϕ and monocytes, release cytokines, and activate T and B cell immune responses, which in some cases, may lead to a dysfunctional strong immune response (Tay et al. 2020). This is evidenced in patients with severe COVID-19 with high plasma levels of Mϕ inflammatory protein 1α (MIP1α), G-CSF, IP-10, MCP-1, TNF-α, IL-1β, IL-2, IL-7, and IL-10 (Huang et al. 2020). Also, IL-6 plasma levels increase over time and are more elevated in non-survivors than survivors (Guirao et al. 2020). Despite that the mechanisms of lung injury and organ failure are still under investigation, these elevated cytokine levels plus Mϕ activation syndrome, elevated C-reactive protein, *d*-dimer levels, and renal dysfunction, suggest that cytokine storm may contribute to the pathogenesis of COVID-19 (Fajgenbaum and June 2020). In the context of Mϕ targeting, some works have recently shown that nanomedicines could mitigate lung injury and reduce lung fibrosis by Mϕ repolarization.

Mechanical ventilation is the standard of care for patients with ARDS including COVID-19 patients. However, the physical forces produced by mechanical ventilation aggravate lung dysfunction through a phenomenon known as ventilator-induced lung injury (VILI) (Slutsky and Ranieri 2013). To mitigate lung injury caused by VILI, AMϕ modestly increase expression of microRNA (miR-146a), (small non-coding RNA that acts as negative posttranscriptional regulators) associated with innate immunity and inflammation. A pre-miR-146a-PEI polyplexes loaded in lipid Np containing the antioxidant tocopheryl polyethylene glycol succinate (TPGS) (160 nm, ζ potential −0.6 mV) increased miR-146a expression in vitro, preferentially accumulated into AMϕ and mitigated lung injury during mechanical ventilation after intratracheal administration to mice (Bobba et al. 2021).

Current clinical evidence supports the possibility that pulmonary fibrosis may be one of the major complications in severe COVID-19 survivors after recovery (Han et al. 2021; Zou et al. 2021). M2 Mϕ polarization contributes to the pathogenesis of pulmonary fibrosis producing TGF-β1 and activating fibroblasts to myofibroblasts. A promising therapeutic target to reduce M2 polarization is the methyl-CpG-binding domain protein 2 (*Mbd2*) that mediates transcriptional repression in methylated DNA regions. Fibrotic lungs of patients with severe COVID-19 exhibited significant M2 Mϕ infiltration and *Mbd2* overexpression (Wang et al. 2021c). Intratracheal administration of *Mbd2* siRNA encapsulated in cationic liposomes (~100 nm, ζ potential +3.2 mV) protected mice from bleomycin-induced lung injury and fibrosis

by a significant reduction in the expression of fibrotic markers (fibronectin, collagen I, and α-SMA) and the M2 Mϕ marker Arg 1 (Wang et al. 2021c).

Systemic (iv hydrocortisone or oral DEX) corticosteroid therapy for 7–10 days in patients with severe COVID-19 is recommend by WHO to reduce mortality. Inhaled corticosteroids however could lower levels of inflammatory markers and improve lung physiology during ARDS, and potentially could inhibit coronavirus replication in host cells (Nicolau and Bafadhel 2020; Yamaya et al. 2020). Nowadays, several clinical trials are ongoing for the use of inhaled corticosteroids to treat or prevent COVID-19. Pulmonary administration of DEX-loaded Np however could specifically target AMϕ that initiate and spread inflammation in the lung, blood, and myeloid and lymphoid tissues, being a strategy to intervene in the sub-acute phase of COVID-19. This would better control Mϕ activation syndrome and cytokine storm, that would help patients recover faster and more efficiently than with free DEX treatment (Lammers et al. 2020).

Most clinical studies for pulmonary administration of Nps use nebulizers to generate aerosols (da Rocha Sandro et al. 2019). The use of nebulizers is the most direct, quick, and easiest way to aerosolize an aqueous suspension, also allowing a quick translation from animals to clinical tests with the same formulation and the same administration device previously evaluated preclinically (Cipolla et al. 2013). Furthermore, liposomes are almost the only Nps found in clinics (liposomal amikacin Arikayce) and in advanced clinical trials and this is mainly due to their high lung biocompatibility. Nebulization is not a gentle process and liposomes may lose their structure (modify vesicle size, lamellarity, membrane fluidity, and the amount of encapsulated drug) due to exposure to shear forces and the air–liquid interface (Carvalho and McConville 2016). However, liposomes with lipid bilayers of transition temperature above the nebulization temperature, such as those containing saturated phospholipids, are more robust to the nebulization process. For instance, Arikayce are unilamellar liposomes of 300 nm composed of dipalmitoylphosphatidylcholine (DPPC) and chol at 2:1 weight ratio.

There are few works studying inhalation delivery of liposomal DEX. In one of the pioneer work, it was shown that intratracheal administration of plain liposomes loaded with DEX increase the retention time of DEX in the lung as well as improve prophylactic efficacy in counteracting LPS-induced lung injury compared to free DEX (Suntres and Shek 2000). An active targeting strategy with DEX loaded in mannosylated liposomes was able to further decrease the production of TNF-α when administered in a model of inflammation induced by LPS in rats (Wijagkanalan et al. 2008). Recently, it was shown that co-delivery of DEX and antioxidants into liposomes effectively reduce lung inflammation. For instance, DEX and *N*-acetyl-cysteine (antioxidant) co-loaded in hyaluronan covalently linked multilamellar liposomes (HA-MLV, soybean phosphatidylcholine (SPC): dipalmitoylphosphatietanolamine (DPPE):chol 75:5:20 molar ratio) reverted animal weight loss to a level similar to that of control mice in a model of pulmonary inflammation and edema caused by exposition to toxic industrial chemicals (Rivkin et al. 2017). On the other hand, DEX disodium phosphate (DEX-P) loaded into plain liposomes (DPPC/1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphoglycerol ammonium

salt, POPG) containing TPGS (~270 nm, ζ potential −15 mV) significantly lowered oxidative stress, IL-1β, IL-6, and TNF-α levels in BALF in a mouse model of acid-ALI (Shah and Banerjee 2019).

None of the above strategies however consider the cytoplasmatic delivery of DEX where its cytoplasmic receptor is located. Novel pH-sensitive nanovesicles (ApH) that incorporated the natural SR-A1 ligand, the archaeolipid PGP-Me (2,3-di-*O*-phytanyl-sn glycero-1-phospho-(3′-sn-glycerol-1′-methylphosphate), were designed to increase the delivery of DEX-P to the cytoplasm of AMϕ. PGP-Me, an archaeolipid extracted from halophilic archaebacteria, can be incorporated into lipidic nanostructures such as vesicles or lipid Nps without any chemical synthesis. Besides, the chemical structure of PGP-Me (sn1,2 glycerol ether having fully saturated polyisoprenoid chains) make archaeolipids resistant to lipolytic enzymes, hydrolytic or oxidative attacks, and too harsh conditions such as nebulization. ApH (~150 nm, ζ potential −40 mV) showed increased cellular internalization than conventional liposomes in AM NR8383 and in J774A.1 cells. Due to their great cellular internalization, ApH were able to improve the anti-inflammatory and antioxidant activity of DEX-P in LPS-activated Mϕ. In addition, ApH were efficiently aerosolized with a vibrating mesh nebulizer, proving to be more stable than high stable [hydrogenated SPC (HSPC): chol 3:1 weight ratio] liposomes to the nebulization process (Altube et al. 2016). In addition, after nebulization, ApH could overcome a pulmonary surfactant barrier and deliver its hydrophilic cargo into J774A.1 cells to a greater extent than conventional liposomes (Altube et al. 2017). These strategies could be further investigated to evaluate their anti-inflammatory capacity in the context of COVID-19.

Overall, local administration via inhalation with stable and biocompatible Np has shown to be the best option for Mϕ targeting to reduce lung inflammation or fibrosis, protecting extremely sensitive siRNA or microRNA, and reducing access to corticosteroids such as DEX to health tissues.

10.2.6 Inflammatory Bowel Diseases and the Role of Macrophages

Inflammatory bowel diseases (IBD) such as Crohn's disease (CD, transmural inflammation in the complete intestine) and ulcerous colitis (UC, diffuse superficial mucosal inflammation in colon) are chronic, relapsing, progressive disabling disorders of the gastrointestinal tract (GIT), that affect millions of persons in the world (Palmela et al. 2015). Intestinal inflammation and epithelial damage induced by the uncontrolled activation of the immune system characterized these diseases.

Mϕ in the lamina *propria* of the intestine are vital for keeping homeostasis and the balance between the commensal microbiota and the host (Bain and Mowat 2014). These Mϕs are TLR-hyporesponsiveness acting as noninflammatory scavengers of microbes, express high levels of IL-10, contribute to the maintenance of

regulatory T (Treg) cells, and stimulate epithelial cell renewal (Bain and Mowat 2014). During intestinal inflammation, neutrophils and monocytes are sequentially recruited to mount a suitable immune response. These cells, following activation by PAMPs, produce IL-12, IL-23, and IL-1β that promote Th1 and Th17 cell responses toward invading microorganisms and produced epithelial damage. In a healthy person, efferocytosis of apoptotic neutrophils induces suppression of pro-inflammatory cytokines production and enhanced IL-10 and TGF-β production switching of M1–M2 Mϕ and starts the resolution phase. M2 Mϕ reduce the Th1 and Th17 responses and are indispensable to regenerate the epithelial barrier. In IBD patients however this process is dysregulated, conducting to accumulation of M1 Mϕ in the inflamed colon, which induces Th17 cells with increased expression of pro-inflammatory markers (IL-23, TNF-α, IL-1b, IL-6, and iNOS), contributing directly to the defective intestinal barrier function (Na et al. 2019). Besides, a defective signaling through TGF-β, which impairs M2 Mϕ recutting and deficiencies in efferocytosis is also involved in the pathogenesis of IBD.

10.2.7 Current Therapeutics for IBD

Treatment of IBD is symptomatic and depends on the stage of the disease. The classic oral drugs include 5-aminosalicylic acid, corticosteroids, and immunosuppressive drugs (azathioprine and methotrexate, MTX). Intravenous anti-TNF-α Mab infliximab and adalimumab, or the subcutaneous certolizumab pegol are used when conventional drugs fail. However, all treatments have limited benefits because of their systemic adverse effects displayed during long-term use. Therapy with Mab is expensive and could lead to serious adverse reactions, such as infection, anaphylaxis, and myelosuppression (Abraham et al. 2017).

10.2.8 Macrophages-Targeted Nanomedicines for IBD

Mϕ-targeted nanomedicines for IBD could reduce or block pro-inflammatory cytokines production, switch M1–M2 Mϕ phenotype, and promote wound healing (Table 10.3). Oral is the most ideal administration route, as it presents great safety, patient compliance, and is cost-effective for production. Oral administration also allows direct access to the intestinal colonic mucosa. However, the success of oral-targeted Nps depends on their ability to remain structurally stable along the GI transit, and on the possibility of accessing Mϕ. If well, the GIT is the most hostile environment in the organism, the differences between the inflamed mucosa of IBD patients and the normal gut can be exploited for Nps-mediated targeted delivery. IBD patients show loss of the inner adherent and the outer mobile mucus layer; infiltration of immune cells such as neutrophils, Mϕ, lymphocytes, and DC (Antoni et al. 2014); accumulation of positively charged proteins such as transferrin (Tirosh

Table 10.3 Summary of macrophage-targeted nanomedicines for the treatment of IBD and RA

Disease	Receptor	Ligand	Nanomedicine	Drug	Aim	References
IBD	MR	Mannose	Polymeric Np (carboxymethyl inulin)	Apremilast	M1–M2 switching	Sun et al. (2018)
		Mannose	Polymeric Np (chitosan)	miR-146b	M1–M2 switching, wound healing	Deng et al. (2019)
	CD44	HA	Polymeric Np (PLGA-chitosan covered)	CD98 siRNA KPV	Anti-TNF-α	Xiao et al. (2016, 2017)
		HA	HA-bilirubin conjugate	Bilirubin	Antioxidant	Lee et al. (2020)
		CS	pH-sensitive natural silk fibroin	Curcumin	Antiinflammatory	Gou et al. (2019)
	MGL	Galactose	Polymeric Np (PLGA-chitosan covered)	TNF-α siRNA	Anti-TNF-α	Huang et al. (2018) and Xiao et al. (2018)
	FR	FA	Pegylated polymeric Np (PLGA-Peg-FA)	6-Shogaol	Anti-inflammatory	Zhang et al. (2018b)
	PepT1	KPV	Polymeric Np (PLGA)	CyA	Anti-inflammatory	Wu et al. (2019)
	SR-A1	PGP-Me	Archaeosomes	DEX and BR	Anti-inflammatory Anti-oxidant	Higa et al. (2017, 2020)
RA	FR	FA	Stimulus-sensitive pegylated polymeric Np (PLGA-Peg-FA)	Mcl-1 siRNA	Macrophages apoptosis	Sun et al. (2019)
		FA	Redox sensitive pegylated AgNp	Ag+	M1–M2 switching	Yang et al. (2021)
	SR	DS	DS-5β-cholanic acid conjugate	MTX	Anti-inflammatory	Heo et al. (2017)
		DS	DS-MTX conjugate	MTX	Anti-inflammatory	Yang et al. (2017)
	CD44	HA	pH-sensitive pegylated HA-5β-cholanic acid	MTX	Anti-inflammatory	Alam et al. (2017)

AO antisense oligonucleotide, *BR* bacterioruberin, *CS* chondroitin sulfate, *CyA* cyclosporine A, *DEX* dexamethasone, *DS* dextran sulfate, *FA* folic acid, *HA* hyaluronic acid, *IBD* inflammatory bowel diseases, *KPV* lysine–proline–valine, *LRP-1* low-density lipoprotein receptor-related protein, *MTX* methotrexate, *PLGA* poly lactide-*co*-glycolic acid, *RA* rheumatoid arthritis, *SR* scavenger receptor

et al. 2009), bactericidal/permeability increasing protein, and antimicrobial proteins (Canny et al. 2002; Ramasundara et al. 2009); and disruption of the epithelial barrier function with the concomitant increase of permeability (Goggins et al. 2013). In this pathological context, macrophages can be found at the luminal side of the inflamed mucosa, favoring its accessibility from the oral route. On the other hand, the local pH is decreased [from 6.8 to 7.2 in normal mucosa to 5.5 to 2.3 in IBD patients' mucosa (Fallingborg et al. 1993)] and the GI transit is enhanced, with frequent diarrhea. Overall, to reach mucosal macrophages in an intact form, oral Nps must overcome low pH, the activity of degradative enzymes, and avoid being trapped within the mucus layer during the transit across the healthy portions of the gut. Negatively charged and small-sized Nps have been reported to accumulate to a high extent into the inflamed mucosa (Lamprecht et al. 2001). Nps can passively accumulate in the inflamed colon sites based on the epithelial enhanced permeability and retention (eEPR) effect (Watanabe et al. 2016; Lamprecht 2010). Np could then release their content; however, only if Np is taken up, loaded drug could be intracellularly released and hence therapeutic efficacy could be enhanced.

Mannose is widely used as a superficial ligand due to its high binding affinity for MR, its simple structure, non-immunogenicity, and for being inexpensive. Mannose-modified chitosan Nps was used to encapsulate microRNA mimic (miR-146b mimic) complexed with PEI to inhibit M1 Mϕ activation and promote wound healing. Oral administration of Nps (213 nm, ζ potential +28.3 mV) switched M1–M2 phenotype by regulating TLR4 signaling pathway resulting in the repression of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) and promotion of intestinal epithelial cells regeneration by regulating STAT3-dependent IL-10 production (Deng et al. 2019). Initial burst release in the upper GIT from polymeric Nps and hydrolysis of the ligand-Nps linkage could be reduced by incorporation into enteric-coated capsules or hydrogels. For example, mannosylated decamethylenediamine-grafted-carboxymethyl inulin Nps loaded with apremilast, a phosphodiesterase 4 inhibitor that switches M1- into M2-phenotype, were freeze-dried and encapsulated into enteric-coated capsules for oral administration. The synthesis of the self-assemble mannosylated amphiphile took several steps: carboxymethylation of inulin, introduction of hydrophobic segments of decamethylenediamine through an acid amide bond, and mannose linkage through a Schiff's base. The mannosylated-Nps (323 nm, ζ potential −11.7 mV, 5% mannose graft) showed great uptake in inflamed Mϕ and large accumulation in inflamed colon of DSS-model (60%); however, in vivo activity was not reported (Sun et al. 2018).

HA and chondroitin sulfate (CS) were also used for superficial Nps modification to target CD44 which is overexpressed on colonic epithelial cells and Mϕ in UC tissues. HA is biocompatible, biodegradable, and has several modification sites; however, HA is degraded by GI hyaluronidases. Nps can be embedded into chitosan/alginate hydrogels that protect Nps along the passage through the upper GIT, and then disassemble in the colon, releasing the loaded Nps. HA-modified PLGA Nps were prepared to simultaneously deliver CD98 siRNA (transmembrane protein complex related to mucosal damage and inflammation) and the antioxidant and anti-inflammatory curcumin. Nps (~246 nm, ζ potential −14 mV) were prepared

by a complex with several steps, double emulsion-solvent evaporation method. Briefly, first CD98 siRNA-spermidine complex was loaded into PLGA-polyvinyl alcohol (PVA) containing curcumin Nps, then chitosan was superficially adsorbed, and finally Nps were functionalized with HA via ester bond formation. Nps embedded in a chitosan/alginate hydrogel prevented mucosal damage and reduced inflammation, inhibiting the DSS-induced overexpression of CD98 and TNF-α in the colon (Xiao et al. 2016). In further work, same Nps were loaded with the naturally occurring anti-inflammatory tripeptide lysine-proline-valine (KPV). Oral administration of HA-Nps-KPV (270 nm, ζ potential −5.3 mV) exhibited a much stronger capacity to prevent mucosa damage and downregulation of TNF-α compared with non-targeted Np (Xiao et al. 2017).

A different strategy based on the self-assembling of an amphiphilic conjugate of hydrophilic HA and the hydrophobic endogenous antioxidant bilirubin that confers hyaluronidase resistance has been recently developed. The conjugate synthesis takes several steps starting from an acid form of HA and an aminoethylene–bilirubin conjugate and the latter conjugation through amine linkage in a ~4 molecules of bilirubin per each 100 kDa HA molecule ratio. The HA-bilirubin Nps (~400 nm, ζ potential −46 mV) accumulated in inflamed colonic epithelium and restored the epithelium barriers in DSS-induced colitis model (Lee et al. 2020). Other self-assembled Nps based on CS-modified natural silk fibroin were used for curcumin delivery by oral and iv routes (Gou et al. 2019). Hydrophobic domains of silk enable the self-assembly of Nps where curcumin is kept trapped using a mild desolvation method, followed by CS superficial conjugation via amide bonds. CS-silk Nps (175 nm, ζ potential −35.5 mV) embedded in chitosan/alginate hydrogel accumulated in colitis tissues after oral administration and undergo internalization by macrophages. Remarkably, due to the pH-sensibility of silk fibroin, curcumin could be released into the cytoplasm upon endocytic uptake. Interestingly, intravenous administration resulted in higher colonic Nps accumulation than oral administration.

Lactobionic acid (LA) was used for modification of chitosan through amidation reaction to target MGL. Galactosylated-chitosan coated PLGA Nps loaded with TNF-α siRNA were prepared by laborious double emulsion-solvent evaporation method. Galactosylated-Nps (~300 nm, ζ potential +12.2 mV) resisted the harsh conditions of the GIT and displayed superior efficacy in TNF-α gene silencing than galactose-negative Np in colon of DSS-mice (Huang et al. 2018). Co-embedded galactosylated-Nps (260 nm, ζ potential −8 mV, galactose content 0.23 mg/g Np) with recombinant IL-22 (inductor of epithelial regeneration) in a chitosan/alginate hydrogel significantly inhibited TNF-α, infiltration of mucosal neutrophils, and promoted the colon epithelia regeneration (Xiao et al. 2018). Chitosan Np could release the siRNA into the cytoplasm since it is known to overcome lysosomal sequestration by membrane destabilization or through a proton sponge effect (Coya et al. 2019).

FA-pegylated PLGA Nps loaded with the ginger active compound 6-shogaol to target both colon epithelial cells and Mϕ were prepared using commercial PLA-PEG-FA block copolymer added to a PLGA polymer/6-shogaol emulsion.

FA-PLGA Nps (250 nm, ζ potential −24 mV) embedded in a chitosan/alginate hydrogel relieved colitis signs and enhanced wound repair in DSS-mice through downregulation pro-inflammatory molecules (TNF-α, IL-6, IL-1β, and iNOS) and upregulation anti-inflammatory (Nrf-2 and HO-1) players (Zhang et al. 2018a).

The tripeptide KPV, additionally being an anti-inflammatory agent, is a ligand of peptide transporter 1 (PepT1) that is highly expressed in inflammatory colon epithelial cells and Mϕ (Wang et al. 2018). Complex method with several steps was used to prepare cyclosporine A (CyA) loaded KPV-PLGA (covalent linked) Nps were further coated with montmorillonite/chitosan for reduced CyA leakage in the upper GIT and mucus adhesion, respectively. Oral administration of Nps (185 nm, ζ potential +30 mV) decreased the levels of inflammatory cytokines and relieved colitis symptoms (Wu et al. 2019).

SR-A1 is involved in the innate immune response in intestinal inflammation (Komai et al. 2017). SR-A1 negative regulates NF-κB signaling and stimulates production of reparative cytokines, shifting Mϕ phenotype (Zong et al. 2018). However, SR-A1 has been sparsely explored as a receptor for Mϕ targeting. Solid lipid nanoparticles (SLN) containing the natural SR-A1 ligand PGP-Me has shown to deliver DEX to Mϕ of inflamed mucosa with enhanced anti-inflammatory activity and reconstitution of the epithelial barrier. Ultrasmall SLN containing PGP-Me (∼67 nm, ζ potential −41 mV) were highly uptaken and significantly reduced the levels of TNF-α, IL-6, and IL-12, compared to SLN without PGP-Me, by macrophages stimulated with LPS (Higa et al. 2017). Besides SLN showed enhanced mucus penetration. Further, incorporation of the high antioxidant C50 carotenoid bacterioruberin into SLN-containing PGP-Me displayed high anti-inflammatory and antioxidant activities on a gut inflammation model made of Caco-2 cells and LPS stimulated THP-1 derived Mϕ reducing TNF-α and IL-8 release and ROS production. Nps also reversed the morphological changes induced by inflammation (normal microvilli, well-defined tight junctions, desmosomes, interdigitations, and F-actin filaments) and increased the transepithelial electrical resistance, partly reconstituting the barrier function (Higa et al. 2020). One of the most important aspect of this work is that after in vitro digestion, the anti-inflammatory activity of Nps was retained, indicating the high structural resistance of Nps prepared with lipids extracted from halophilic archaebacteria.

Overall, oral administration with highly stable or embedded into protective capsules, for GI degradation and drug leakage reduction, active targeted Nps have shown to be a good option for Mϕ targeting to reduce intestinal inflammation, oxidative stress, and promoted wound healing in IBD. Some receptors used for targeting however, such as CD44 and PepT1, are not only expressed on inflammatory Mϕ but also on epithelial cells. Additionally, mucus penetration and retention of Nps in inflamed intestine were scarcely studied.

10.2.9 Rheumatoid Arthritis and the Role of Macrophages

Rheumatoid arthritis (RA) is a systemic, chronic, autoimmune disease with a high world prevalence (0.5–1%) that causes long-term disability and low quality of life (Davis and Matteson 2012). RA cause progressive destruction of joints and vascular, metabolic, osseous, and psychiatric comorbidities. Multiple causes are associated with RA pathogenesis, but all generate inflammatory cell infiltration, synovial hyperplasia, autoantibodies production, and excess of synovial fluid. These in turn cause joint swelling, pain, progressive stiffness, joint destruction, and bone erosion. During this process, principally Mϕ produce pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), which stimulate synovial fibroblast (synoviocytes) to produce MMP that degrade the joint and activate osteoclast which produce bone erosion (McInnes and Schett 2017). Besides the inflammatory mediators, ROS and RNS produced by Mϕ play a key role in RA pathogenesis. Due to the hypoxia-inducible factor (HIF-1α) expression and the high ROS level, in arthritic joints Mϕs are M1 subtype (Peiser and Gordon 2001).

10.2.10 Current Therapeutics for RA

Current therapeutic agents are divided into four categories: disease-modifying anti-rheumatic drugs (DMARD, such as MTX, hydroxychloroquine, and sulfadiazine), glucocorticoids (DEX, hydrocortisone, prednisone), nonsteroidal anti-inflammatory drugs, and biological agents (anti-TNF-α Mabs such as adalimumab, certolizumab and infliximab, the anti-IL-6 Mab sarilumab and the anti-IL-17 Mab ixekizumab). Even though these treatments are effective up to a certain level, there are still numerous limitations. For example, many patients do not respond to DMARD and all drugs showed numerous adverse effects. Mabs to some degree reverse the progression of RA however ~50% of patients who respond at the beginning, stop responding after 1 year (Singh et al. 2011). Additionally, these patients are prone to acquire local or systemic infections, such as tuberculosis or tumors, and the cost of treatments with Mabs are extremely high.

10.2.11 Macrophages-Targeted Nanomedicines for RA

M1 Mϕ should be eliminated/switched to M2 phenotype to alleviate synovial inflammation. Vascular permeability in RA sites is high, which allows passive accumulation of pegylated Nps through so-called ELVIS effect (Extravasation through Leaky Vasculature and subsequent Inflammatory cell-mediated Sequestration) (Wang and Goldring 2011) like the EPR effect observed in solid tumors. For instance, DEX loaded into pegylated micelles or pegylated liposomes showed

accumulation into inflamed joints and reduced inflammation. DEX loaded into polymerized stealth liposomes suppressed pro-inflammatory cytokines (TNF-α and IL-1β) in joint tissues, decreasing the swelling of inflamed joints in the adjuvant-induced arthritis rat (AIA) model (Fang et al. 2020). Since pegylation reduces Mϕ uptake of Np, accumulated Nps in the inflamed joints act as depots where DEX should be released, active targeting to Mϕ could enhance the intracellular accumulation of the loaded drug and improve therapeutic efficacy.

FA, dextran sulfate, and HA were used as ligands of Nps for targeting FR-β, SR-A, and CD44 receptors on Mϕ, respectively, to treat CIA mice or AIA rat by the iv route (Table 10.3).

Myeloid cell leukemia-1 (Mcl-1) is an anti-apoptotic signal overexpressed in Mϕ from RA joints. Polymeric Nps composed of a commercial FA-Peg-PLGA as targeting ligand, a novel polyketal (PK3) as a pH-sensitive polymer, and a Mcl-1 siRNA/DOTAP (dioleoyloxy)propyl-trimethylammonium methyl-sulfate) lipoplexes core (143 nm, ζ potential 3.6 mV) was shown to be taken up by Mϕ, while the siRNA was released into the cytosol. Nps showed to be accumulated in inflammation zones and high efficacy in the AIA rat model (Sun et al. 2019).

In a different strategy, glutathione-sensitive FA modified silver Np (FA-AgNps) was used to induce M1 Mϕ apoptosis and M2 switching. FA-AgNps were obtained in several steps. Briefly, heterofunctional lipoyl-FA-Peg (LA-Peg-FA) was synthesized through DCC/NHS coupling chemistry (~45% of LA-Peg modification) and then LA-Peg-FA was attached through Ag-sulfide bond on the AgNps surface. After entering cells, FA-AgNps (30 nm, ζ potential −6 mV) released Ag^+ in response to intracellular glutathione (~1000-fold higher than in extracellular fluids), and induced M1 Mϕ apoptosis and scavenged ROS causing M2 polarization. FA-AgNps displayed long-circulation life and showed biodegradability. FA-AgNps accumulated in inflamed joints decreased clinical score and showed better end outcomes, than MTX and MTX-AgNps in CIA mouse. FA-AgNps reduced TNF-α, IL-1β, and IL-6 almost to a normal level, reduced M1, and increase M2 Mϕ-specific biomarkers in inflamed joints (Yang et al. 2021).

Mϕ and synoviocytes of patients with RA overexpressed the SR-A that is in part responsible for pro-inflammatory cytokines and MMP generation. Dextran sulfate (DS) is a hydrophilic biocompatible and biodegradable polysaccharide ligand of Mϕ (SR-A). Self-assembled Nps of DS-5β cholanic acid and MTX-DS showed to be accumulated in inflamed joints and reduced the pro-inflammatory status. An amphiphilic DS derivative was synthesized by a simple two-step procedure where 9 hydrophobic 5β-cholanic acid were conjugated to 100 sugar residues of DS to obtain self-assembled Nps (220 nm) where MTX was loaded by dialysis. Fluorescently labeled Nps showed accumulation in inflamed knee and ankle of CIA mice; however, substantial fluorescence was found in liver and kidney. Despite rapid MTX released from Nps in the initial 3 h (~50%), MTX-Nps showed higher efficacy against CIA mice compared to free MTX alone (Heo et al. 2017). To reduce the MTX leakage, MTX was covalently linked with DS through condensation reaction. The amphiphilic DS-MTX conjugate obtained self-assembled into ~100 micelles and accumulated at inflamed site after iv administration; however, organ distribution

was not shown and 60% of MTX was released in the first hours. DS-MTX micelles mitigated synovitis and protected articular cartilage (Yang et al. 2017).

CD44 is overexpressed in the early stages of inflammation to recruit immune cells. Self-assembled pegylated HA-5β-cholanic acid was used for MTX delivery to Mϕ in CIA mice. Peg was linked to amphiphilic HA-5β-cholanic acid conjugate (11 5β-cholanic acid moieties per 100 sugar residues of HA). Pegylated-HA acts as the hydrophilic shell and 5β-cholanic acid as the hydrophobic core, where MTX was encapsulated. Calcium phosphate was further loaded into HA to selectively release MTX in acidic media (50% at pH 5). Nps (~220 nm) showed accumulation in arthritic paws and reduction of inflammation safety with a high dose of MTX, in CIA mice (Alam et al. 2017). However, the nonspecific uptake by hepatic sinusoidal endothelial cells and the low stability in physiological conditions of HA-based Nps should be considered (Choi et al. 2012).

Overall, iv administration of active targeted Nps has shown to reduce joint inflammation in RA. However, a great accumulation of Nps into Mϕ of the MPS and the use of receptors that are also expressed in other tissues such as MR or CD44, or receptor that could be overexpressed by comorbidities could reduce the effectivity and increase the potential off-target toxicity of these strategies.

10.3 Macrophages-Targeted Nanomedicines for Infectious Diseases

Mϕ-targeted nanomedicines have been used to intracellular co-localize antimicrobial drugs with infections agents like leishmanial parasites, tuberculous, and nontuberculous mycobacterium.

10.3.1 Leishmaniasis and the Role of Macrophages

Leishmaniasis, caused by the protozoa parasite *Leishmania*, affects around one million persons annually and causes 20,000–30,000 deaths. Most of the leishmaniasis cases occur in Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan.

There are three clinical manifestations of leishmaniasis: cutaneous (CL), mucocutaneous (MCL), and visceral (VL). Leishmania promastigotes enter the skin by the bite of sandflies and invade local phagocytic cells. Promastigotes transform into amastigotes and survive into the phagolysosomes, where they multiply. After being released, promastigotes are distributed to local or distant phagocytes. VL is characterized by parasites colonization of liver, spleen, and bone marrow Mϕ, skin Mϕ. CL is characterized by Langerhans cells and DC colonization, while in MCL, lymph nodes and mucosal cells are also colonized.

10.3.2 Current Therapeutics for Leishmaniasis

Pentavalent antimonials (Sb^v) were the first antileishmanial agents used, but given their toxicity, treatment evolved depending on the clinical manifestation to parenteral liposomal amphotericin B (AmB) (AmBisome) and paromomycin, and oral miltefosine. The iv infusion of AmBisome (plain unilamellar liposomes of 80 nm) is a standard treatment for the lethal VL and clinical efficacy in CL patients was shown (Wijnant et al. 2018; Wortmann et al. 2010). However, all treatments show disadvantages such as variable cure rates, toxicity, high costs, and emerging resistance.

10.3.3 Macrophages-Targeted Nanomedicines for Leishmaniasis Treatment

Since liver and spleen Mϕ are targets of both leishmania parasites and iv administered plain Nps, in a seminal work it was shown that Sb^v-liposomes eliminate 99.8% of the parasites in vivo but with a 100-fold lower dose than free Sb^v (Alving et al. 1978). Almost 20 years later, FDA approved AmBisome for the treatment of VL. AmBisome alters PK, BD, and PD properties of AmB resulting in improved efficacy, tolerability, and reduction of the nephrotoxicity associated with conventional AmB deoxycholate administration (Fungizone). The main limitation of AmBisome however is its high cost, even a single dose (5 mg/kg 97.5% cure rate in VL patients in India) is costly. Besides, AmBisome is unstable above 25 °C (Croft and Olliaro 2011), increased size and decreased AmB content have been reported after 72 h storage at room temperature (Zia et al. 2017). Even slight changes in the AmB to phospholipids molar ratio or modifications in the manufacturing procedure affect the efficacy and toxicity of liposomal AmB (Olson et al. 2008).

Several different types of Nps (liposomes, polymeric Nps, SLN) encapsulating diverse clinically approved or preclinical antileishmanial drugs have shown promising results in experimental models of VL and CL as recently been reviewed (Singh et al. 2019, 2020; Nafari et al. 2020; Sousa-Batista and Rossi-Bergmann 2018; Espuelas et al. 2016). However, it should be relevant to find only one or a few doses of treatment that could be economically affordable. Here, only the more recent Mϕ targeting strategies that employed approved drugs and with the best chances of translation will be described.

Immunomodulation could be a way to improve the current therapy by enhancing efficacy and/or reducing drug intake. The *Leishmania* parasite has developed several strategies, which can inhibit Th1 response by diverting DC to a state that induced parasite-infected Mϕ toward anti-inflammatory Th2 response. These lead to reduced production of pro-inflammatory cytokines (TNF-α, FN-γ), ROS, and RNS. Additionally, these early controls in Th1 response may help in the early control of innate immunity that eventually leads to compromised adaptive immunity characterized by decreased proliferation of CD4+ and CD8+ T cells and enhanced Th2 response by

means of anti-inflammatory cytokines (IL-4, IL-10, and TGF-β). Co-loading of an immunomodulatory drug with a leishmanicidal drug into Mϕ targeting nanomedicines could reverse the immune bias from Th2 to Th1 response. However, excessive inflammatory response may worsen the prognosis of CL, whereas restoration of Th1 response is necessary to cure VL patients (Murray et al. 2000).

MR, MGL, SIGN-R1, DCSIGN, and other cell surface Mϕ receptors that recognize polysaccharide residues on parasite could lead to immune stimulation. For instance, AmB loaded into mannan-PLGA nanospheres and mannosylated-chitosan Nps have shown immunomodulation in the treatment of VL. High expression of MHCII and co-stimulatory molecules (CD40, CD80, and CD86), induction of a pro-inflammatory response (IL-6, IL12p40, and TNF-α) on Mϕ and higher in vivo efficacy was shown by AmB-loaded mannan-PLGA nanospheres against *L. infantum* infection in comparison with Fungizone (Barros et al. 2015). Interestingly both empty and AmB-loaded mannosylated-chitosan Nps (~200 nm, ζ potential +31.7 mV) induced high expression of pro-inflammatory mediators (IFN-γ, IL-12, and TNF-α), suppress levels of immunosuppressive cytokines and increased iNOS production in *L. donovani* infected hamsters. However, AmB-loaded mannosylate-Nps significantly reduced splenic parasite burden (~90%) compared with non-targeted Np (Asthana et al. 2015a). AmB was also loaded into lactoferrin-coated PLGA Np (~200 nm, ζ potential +21.7 mV). Lactoferrin binds to multifunctional glycolytic protein (GAPDH) but also to MR and DCSIGN. Infection results in overexpression of GAPDH to accomplish iron requirement of parasite. Lactoferrin-Nps has shown to increase production of pro-inflammatory mediators while downregulated disease-stimulating cytokines in *L. donovani*-infected hamsters, resulting in higher reduction of splenic parasite burden (~88%) compared with AmBisome (~68.8%) and fungizone (~55.6%) (Asthana et al. 2015b). More recently, galactofuranoside containing liposomes showed to produce a mixed polarization profile into Mϕ (induction of genes encoding M1 pro-inflammatory cytokines (IL-12, IL-1β, and TNF-α and iNOS) and the M2 cytokine IL-10). Galactofuranoside-liposomes enhanced Th1 immune response showing induction of crucial pro-inflammatory cytokines and iNOS in target organs, but reduced serum inflammatory cytokines in mice. Treatments however only modestly reduce parasite loads in liver and spleen, compared with AmBisome, against *L. donovani* infection (Guegan et al. 2019).

The oral route is suggested for CL and VL however because of their poor aqueous solubility and bioavailability, most drugs, except miltefosine, are administered by parenteral routes. New approaches intended to change the administration route have recently been described. For instance, to enhance AmB oral absorption and minimize its side effects, three formulations (cochleates, chitosan Nps, and self-emulsifying drug delivery systems, SEDDS) are in clinical trials (Serrano and Lalatsa 2017). While cochleates and SEDDS are based on conventional pharmaceutical technology AmB-chitosan Np allows oral targeting to Mϕ of lung, liver, and spleen, but avoids delivery to kidneys. AmB was encapsulated in core-shell Nps (200 nm) made of palmitoyl-methyl dimethyl-trimethyl-6-*O*-glycol chitosan (GCPQ). The palmitoyl chains of GCPQ form a nanocomplex with AmB in the core while the hydrophilic

quaternary ammonium groups interact with carboxylate group of AmB and form the particle shell. The AmB-GCPQ Nps obtained are very stable and can be reconstituted from a dry powder. AmB-GCPQ Nps are taken up by enterocytes and Peyer's patches that enable the translocation of AmB-GCPQ Np. High levels of AmB were found in liver, lung, spleen, and bone marrow, as a result of Mϕ phagocytosis of Nps. Lower AmB concentrations were found in target organs after oral administration of AmB-GCPQ Nps compared to iv AmBisome; however, there were no differences in the efficacy of both formulations in *L. infantum*-infected Balb/c mice (Serrano et al. 2015).

Topical treatment of CL should be advantageous compared to parenteral treatments since it could eliminate the local parasites preventing the risk of dissemination, reduce scar formation and disfigurement, reduce the toxicity of parenteral drugs, improve patients' compliance, and reduce treatment costs. However, the location of infected Mϕ in the border of the lesions with epidermal thickening difficult drug penetration. Nps could enhance drug permeation and target drug intracellularly, besides Nps could have immunomodulatory or wound-healing properties.

Several works show the effectivity of Sb^V or miltefosine-loaded liposomes for the topical treatment of CL. For example, deformable liposomes loaded with Sb^V (195 nm, ζ potential +32.8 mV) showed tenfold higher skin retention in the deeper skin layers than free drug, without the use of classical permeation enhancers, and reduced parasite burden in *L. tropica* infected Balb/c mice (Dar et al. 2018). Recently, deformable liposomes co-loaded with miltefosine and the polyphenol apigenin (120 nm) showed 3.2-fold higher skin permeation compared with free drug and a 9.5-fold reduced parasitic burden in *L. mexicana*-infected Balb/c mice (Dar et al. 2020). Stearylamine (with per se antileishmanial activity)-bearing liposomes loaded with Sb^V improved the Sb^V permeation compared with Sb^V cream and reduced lesions size in *L. major* infected Balb/c mice (Moosavian et al. 2019).

The use of AmB-loaded Nps for topical treatment of CL however is more complex. On one hand, the structural properties of drugs (MW and hydrophobic/hydrophilic balance) directly impact skin penetration and their leishmanial activity. On the other hand, susceptibility differences of leishmania species and their lymphatic nodule dissemination could contribute to this problem. The low permeation of AmB (high MW and insolubility in water) through uninfected and infected skin explained the unsuccessful topical AmB treatment on *L. major* infected mice (El-On et al. 1984). Liposomal AmB with increased skin permeation has shown different efficacy depending on the leishmanial strain. SinaAmpholeish 0.4% gel is a semisolid formulation of liposomal AmB (80 nm) produced by Exir Nano Sina (Tehran, Iran) for topical treatment of CL. It is claimed that sinaAmpholeish permeates *stratum corneum* and reaches the dermal and epidermal macrophages. SinaAmpholeish was effective against *L. major* using a Balb/c back rump infection model (US Patent US 20150147382A1). Clinical studies show that SinaAmpholeish has a 95% effectiveness for rural leishmaniasis with *L. major* and 30% for urban leishmaniasis with *L. tropica*. However, topical SinaAmpholeish was unable to cure a murine *L. mexicana* infection model that closely resembles clinical disease

(Varikuti et al. 2017). The virulence of this leishmania strain might require a higher dose, an earlier or a more prolonged treatment.

Interestingly, recently it was shown that iv administration of AmB-loaded chitosan-TPP Nps (70 nm, ζ potential +25.5 mV) reduced lesion size and parasite load in *L. major* infected Balb/c mice, with more efficacy than AmBisome. However, poor AmB permeation into and through mouse skin showed that AmB-loaded chitosan Nps are not appropriate candidates for topical treatment of CL (Riezk et al. 2020). These Nps release the AmB on the skin, which then should permeate into and through the skin in the free form. In the same sense, topical chitosan-coated poly (isobutyl cyanoacrylate) Nps (187 nm, 53.8 mV), gelled with pluronic F127 resulted in partial and incomplete healing lesions in *L. major* infected Balb/c mice (Malli et al. 2019).

Overall, if well for more than 20 years AmBisome has shown to be effective for leishmaniasis treatment, we are still searching for inexpensive treatments that could be orally or topically applied.

10.3.4 Tuberculosis and the Role of Macrophages

Tuberculosis (TB) is a life-threatening disease caused by *Mycobacterium tuberculosis* (*Mtb*). TB is one of the top 9 causes of death worldwide. Mycobacteria, transmitted by the air, enter the respiratory tract, and are phagocytized by AMϕ, where they spread across the lungs. Weeks later, mycobacteria could distribute to liver and kidneys. Inside Mϕ, mycobacteria multiply within phagosome arresting their fusion with lysosomes. In this way, Mtb subvert host immune responses and utilize the lung Mϕ as a niche for growth and proliferation.

10.3.5 Current Therapeutics for TB

Although the BCG (Bacille Calmette–Guérin) vaccine prevents childhood TB, it fails to protect adults already infected or sensitized to mycobacteria. Six-month treatment with 4 drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) is the standard chemotherapy for drug-susceptible TB. Prolonged treatments (up to 24 months) with pyrazinamide combined with second-line drugs (e.g., fluoroquinolones, ethionamide, cycloserine, capreomycin, or prothionamide) are required for multidrug-resistant strains or low proliferating phases. Limited bioavailability and poor absorption; rapid degradation or excretion; systemic distribution; toxicity and high costs of these drugs, as well as low patient compliance to the long-lasting treatments, are drawbacks of the current therapies.

10.3.6 Macrophages-Targeted Nanomedicines for TB

Passive and active targeting is possible for Np delivery in TB by the iv route. Pegylated Nps could be accumulated in granulomas by the recruitment of Mϕ that phagocytosed the Nps (Trousil et al. 2019) or via a process that resembles EPR effect (Fenaroli et al. 2018). Several studies demonstrating in vivo achievements using passively targeted Nps against TB have recently been reviewed (Hussain et al. 2019; Donnellan and Giardiello 2019). Although great number of works, from more than 10 years, have focused on active targeting Mϕ demonstrating enhanced uptake of the targeted Nps relative to non-targeted formulations, only a small portion of works shows in vivo results (Baranyai et al. 2021). Besides, biodistribution studies corroborated that KC of the liver is the cell population that concentrates the highest proportion of the injected Nps dose. Additionally, a change of the currently used oral administration route by iv administration should ensure the complete elimination of Mtb with few doses.

The inhalation route appears the most promising drug entry to achieve high local concentrations of the drug in the infected Mϕ, since ~75–80% of infections persist localized in the lungs. Besides, local administration could reduce dose level and prevent adverse reactions, avoiding GI degradation and first-pass metabolism. Despite this, very few studies have focused on Mϕ targeting nanomedicines for inhalation therapy against TB. Spray drying of Nps using suitable carriers can preserve Nps during dehydration and can provide microparticles (MP) with aerodynamic diameter (daer) optimal for alveolar deposition (1–5 μm). Upon contact with pulmonary fluid, these MP could dissociate and release the Nps for further Mϕ uptake. For example, inhaled mannitol MP encapsulating SLN loaded with rifabutin with daer of 4–5 μm delivered higher amounts of rifabutin in lungs compared with free drug in mannitol MP, additionally relevant quantities of drug were also detected in liver and spleen in mice. This system efficiently reduced the bacterial burden in lung, spleen, and liver of Mtb-infected mice (Gaspar et al. 2017). Mannosylated-SLN and liposomes were also designed for powder inhalation. Mannosylated-SLN [containing hexadecanoic acid (aminoethyl α-D-mannopyranoside)amide] achieved a respirable particle fraction of 30–50%, showed high Mϕ uptake even in the presence of a commercial replacement of natural pulmonary surfactant and showed high rifampicin retention in lungs after intratracheal powder aerosolization in mice (Maretti et al. 2019a, b; Truzzi et al. 2020). Mannosylated-liposomes loaded with moxifloxacin co-spray drying with dextran improved liposomal physical stability, achieved a respirable particle fraction of more than 75%, and showed deep lung deposition after intrapulmonary administration using dry powder inhaler in rats (Hamed et al. 2019). The efficacy of these two approaches on TB models remains to be tested.

Self-assembling hydrophobized HA-nanogels (500 nm, 2.4 mV) loaded with an antimicrobial peptide (LLKKK18) were nebulized to *M. avium* or *Mtb*-infected mice. HA-nanogels were highly internalized by Mϕ and reduced the intracellular levels *M. avium* and *Mtb* in vitro, together with reducing pro-inflammatory cytokine

levels (IL-6 and TNF-α). Intratracheal administration using a MicroSprayer® aerosolizer of peptide-HA-nanogels significantly reduced bacterial levels in the lungs (Silva et al. 2016).

If well only recently has been addressed, local administration via inhalation with passive or active targeted Np could be a good option for Mϕ targeting to increase antimicrobial activity, while reducing access of antibiotics to healthy tissues.

10.3.7 Nontuberculous Mycobacterial Disease

Pulmonary nontuberculous mycobacterial (*Mycobacterium avium* complex [MAC]) disease is a chronic, frequently progressive infection characterized by necrotizing inflammation, bronchiectasis, associated irreversible lung damage, and increased mortality. In 2018, the FDA approved liposomal amikacin for inhalation (LAI; Arikayce) to treat refractory MAC lung disease, becoming the first liposomal formulation specifically approved to be administered by the inhalation route. Arikayce are neutrally charged liposomes (~300 nm) administered via a PARI eFlow vibrating mesh nebulizer. Arikayce improved the efficacy of conventional treatment (macrolide, ethambutol, and rifamycin) for MAC lung disease in terms of microbiological results, although a clinical benefit has not yet been established (Olivier et al. 2017; Shirley 2019). Treatment of nontuberculous mycobacteria infections can be challenging because they can persist in biofilms or as intracellular infections within Mϕ. One of the main advantages of inhaled LAI treatment versus conventional amikacin-free intravenous administration is its ability to target pulmonary Mϕ. In vitro, LAI can improve amikacin uptake by ~fourfold into THP-1 Mϕ compared with free amikacin. In rats, nebulized LAI increased amikacin concentrations in pulmonary Mϕ by eightfold at 24 h post-dose relative to free amikacin. Furthermore, compared to iv-free amikacin, LAI increased 274-fold the mean AUC-time curve in Mϕ (Zhang et al. 2018b). Consequently, LAI can improve lung retention time while minimizing systemic exposure, compared to iv administration of free drug.

10.4 Conclusions and Prospects

AmBisome, one of the first FDA-approved nanomedicine in the 90s, and the recently approved Arikayce, are based on plain liposomes that are naturally highly uptaken by MPS Mϕ and AMϕ to deliver massively and specifically the loaded amphotericin B and amikacin by the iv or inhalatory routes, respectively, to the infected macrophages. AmBisome and Arikayce significantly reduced the toxicities of free drugs (nephrotoxicity of AmB and renal and auditory toxicities of amikacin) and improved the efficacy of conventional treatments. As we have described throughout this chapter, the active Mϕ targeting nanomedicines to selectively

eliminate them, switching their phenotype, decreasing production of pro-inflammatory mediators, increasing antimicrobial activity, and modulating their immune response could improve the treatment of several diseases. However, currently, no translation studies have been done. Some crucial challenges should be addressed to accelerate clinical studies.

The first issues are related to the selection of the administration route that is fundamental to get access to macrophages and imposed the structural design of nanomedicines. For instance, direct access of nanomedicines to intestinal Mϕ or AMϕ could be achieved by oral or pulmonary routes, respectively. However, not only Np should be structurally resistant, but ligands and linkages should not be degraded during GI transit or the stress of aerosolization. In addition, for iv-administered Np, stability during blood circulation is fundamental to have the chance for reaching the inflammation sites. Colloidal instability, aggregation, and opsonization produce drug leakage, uptake by Mϕ of the MPS, and reduce targeting opportunities.

The second issues are related to the selection of the ligand and its binding method to Nps that are key to minimized off-target effects and for translation of results. On one hand, the selected receptor should be overexpressed on activated Mϕ and no in other cells or rest Mϕ, to reduce the off-target effects. For example, although CD44 is typically overexpressed by inflammatory Mϕ HA-targeted Np in principle can also accumulate where HA catabolism takes place such as in the skin and liver, and this has a clearly negative effect on therapeutic efficacy. Besides, receptor expression in comorbidities may hamper its effective translation. On the other hand, ideally, ligands should be highly specific, with a simple structure, readily available, economic, and stable. Usually, highly specific ligands, such as antibodies, are structurally complex and highly instable. The methods for binding ligands (pre- or post-preparation Nps) usually include several steps or are performed in organic solvents and under harsh reaction conditions, resulting in potential toxicity and high cost. Besides, it should be considered that structural heterogeneities in Np population obtained can reduce batch-to-batch consistency, and the accuracy of its chemical characterization, which leads to unwanted unpredicted BD and activities after in vivo administration.

Surface functionalization and excessive structural complexity are two factors that will delay future market implementation. In this sense, the use of natural ligands that could be incorporated as one component of Nps, that could not require chemical synthesis and that could be easily quantified, could be advantageous. In addition, sufficient data on the best density of ligands to insurance effective targeting is lacking, and examples show that excessive ligand density could be conduced to off-targeting.

The third issues are related to the in vivo models used to preclinical test Mϕ targeted nanomedicines. Animal models of inflammation are easy to use but are not particularly reflective of the mechanisms of action in human disease since they do not recapitulate complete human diseases. For example, DSS-colitis models are characterized by acute instead of chronic inflammation probably with a higher

eEPR effect than that present in patients, and hence site-specific accumulation of nanomedicines could be artefactually magnified (Danese et al. 2016). Besides, in recent years, it has been noticed that in cancer the EPR effect is highly heterogeneous, changing during tumor growth, changing among tumor types of the same origin, and between tumors and metastases in the same patient, and varying between mouse models and patients (Tanaka et al. 2017; Golombek et al. 2018). However, eEPR effect was not fully characterized in patients with inflammatory diseases.

The fourth issues are related to potential nanotoxicity of iv-administered nanomedicines. It is well known that because of their size and high specific surface, Nps may be recognized as foreign bodies by the immune system, by macrophage uptake and/or complement activation. Besides, nanomedicines can induce the production of binding and/or neutralizing antibodies that hamper or suppress pharmacological activity such as the accelerated blood clearance effect (Moghimi 2018; Szebeni 2018). Besides, the iv infusion of certain nanomedicines, for instance, AmBisome and Doxil (liposomal doxorubicin) are known to induce idiopathic hypersensitivity (HSR) (Jiskoot et al. 2009). Additionally, data on how Nps modulate the innate immune response is mostly unknown. For example, plain and mannosylated-chitosan Np control the expression of cell-cycle-related genes, inflammation, and upregulate stress response genes on Mϕ (Coya et al. 2019). Besides, the effects of Mϕ repolarization on autoimmune or inflammatory diseases promotion have been not addressed at all (Ardura et al. 2019).

Finally, counting with a production method that could be industrially scalable and cost-effective is fundamental for translation. For instance, there are no available industrial methods for the solvent precipitation method predominantly used to prepare polymeric Nps at the laboratory scale (Khayata et al. 2012). It is important to underly that, because of their non-biological complex drug (NBCD) nature, switching from lab scale to industrial production methods is expected to alter the structural features of the final product, modifying its therapeutic performance and toxicity (Smith et al. 2016; Coty and Vauthier 2018). The scalable, controlled, and reproducible production of nanomedicines under good manufacturing practice (GMP) conditions still present unique challenges (Paliwal et al. 2014; Agrahari and Agrahari 2018). The operative challenges involved in their industrial production have been already solved for liposomes. However, industrial manufacture of Np exhibiting major structural complexity, such as displaying surface protein ligands, remains troublesome (Paliwal et al. 2014). This is reflected in the pipeline of products in clinical trials and in the market; most of them are plain or sterically stabilized liposomes, while targeted nanomedicines constitute a minor fraction of the total.

Overall, Mϕ targeting, different from cancer targeting nanomedicines, has been only recently address, but it could be a straight way to modulate the behavior of these versatile cells involved in a considerable number of diseases. Plain and Mϕ targeted liposomes have more promising translational opportunities compared with other Nps.

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