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Macrophage-targeted nanomedicine for chronic diseases immunotherapy

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ABSTRACT

Macrophage is the key innate immune effector in first-line defense against the pathogens, and can be polarized into different phenotypes to regulate a variety of immunological functions. However, the plasticity of macrophage is extraordinarily recruited, activated, and polarized under pathological conditions, playing paramount roles in occurrence, development, and prognosis of various chronic diseases, such as rheumatoid arthritis (RA), atherosclerosis (AS), and cancer. To this end, macrophage has become an important therapeutic target for etiological treatment of these diseases. Meanwhile, with the development of nanotechnology, various nano-drug delivery systems have been explored to target macrophages for disease modulation, displaying unique advantages to address both pharmaceutic and biopharmaceutic limitations of various drugs. This review aims to summarize the recent progress of macrophage-targeted nanomedicine for chronic diseases immunotherapy. First, the origin, polarization and biological functions of macrophages have been introduced, in which macrophages can differentiate into different phenotypes in response to physiological stimuli to play various immunological roles. Then, the macrophage disorder has been reviewed in related with various chronic diseases, and several representative diseases, including AS, RA, obesity, and cancer, have been discussed in detail to elucidate the pathological contributions of macrophages for disease progress. Next, strategies to regulate macrophages for diseases immunotherapy, such as macrophages depletion, macrophage reprograming, inhibition of macrophage recruitment, are summarized, and particular attention has been paid on bio-functional nanomaterials to engineer macrophages *via* different mechanisms. Further, methods for macrophage-targeting delivery nanosystems are discussed based on both passive and active targeting approaches. Finally, the perspective is speculated for potential clinical translation, and there still has significant room for the development of novel macrophage-targeting nanomedicine for precise, effective, and biosafe therapy.

1. Introduction

The immune system exerts physiological protective functions by recognizing and eliminating xenogenous/foreign substances to maintain physiological balance. Basically, the immune system functions in three aspects: immune defense, immune homeostasis and immune surveillance. The immunological disorders, however, could result in various autoimmune or immunodeficiency diseases, for example, rheumatoid arthritis (RA), atherosclerosis (AS), and cancer. To this end, immunotherapy presents an etiological therapy to mechanistically conquer these diseases. Immunotherapy refers to disease treatment *via* intervention or adjustment of immune functions. Indeed, the immunotherapy has experienced for more 2000 years, and the earliest one is the vaccines to prevent disease. In the 1890s, Dr. William B. Coley has pioneered to harness immunotherapy for managing cancer [1]. More recently, we have witnessed a surge in the development of immunotherapy for the treatment of various disease [2,3], and the most notable advancement would be cancer immunotherapy (which has won the Nobel Prize in 2018).

Macrophages, a type of immune cells, play critical roles in different immune responses (both innate and adaptive) due to its capability of cell engulfment, antigen presentation and cytokine secretion [4]. The macrophages are initially thought to be derived from monocyte in bone marrow, while some recent studies indicate that tissue-resident macrophages are embryo-derived rather

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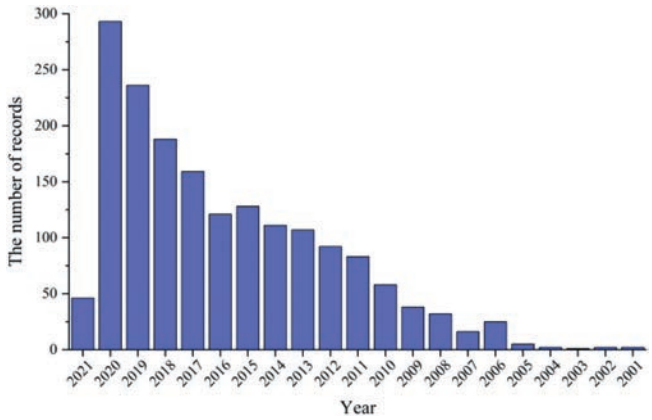

Fig. 1. The reports involving macrophage-targeting NPs have increased over past 20 years. The records were obtained by Web of Science.

than monocyte-derived [5,6]. Physiologically, pathogen-associated molecules and apoptotic cells-derived endogenous ligands are recognized by macrophages *via* receptor-mediated recognition patterns, resulting in pathogen clearance by phagocytosis and consequent infection alleviation. Besides, macrophages express an impressive amount of different receptors (*e.g.*, Fc, complement, scavenger and lectin receptors) to bind a wide range of ligands and molecules [7]. However, inappropriate activation of macrophages could disrupt their homeostatic functions with uncontrollable inflammatory response, and such abnormality of macrophages is related to the occurrence and progression of various chronic diseases, including malignant tumors [8–10], inflammatory diseases (*e.g.*, RA [11,12]), metabolic diseases (*e.g.*, AS [13,14], diabetes [15] and obesity [16]) and severe infections like acquired immune deficiency syndrome [17,18]. To manage these diseases, various immunotherapies have been developed to repair the functions of macrophages, such as removing the diseased macrophage, repolarizing macrophage, and inhibiting macrophage infiltration [19–22]. Therefore, macrophages are important targets for immunotherapy.

However, just like conventional drug treatment, the macrophage-related immunotherapy also inevitably meets several deficiencies, such as systematic toxicity, low patient compliance, incomplete treatment and even the uncontrolled excessive immune-response [23]. To address these issues, the nanotechnology provides unique solution. Nanoparticles (NPs) have been widely used as carriers to deliver antigens [24], imaging labels [25], and therapeutic drugs [26–28], which could improve the pharmacokinetics and bioavailability of drugs. After the first nano-formulation being approved by the Food and Drug Administration in 1989, many NP formulations of existing drugs have been commercially available [28,29]. Compared with traditional immunotherapy, NPs can be equipped with targeting molecules to increase the accumulation at targeted sites and minimize toxicity and off-target effect. As macrophages display specific ability to engulf xenogenous substances, the macrophages-targeting NPs can be designed by tuning their intrinsic properties (such as shape, size and surface charge), and surface modification of specific molecules to recognize surface markers on the membrane of macrophages.

Over the past two decades, the fundamental research on macrophages-targeting NPs has gained particular attention, with increasing search work being published over the past two decades (Fig. 1). Regrettably, these intensive fundamental researches have made limited clinical impact, with only 2 cases entering clinical trials by searching the National Institutes of Health/Clinical Trials government website, both of which used Ferumoxytol as a contrast agent to enhance the effect of magnetic resonance image. Therefore, it is critical important to think back of the progresses and issues in the field to provide information for future direction. Recently, several related Reviewer papers have been published, which however only focused on specific diseases (*e.g.*, AS, metabolic diseases and tumor) or NPs (*e.g.*, metal or metal oxide NPs) [30–35]. In this critical Review, we make a comprehensive summary of the macrophage-targeting nanomedicine for chronic diseases immunotherapy. The biological functions of macrophages were first introduced, followed by the chronic diseases related to the macrophages disorder. Then, the strategies to regulate macrophages for diseases treatment were discussed, accompanied by various macrophages-targeted nanomedicines. Finally, the current problems of the field were discussed, and future perspective was speculated.

2. Polarizations and biological functions of macrophages

Macrophages are an important class of antigen-presenting cells (APC), which function by phagocytosis, antigen processing and presentation to activate T cells for adaptive immunity [36–38]. Interestingly, macrophages show unexpectedly high level of heterogeneity in their origins and functions, which is highly associated with environmental factors in their functional specialization (Fig. 2). As a type of highly plastic cells, macrophages can respond to different stimuli and secrete various chemokines and cytokines, making them of great importance in the pathogenesis of many diseases. Based on the microenvironment signals they received, macrophages can polarize to various phenotypes with diverse functions, such as pro-inflammatory, antibacterial, immunomodulatory, tissue remodeling, and cancer-promoting effects [39]. In most cases, the macrophages can differentiate to two phenotypes: pro-inflammatory macrophages (classical activated macrophages, M1) to kill pathogens, resist tumor and even damage tissues; and anti-inflammatory macrophages (alternative activated macrophages, M2) for anti-inflammation, tissue repairing, remodeling and promoting tumor growth [4,38,40]. In addition, there are still several other types of macrophages that cannot be assigned to either classification (*e.g.*, Mhem [41], Mox [42] and M4 [43]).

M1 and M2 macrophages represent two extremes on a continuum, while both two can switch to each other [44]. Macrophages polarization is regulated by various transcription factors, for instance, nuclear factor kappa-B (NF-κB), signal transducer and activator of transcription 1 (STAT1) and interferon regulatory factor 5 (IRF5) involving in the M1 pathway, while STAT6, c-Myc, peroxisome proliferators-activated receptors γ (PPARγ) and krueppel-like factor 4 (KLF-4) in the M2 pathway [45–47]. M1 macrophages can be induced by interferon-γ (IFN-γ), the granulocyte macrophage colony stimulating factor (GM-CSF), lipopolysaccharide (LPS) or other signals binding with toll-like receptors (TLRs) [48,49]. In response, M1 macrophages secrete inflammatory chemokines (such as C-C motif chemokine ligand 2 (CCL2), CCL3 and C-X-C motif chemokine ligand 9 (CXCL-9)), cytokines (*e.g.*, interleukin-1β (IL-1β), IL-6 and tumor necrosis factor α (TNF-α)) and other substances to inhibit infection [38,49,50]. Meanwhile, there is a lot of lysosomes in M1 macrophages, which can produce reactive oxygen intermediates (ROI), nitric oxide (NO) and lysosomal enzymes to help kill pathogens [50]. On the other hand, cytokines secreted by T helper type 2 (Th2), such as IL-4 or IL-13, and the macrophage colony-stimulating factor (M-CSF) can stimulate the macrophages differentiating into M2 phenotype [49,51]. M2 macrophages could further be subdivided in three subsets: M2a induced by IL-4 or IL-13, M2b promoted by immunoglobulin complex and M2c derived by IL-10 or transforming growth factor-β (TGF-β). Different from M2a and M2b that promote type II immune response, M2c is an exception that suppresses immunity and repairs

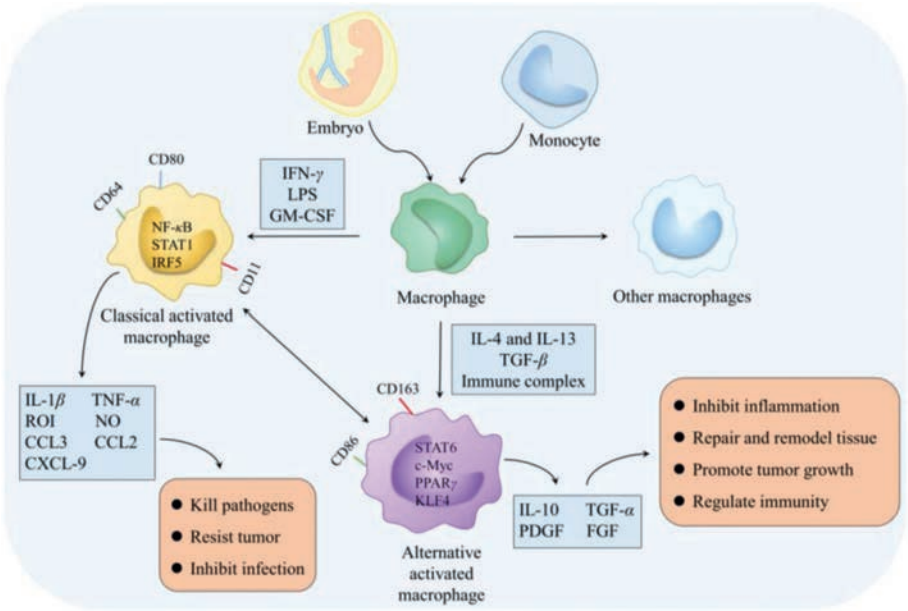

Fig. 2. The origin, polarization and biological functions of macrophages. Based on their origin, macrophages can be divided into monocyte-derived and embryo-derived. Macrophages transform to different phenotypes by the effect of substances in micro-environment and play different biological functions.

tissue. When stimulated, M2 macrophages can secrete IL-10, TGF-β, platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF), and then inhibit inflammation, promote the growth of tumor, regulate immunity and involve in the repair and fibrosis of damaged tissue [39,52–54]. M1 phenotypic markers include cluster of differentiation 80 (CD80), CD38, and CD11, whereas M2 markers include CD163 (a type of scavenge receptor), C-type mannose receptor 1 (CD206), CD204 and CD209 [48,55–57]. Besides, CD86 is the marker for M1 and M2b that can discriminate M2b from the other subtypes of M2 macrophages [58]. Based on the differences of the phenotypic surface level and the cytokines and chemokines production, the phenotype of macrophages can be identified.

Other than these two main phenotypes, kinds of other phenotypes have also been described, such as MFe^{hi} macrophages [59], M4 macrophages [43], Mox and Mhem populations [41,42]. MFe^{hi} macrophages have been found in adipose tissue (AT), which contains elevated level iron content and iron handling genes inside cells. Interestingly, while high iron level is beneficial to maintaining M1 macrophage polarization, MFe^{hi} macrophages display a strong M2-like phenotype with biomarker expression of CD163 and mannose receptor [31]. In human, M4 macrophages are induced by CXCL4, which display some characteristics of both M1 and M2 phenotypes, but they lack the capacity for phagocytosis [60]. In addition, M4 macrophages prevent the development of macrophages towards Mhem phenotype [43]. Mox and Mhem populations were first found in atherosclerotic plaques, which are M2-like macrophages with high expression of CD163, haptoglobin/hemoglobin receptor, and mannose receptor. Overall, the macrophages polarization is highly important for their normal functions as well as the pathogenesis of different diseases, and thus reprogramming the macrophages could provide a novel approach to manage related diseases.

3. Macrophage disorder and diseases development

While macrophages play critical roles in biological functions, their disorder also contributes to the pathological process of many diseases. It has been reported that macrophages exert distinct and diverse activities in different phases of diseases, and intensive research attention has been paid to study the relationship between the phenotype switch and disease process. In this section, we will discuss several representative macrophage-related diseases, and the roles of macrophages for disease development, including AS, RA, obesity, and cancer.

3.1. Atherosclerosis (AS)

AS is the leading cause of death worldwide, and it is a type of chronic inflammatory cardiovascular diseases caused by lipid metabolism disorder. Because of their capability to maintain the homeostasis of lipid metabolism, macrophages are the key player for AS evolution (Fig. 3). In normal state, the lipid (mainly the low-density lipoprotein, LDL) can be absorbed by macrophages *via* the scavenger receptors and hydrolyzed into free cholesterol (FC) in lysosomes. Part of FC finally effluxes by the cholesterol efflux transporters and the other intracellular FC converts into cholesteryl ester (CE). Once the metabolism of lipid is disturbed, more lipids are accumulated into macrophages, and that facilitates the accumulation of macrophages into subendothelium and neointima. Then, the macrophages develop into foam cells, a hallmark of atherosclerotic lesion [61]. In addition, excessive CE also promotes the translation of macrophages into foam cells. The macrophage-derived foam cells secrete lots of inflammatory factors such as IL-1β, IL-6 and IL-12 to promote the formation of plaque, which consists of necrotic foam cells and fibrous cap [62]. Besides, they may contribute to plaque instability, including the fibrous cap rupture and intra-plaque hemorrhage. In early lesions, the clearance of overloaded apoptotic cells by macrophages is protective against AS progress. However, in advanced lesions, the defective efferocytosis and autophagy of macrophages can promote plaque necrosis and inflammation to increase the lesion area, leading to heart attack or stroke. Recently, several other macrophage populations have been discovered in AS, such as Mhem or M(Hb) macrophages induced by hemoglobin-haptoglobin complexes and haem, Mox macrophages induced by oxidized phospholipids, and M4 macrophages induced by chemokines CXCL4 [41–43].

3.2. Rheumatoid arthritis (RA)

RA is a chronic inflammatory autoimmune disease with multiple joint synovium, which is characterized by the pannus forma-

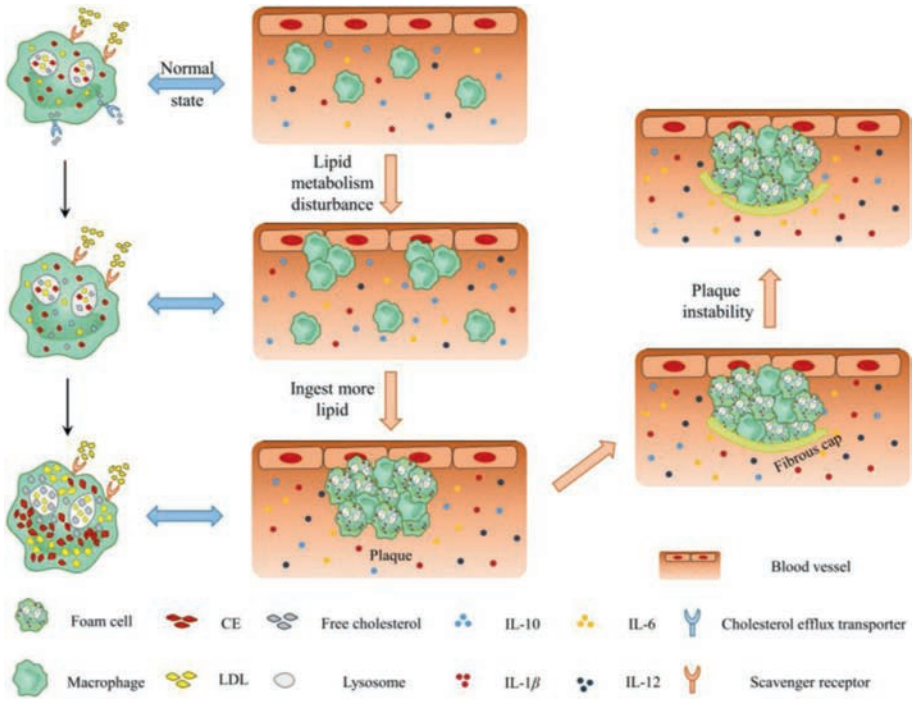

Fig. 3. Schematic illustration of the roles of macrophages at different stages in AS. Normally macrophages ingest lipid (such as LDL), and lipid can be hydrolyzed into free cholesterol and then efflux, or convert into cholesteryl ester (CE) and accumulate in macrophage. Due to the disturbance of lipid metabolism, macrophages ingest excessive lipids and then transform into foam cells. These cells secrete various pro-inflammatory cytokines including IL-6, IL-1β and IL-12, to promote the formation of plaque. With the progress of AS, the plaque can be instable and lead to heart attack or stroke.

tion and synovial hyperplasia, leading to joint damage and loss of function [63]. The mechanisms of RA have not been clearly elucidated, while it has been known that the development of RA can be related to various genetic and environmental factors, and macrophages play critical roles in its pathogenesis. In the early stage of RA, an increased number of macrophages are infiltrated into synovial fluid, which predominantly polarize into M1-like phenotype [64]. The M1 macrophages secrete large amounts of cytokines (including TNF, IL-6 and IL-1β) to drive other immune cells (T cells and B cells) activation and infiltration, which in turn product immunoglobulins, rheumatoid factors and positive feedback factors of macrophages to promote inflammation [65]. Meanwhile, the synovial fibroblasts are activated by TNF and other cytokines secreted by macrophages and T cells, and produce GM-CSF and M-CSF, which is essential to the infiltration of macrophages and osteoclasts [66]. Osteoclasts activity in RA is chronically induced and can cause severe bone erosion. Furthermore, GM-CSF further exacerbates arthritis as they can enhance the functions and survival of neutrophils and macrophages [67]. Since the cytokines play extremely pleiotropic effects in different types of cells, the effect of cytokine network is highly complex in RA (Fig. 4). Overall, macrophages drive the vicious cycle of chronic inflammation and tissue damage in RA, which recruit immune cells, produce large amounts of inflammatory cytokines, promote angiogenesis and proliferation of synovial fibroblasts, and finally lead to the erosion of cartilage sand bones.

3.3. Obesity

Obesity, a chronic metabolic disorder characterized by excessive accumulation of white AT and low-grade chronic inflammation, is one of the main contributors of insulin resistance and type 2 diabetes, and other related diseases (*e.g.*, asthma, hypertension and cancer). Macrophages are one of the major immune cells in AT (Fig. 5), accounting for 40%–60% of all immune cells of AT in obese mice and 10%–15% in human [68,69]. Normally, adipose tissue macrophages (ATMs) participate in lipolysis, tissue remodeling, clearing cellular debris, immune surveillance and resolving inflammation to maintain adipose tissue homeostasis [70–72]. However, the metabolic disturbance in obese state expands the AT, which leads to hypoxia and death of adipocytes resulting in excessive leptin, cytokine and chemokine secretion. As a result, a large number of macrophages were recruited into AT site and activated by the effect of monocyte chemoattractant protein-1 (MCP-1) and leukotriene B4 (LBT-4), as well as activation of pattern recognition receptors (PRRs) of macrophages (*e.g.*, TLRs and NOD-like receptors (NLRs)) in obesity [73–75]. Moreover, high level of free fatty acid (FA), saturated FA and cholesterol may also increase the number of macrophages, which plays a key role in obesity and leads to inflammation and insulin resistance [76]. M1-like ATMs produce pro-inflammatory cytokines including TNF-α, IL-6 and IL-8, among which TNF-α stimulates pro-inflammatory protein kinase to mediate AT remodeling and angiogenesis, promote insulin resistance, unchecked basal lipolysis, and ectopic lipid storage in other metabolic tissues [31,72]. While the function of ATMs is regarded to be pro-inflammatory, the phenotype may also be transfered to M2-like by unsaturated FA to help lipid clearance. In addition to the activation and inflammatory profile of macrophages in the obese state, ATMs are highly adaptive to lipid-rich environment that increases their adiposity by activating lysosomal lipid metabolism to maintain AT homeostasis [77]. Besides, as functional antigen presenting cells, ATMs phagocytose and process antigens for presentation, express co-stimulatory molecules and contribute to the proliferation and maintenance of T cells in AT [78,79].

Macrophages in AT are highly heterogeneous, which cannot be simply classified as M1 phenotypes or M2 phenotypes (Fig. 5). A new population of ATM called metabolically active macrophages (MMes) was identified in AT recently that promote inflammation and dead adipocyte clearance through lysosomal exocytosis [80,81]. While they secrete pro-inflammatory cytokines, MMes ex-

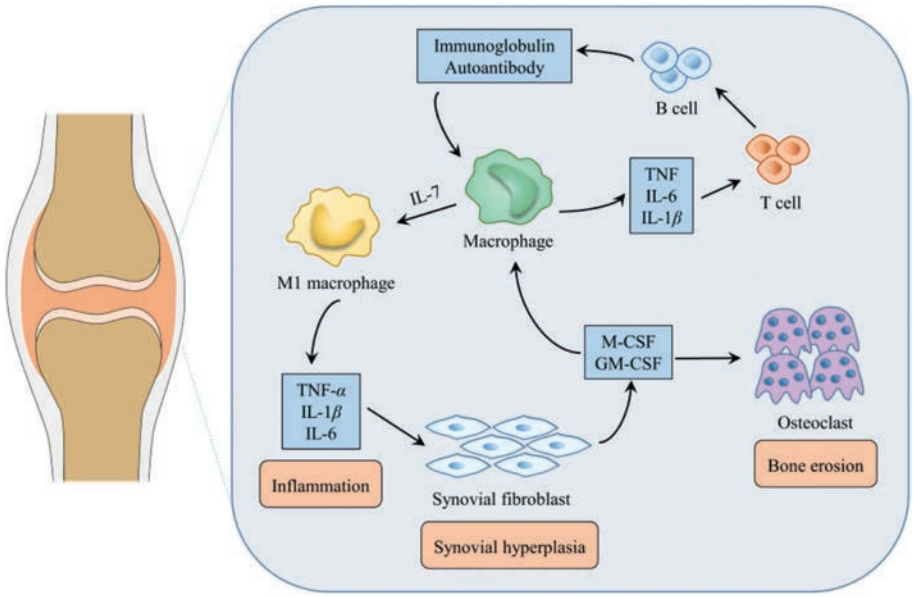

Fig. 4. Schematic illustration of cyclized macrophage-related pathogenesis of RA. Tissue-resident macrophages in synovium can be polarized into M1 phenotype by the stimulation of IL-7. M1 macrophages produce lots of pro-inflammatory cytokines leading to inflammation and the activation of the synovial fibroblasts. Synovial fibroblast secretes various cytokines to cause synovial hyperplasia, of which M-CSF and GM-CSF are crucial to osteoclasts and macrophages and promote the progress of RA. The other cycle is that M1 macrophages involve the activation and infiltration of T and B cells, and the productions of B cells work as positive feedback factors to macrophage activation and inflammation progress.

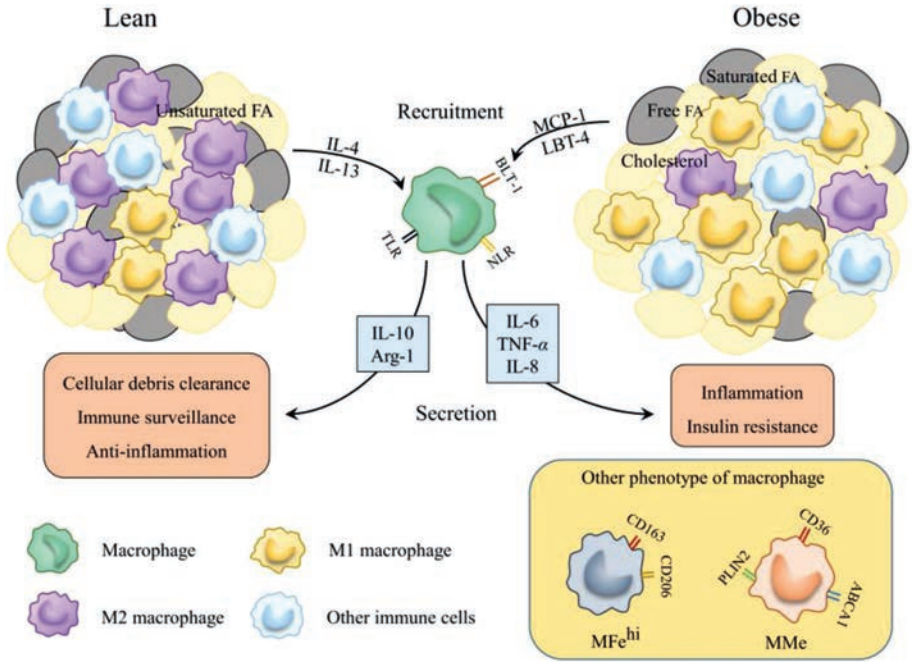

Fig. 5. Schematic illustration of diverse macrophages composition and their function in lean state and obese state. Macrophages can be recruited into AT by IL-4 and IL-13, and most of them polarize to M2 phenotype with beneficial functions including anti-inflammation, cellular debris clearance and immune surveillance in lean state. In obesity, MCP-1 and LBT-4 also recruit macrophages into AT. Unlike the lean state, M1 macrophages are dominated, which promote inflammation and even lead to insulin resistance. Besides, other phenotypes of macrophages are also identified in obese tissue, such as MFe^{hi} and MMe.

press M2 macrophages surface markers including ATP-binding cassette transporter A1 (ABCA1), CD36 and Perilipin2 (PLIN2), making them differentiate from M1 and M2 macrophages. Moreover, another iron handling macrophages called MFe^{hi} were found in AT, which have high level of iron content and iron-handling genes [59].

3.4. Cancer

In tumor stroma, there are numbers of inflammatory cells (accounting for 30%–50% of all cells) [82], which play critical roles as orchestrators of cancer-related inflammation [83,84]. Among them, tumor-associated macrophages (TAMs) are the most abundant subtype. Circulating precursors, including conventional inflammatory monocytes and myelomonocytic cells, are recruited into tumor and then differentiate into TAM [83]. In some tumors, TAM may be derived from tissue-resident macrophages of embryonic origin. Moreover, the macrophages are directly attracted by the CCL2 and M-CSF (CSF-1) that is produced by the elicitation of high HIF-1 level in the hypoxia tumor [85].

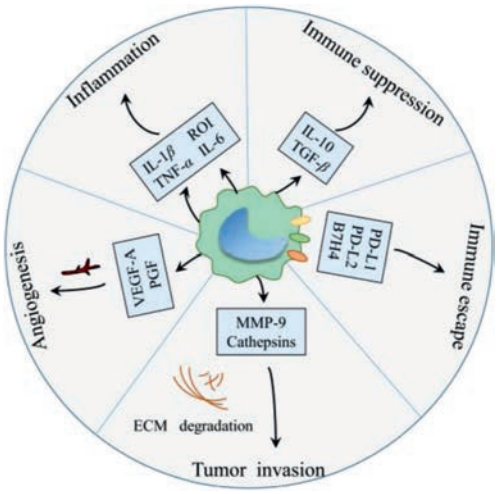

Fig. 6. The functions of macrophages in tumor. By the secretion of inflammatory cytokines including IL-1β, IL-6 and TNF-α, tumor-associated macrophages express pro-inflammatory and anti-tumor function in early phase. But in the anaphase, they release immunosuppressive cytokines, growth factors and some enzymes to promote tumor growth and invasion. The high levels of the ligands for checkpoint molecules, including PD-L1, PD-L2 and B7H4, can cause immune escape and promote tumor growth.

The TAMs exhibit diverse phenotypes in different kinds tumor and different tumor stages, which also play contradictory roles (Fig. 6) [86]. The TAMs express M1-like function in the early stage that produce various kinds of pro-inflammatory cytokines, chemokines and molecules, like TNF-α, IL-12, IL-23, MHC-II, ROI and NO, which can activate Th1 immune response to exert anti-tumor effect such as prompting the destruction of tumor cells, recruiting tumor-killing leukocytes, and directly engulfing tumor cells [87,88]. Induced by lipopolysaccharide (LPS) or interferon C in vitro, M1 macrophages secrete nitric oxide, inflammatory factors, and chemokines, like IL-12, IL-23, MHC-II and B7 family members like B7-1 (CD80) and B7-2 (CD86), whose primary function is activating Th1 immune response to exert anti-tumor effect. However, in the anaphase, TAMs tend to transform into M2 type and functionally participate in tumorigenesis, development, invasion, and metastasis *via* producing immunosuppressive cytokines, such as IL-10 and TGF-β [89]. In addition, the growth factors secreted by M2-like TAMs, such as vascular endothelial growth factor A (VEGF-A) or placental growth factor (PGF), promote the tumor endothelial cells to form blood vessel, which supplies nutrient to support tumor growth [85,90]. M2-like TAMs also secrete enzymes of matrix metalloproteinase-9 (MMP-9), serine proteases and cathepsins to degrade the extracellular matrix (ECM), facilitating tumor invasion to adjacent organs, as well as tumor metastasis [91,92]. Moreover, M2-like TAMs express high levels of the immune checkpoint molecules, including programmed death ligand 1 (PD-L1, also called B7H1), PD-L2 (B7DC) and B7H4, which cause immune escape [93–95]. Recent studies have also suggested that M2-like TAMs can suppress NK and T cells activity by preventing immune cells from identifying cancer cells [91]. All these evidences demonstrate the close relationship between TAMs and tumor growth, and macrophage-centered tumor therapy has become a hot topic in the field, which is mainly involved in activating antitumor activity and inhibiting their recruitment and functions related to tumor promotion.

4. Strategies to regulate macrophage by nanomedicines for diseases treatment

In view of the relevance between various diseases and macrophages, macrophages have become an important therapeutic target. In different diseases, macrophages play different roles, so they need to be regulated from different aspects, such as macrophage depletion, macrophage re-polarization, and inhibition of macrophage infiltration. In response to these strategies, there have been a variety of corresponding nano formulations reported. The use of nanostructures to encapsulate drugs has shown a number of advantages, such as overcoming solubility and stability problems, reducing side effects, and prolonging the circulating time in the body. In addition, a number of co-delivery systems have been proposed to deliver multiple drugs toward the same target for synergistic effect with improved efficiency. In this section, we will describe different macrophage regulation strategies, and focus on the nano-drug delivery systems.

4.1. Macrophage depletion

The accumulation of macrophages in the lesion has an important influence on the development of the disease. M1 macrophages accumulate in inflammation, leading to an imbalance of inflammation and aggravation of the disease. The accumulation of M2 macrophage in immunosuppressive diseases (such as tumors), on the other hand, is closely related to disease development and poor prognosis. As a result, macrophage depletion is the most direct and effective treatment strategy. In this section, the consumption strategies of M1 and M2 macrophages are respectively described.

4.1.1. Depletion of M2 macrophages

The elimination of M2 Macrophages (TAMs) is a strategy to eradicate macrophages at tumor sites, aiming to interrupt the signals network [96]. Advanced methods to deplete TAMs include small molecule inhibitors or antibodies, bisphosphonate and anti-cancer chemotherapy drugs. The depleting small molecule inhibitors mainly target the colony-stimulating factor 1 (CSF1)-CSF1 receptor (CSF1R) pathway, which recruits TAMs to tumors and promotes TAMs differentiation toward pro-tumoral phenotypes. Inhibition of CSF1-CSF1R signaling could inhibit TAMs recruitment and induce their apoptosis, while bisphosphonate and anti-cancer chemotherapy drugs directly deplete TAM efficiently.

CSF-1R is a transmembrane tyrosine kinase class III receptor that presents on the surface of most macrophages. It regulates the differentiation, proliferation and survival of macrophages by recognizing two ligands: CSF-1 and IL-34 [97]. Thus, blockade this pathway has become an interesting strategy to halt recruitment of TAMs and promote their depletion. The small molecular inhibitor BLZ-945 can prevent CSF-1 signal transduction in TAMs and also improve cytotoxic T cell infiltration in tumors by inhibiting the tyrosine kinase activity of CSF-1R [98–101]. However, systemic administration of BLZ-945 only showed moderate tumor suppression effect. Therefore, the effective targeted drug delivery strategy is necessary to enhance its therapeutic efficacy. Rong Zhu *et al.* wrapped BLZ-945 and SN_{38} (a kind of anti-cancer prodrugs) in a type of dual biologically responsive nanogapped gold nanoparticles (AuNNPs), which can achieve chemo-immunotherapy for both primary and metastatic tumors (Fig. 7). AuNNPs are composed of multiple gold NPs, and the adjustable gap between the Au core and the shell of two adjacent AuNPs is 1–10 nm. Its main function is to enhance the plasma coupling effect between the core and the shell, making it easier for imaging, thereby improving the precision of anticancer theranostics. BLZ-945 was loaded in AuNNP@PEG/PSN_{38}VP, which were manufactured by self-assembly of amphiphilic AuNNP, hydrophilic polyethylene glycol (PEG) and copolymerized prodrug poly(SN_{38}-*co*-4-vinylpyridine) (Fig. 7A). Vesicles accumulated at tumor site through the EPR effect (detailed in section 5), and then acidic tumor environment promoted the dissolution of vesicles into individual AuNNP@PEG/PSN38V to release TAM-targeted BLZ-945 (Fig. 7B). Because of the small size, the

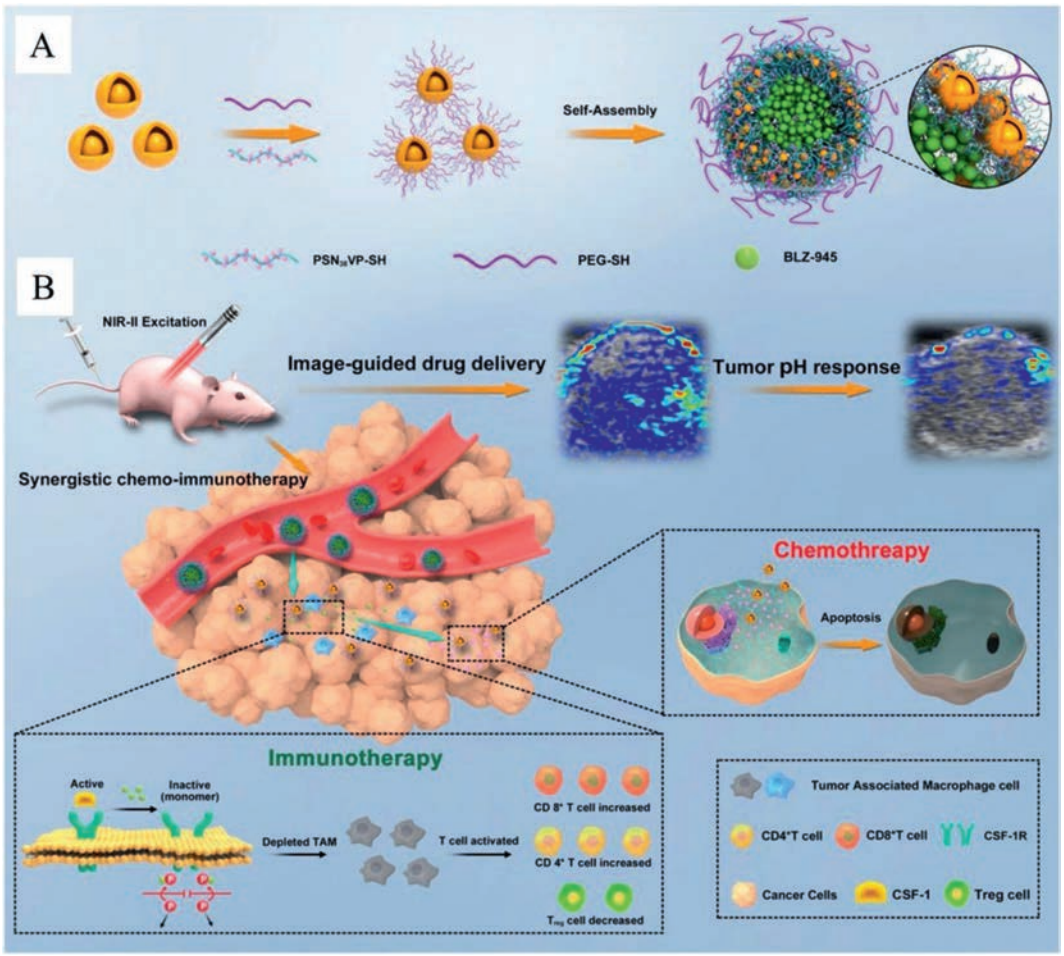

Fig. 7. (A) Preparation of BLZ-945-loaded AuNNP@PEG/PSN$_{38}$VP. (B) Schematic illustration of chemo-immunotherapy by the BLZ-945-loaded vesicles. Vesicles accumulated at tumor site through the EPR effect, and acidic tumor environment promoted the dissolution of vesicles into individual AuNNP@PEG/PSN38V to release TAM-targeted BLZ-945. The single AuNNP@PEG/PSN$_{38}$VP NPs can release SN$_{38}$ in deeper tumor tissues, leading to the apoptosis of tumor cells. Copied with permission [102]. Copyright 2020, American Chemical Society.

single AuNNP@PEG/PSN$_{38}$VP NPs can release SN$_{38}$ in deeper tumor tissues, leading to the apoptosis of tumor cells [102].

Bisphosphonates (*e.g.*, clodronate, alendronate) are a type of mononuclear cells specific cytotoxic drugs, which have been widely used as TAMs depletion [103]. Their mode of action is to inhibit prenylation of proteins such as Ras, leading to cell apoptosis. Bisphosphonates have been widely used to treat several diseases such as osteoporosis, bone metastasis and Paget's disease. However, most bisphosphonates generally cannot achieve satisfactory tissue concentration to exert their pharmacological effects because of their short circulating half-life [104]. To this end, nanotechnologies provide powerful solutions to improve the pharmacokinetic characteristics of drugs. Currently, bisphosphonates-loaded liposomes have been commercialized, which showed enhanced TAM depletion efficiency. In one work, the efficacy of clodronate-filled liposomes (CLD) was evaluated on colon cancer bearing mice model by analyzing the polyp load, inflammatory cytokines and macrophage markers [105]. After treatment, overall polyp number was significantly reduced, accompanied by the decrease of macrophage marker F4/80 and anti-inflammatory cytokines (such as CCL-17, IL-10, TGF-β and IL-13) associated with M2 macrophage, indicating the benefit of tumor growth inhibition *via* TAMs depletion.

Cytotoxic drugs such as doxorubicin (DOX) have also been utilized to deplete TAMs. Niu *et al.* developed DOX-loaded poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles, which were acid-sensitive PEGylated and mannose-modified, showing great TAMs depletion ability [106]. In their system, nanoparticle core was formed by PLGA with surface modification of mannose, and then the acid-sensitive PEG amphiphile of PEG-hydrazone-C18 was applied for PEGylation. It was later demonstrated that the long flexible PEG chain was able to protect mannose from the nonspecific cell attachment and phagocytosis during circulation [107]. When reaching the tumor site, the acidic environment promoted the hydrolysis of the hydrazone bond to liberate PEG chains, which in turn exposed mannose to recognize the mannose receptor on the surface of TAMs for target binding. The nanoparticles achieved specific depletion of TAMs in tumor while has little effect of macrophages in liver and spleen.

4.1.2. Depletion of M1 macrophages

Increasing evidence has indicated that M1 macrophages are closely related to the development and/or maintenance of chronic inflammation [108]. However, there still lacks relevant research on direct depletion of M1 macrophage. Fortunately, RNA interference (RNAi) is considered as a potential strategy to post-transcriptionally silence gene expression, down-regulate inflammatory cytokines such as TNF-α, which reduces the number of M1 macrophages for the treatment of inflammatory diseases [109]. The RNAi is mediated by the small interfering RNAs (siRNA) and its feasibility largely depends on the carrier and delivery system. It has been reported that a kind of Fab'-bearing TNFα-siRNA-loaded NPs can specifically eliminate M1 macrophages for the therapy of in-

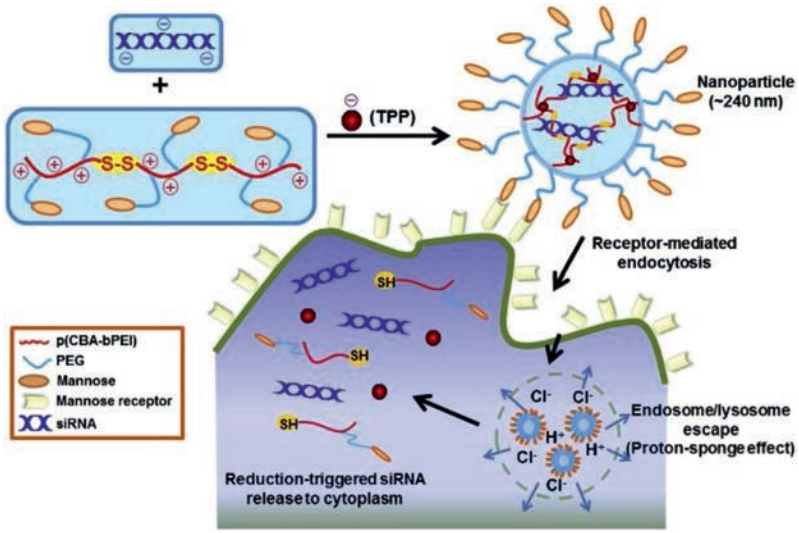

Fig. 8. Schematic illustration of the preparation of TPP-PPM/siRNA NPs, and its macrophage targeting delivery for stimulus responsive release of the small interfering RNAs (siRNA). The TPP-PPM/siRNA NPs were composed of bioreducible PPM conjugate, sodium triphosphate (TPP) and siRNA. Bioreducible NPs are decomposed inside macrophages with a reducing environment to release siRNA that can reduce TNF-α expression and exert anti-inflammatory effects. Copied with permission [111]. Copyright 2013, Elsevier.

flammatory bowel disease (IBD) [110]. Patients with IBD have significant side effects when they receive systemic medication. Therefore, there is a great need to design a nanoformulation that can target therapeutic molecule-TNFα siRNA to inflammatory tissues. In the research of Hamed Laroui *et al*., TNFα siRNA was loaded into NPs constituted of poly(lactic acid) and poly(ethylene glycol) block copolymer (PLA-PEG), and the Fab' part of F4/80 was attached on the surface of NPs to improve the targeting kinetics of NPs. The results showed that the direct binding between macrophages and Fab'-bearing NPs effectively improved the targetability of drugs, resulting in significantly reduced colitis. In addition, Xiao *et al*. [111] prepared a nano-assembly using a mannosylated bioreducible cationic polymer (PPM), which was assembled with the help of sodium triphosphate (TPP) to load siRNA *via* electrostatic interactions (Fig. 8). The nanoparticles could target M1 macrophages, and release siRNA in reduction-triggered manner owing to di-thiol bond in PPM structure, thus achieving down-regulation of TNF-α for inflammatory bowel disease therapy.

4.2. Macrophage re-polarization

Macrophages undergo different polarizations in presence of different external stimuli. Under pathological conditions, macrophages can be induced by pathological factors to polarize toward the direction of disease progression, forming a positive feedback loop to accelerate the progression of the disease. It is worth noting that the polarized macrophages still have good elasticity and can be polarized again. For this reason, re-polarization of macrophages towards the opposite direction of disease is a direct strategy for disease treatment. For example, M1 macrophages can be polarized toward M2 for inflammatory diseases (such as RA and AS), and M2 macrophages can be polarized toward M1 for immunosuppressive diseases (such as tumors). In this section, examples of nanomedicines to induce macrophages repolarization will be reviewed.

4.2.1. Re-polarization the macrophage from M1 toward M2 phenotype

Persistent inflammation can promote the progression of various chronic diseases, while traditional therapies mainly concentrated on the inhibition of acute proinflammatory mediators, for instance, glucocorticoids, antibodies against cytokines and COX inhibitors [112]. During the inflammation development, monocyte/macrophage lineage plays an essential role [113,114], in which M1 macrophages produce proinflammatory cytokines and oxidative metabolites to promote host defense and removal of damaged tissue. However, extraordinary activated M1 macrophages promote the progress of inflammatory diseases, such as RA and AS. Therefore, the reprogramming of M1 macrophages into M2 macrophages has become an effective strategy for the therapy of inflammatory diseases.

CD163, a member of the scavenger receptor family, has been used to induce M2 macrophages polarization by promoting its expression. In one design, CD163 plasmid was loaded into polyethylenimine (PEI) NPs with surface modification of mannose ligand to target monocytic cells *via* mannose receptors [115]. The THP-1 and primary human macrophages were pre-treated with LPS to allow M1 polarization, while after incubation with the NPs to induce CD163 over-expression, the macrophages displayed significantly reduced levels of TNF-α, MCP-1 and increased levels of IL-10 and IL-1ra. Consequently, such NPs were able to target M1 macrophages, and reprogram M1 macrophages to M2-type, resulting in the release of anti-inflammatory factors to alleviate inflammatory diseases.

IL-10 is a type of cytokine with anti-inflammatory properties mainly secreted by lymphocytes, monocytes/macrophages and T cells [116,117]. IL-10 encoding plasmid was loaded into alginate NPs with surface decoration of tuftsin peptide to actively target macrophages [118]. Tuftsin is a four amino acid peptide that can facilitate phagocytosis by recognizing Fc and neuropilin-1 receptors on the surface of macrophages [119,120]. Upon intra-peritoneal injection, macrophages are able to behave as Trojan horse vectors to mediate the accumulation of NPs into inflammation-arthritic joints. After therapy, total synovial M2 macrophages in arthritic rats markedly increased from 9% to 66%, accompanied by remarkable decrease of pro-inflammatory cytokines such as TNF-α and IL-1β at both systemic and joint tissue, thus preventing the development of joint inflammation.

4.2.2. Re-polarization the macrophage from M2 toward M1 phenotype

M2 macrophages play an essential role in tumor immunity by heightening the number of suppressor cells such as myeloid-derived suppressor cells (MDSCs) and immature monocytes. The accumulation of M2 macrophages is closely related

to disease progression and poor prognosis [105]. Reprogramming M2 macrophage to M1-type is a promising tumor therapy method. Many cytokines, immune-agonists, and inhibitors against M2 macrophage (including IL-12 [121], TLR agonists [122–127] and miR-125b [128]) have been explored to reprogram the macrophage from M2 toward M1 phenotypes.

IL-12, a kind of heterodimeric cytokine, can induce transformation of TAMs to M1 type and contribute to anti-tumor response [129]. However, its instability and systemic side effects limit biological applications. To address these limitation, Wang *et al*. encapsulated IL-12 in the tumor microenvironment-responsive poly(β-aminoester) copolymer, which was synthesized by using hydrophobic monomers, pH-responsive monomers, and hydrophilic monomers *via* Michael addition [130]. Compared with free IL-12, the nanoparticles injected through the tail vein could be effectively enriched at the tumor site through the EPR effect, and release IL-12 in response to mild acidic environment of the tumor. The release of lL-12 converted M2 macrophage into M1 phenotype, which improved the therapeutic effect with minimal side effects.

TLRs, a kind of pathogen identification receptors overexpressed on antigen-presenting cells, have great influences on innate immunity [131]. The use of TLR agonists has gained increasing attention to promote TAM conversion into pro-inflammatory phenotype [132]. Imidazoquinoline small molecule compounds, such as imiquimod (R837) and resiquimod (R848), can bind TLR7/8 that usually highly expressed in human antigen-presenting cells (*i.e.* monocytes, macrophages, dendritic cells and B cells) [133–135]. Recognition of TLR7/8 activates intracellular signaling pathways to secrete pro-inflammatory mediators and promote the inflammatory process. However, these agonists can trigger systemic toxicity [136–139]. So it is necessary to develop a targeted delivery system to improve its pharmacokinetic characteristics. Recent research showed that dissolving TLR 7/8 agonist (TLR7/8a) into nanoemulsions (NE) can strongly reduce toxicity. Since R837 and R848 are insoluble in water, they were dissolved in squalene by oleic acid, and then the mixture was dispersed in a PBS solution containing Span 85 and Tween 80 to obtain NE. The NE could not only reprogram TAMs, but also recruit T cells to inhibit tumors (Fig. 9A) [140]. As an agonist of TLR9, CpG oligonucleotides can also act on the TLR signaling pathway to effectively stimulate immune responses, so it can be regarded as a powerful immune adjuvant for anti-infection and anti-tumor therapy [141]. However, as a nucleic acids-based hydrophilic polymer, it cannot penetrate cell membrane on its own. Therefore, the development of stable, highly active and biocompatible carriers to deliver CpG is critically important. Huang *et al*. designed pH-sensitive polymer NPs, which can simultaneously deliver CpG oligonucleotides (ODN) as well as anti-IL10 antibodies against TAMs (Fig. 9B) [142]. Owing to overexpression of galactose-type lectin (Mgl) on the surface of TAMs, such galactose-modified NPs displayed TAMs targetability. After reaching the acidic tumor microenvironment, the pH-responsive coating (PEG-histidine-modified alginate, PHA) detached from NPs, and galactose was exposed for TAMs targeting delivery. The CpG oligonucleotides effectively increased secretion of anti-tumor cytokine IL-12 and anti-IL10 antibodies, and inhibited the IL-10 signaling axis, achieving the reprogramming of TAMs [125]. In addition, some studies have indicated that activating multiple TLR signals can result in more effective anti-tumor responses. For example, Zheng *et al*. [143] have found that using engineered Salmonella typhimurium strains to stimulate TLR4 and TLR5 simultaneously promotes the repolarization of M2 TAM to a greater extent, inducing more effective anti-tumor immune responses with no obvious toxicity.

Certain microRNAs (miR), a type of endogenous noncoding nucleic acids, can also promote reprogramming of M2 macrophages and play an essential role in immune regulation [144,145]. For example, miR-125b, which is overexpressed in macrophages, has been confirmed to regulate macrophage activation [146]. However, just like other nucleic acids-based therapeutics, miR is prone to degradation under biological conditions, and cannot freely penetrate into cell membrane for intracellular delivery. To this end, effective vectors for miR is critically important for its *in vivo* applications. In one study, hyaluronic acid-polyethyleneimine (HA-PEI) NPs were fabricated to deliver miR-125b, which achieved over 6-fold growth in the M1 to M2 macrophage ratio as well as 300-fold growth in the inducible nitric oxide synthase (iNOS) (M1 marker)/Arg-1 (M2 marker) ratio in tumor, demonstrating the excellent ability of microRNA (miRNA)-125b to convert TAM into M1 macrophages [128].

4.3. Inhibition of macrophage infiltration

Recruitment of monocytes and macrophages to diseased sites is a vital feature of host immunity. However, due to their organizational destructiveness, excessive recruitment is harmful to the organism. Several inhibitors, including inhibitors of C-C motif chemokine ligand 2 (CCL2)/C-C chemokine receptor 2 (CCR2), CCL5/CCR5, and VEGF have been demonstrated to prevent the recruitment of macrophages for disease therapy [147,148].

As a powerful chemical attractant, CCL2 plays an important role in the recruitment of myeloid cells. Since $CCR2^+$ monocytes are the precursors of TAMs, inhibiting the CCL2/CCR2 signal axis could effectively reduce the number of macrophages at lesion site. As a proof-of-concept, a CCR2 silencing siRNA was loaded into lipid NPs. The NPs accumulated in bone marrow after intravenous injection and actively targeted monocytes through receptor-ligand binding, resulting in a significant reduction of tumor volume and the number of TAMs [149]. Ban *et al*. proposed a similar approach to target the CCL5-CCR5 axis, in which CCL5 silencing siRNA was loaded in biodegradable mesoporous silicon NPs (MSVs) [149]. Intravenous injection of CCL5-siRNA-loaded-MSVs achieved the repolarization of immunosuppressive myeloid cells in the bone marrow, thereby causing an obvious inhibition of tumor progression and increase of CD8+ T-cell accumulation at the lesion site.

VEGF is a highly specific vascular endothelial cell growth-promoting factor, which can promote the increase of vascular permeability, the migration of vascular endothelial cells and the formation of blood vessels. In tumor tissue, VEGF is highly expressed in TAMs to facilitate cancer progression and metastasis by promoting angiogenesis [150–152]. Using VEGF siRNA to silence VEGF mRNA in TAMs to enhance tumor inhibition may be a feasible treatment strategy. Conde *et al*. developed PEGylated gold NPs to deliver VEGF siRNA, and they immobilized a ligand M2 peptide on the surface that can specifically recognize TAMs [153]. The results indicated that nanoparticles could successfully prevent the recruitment of TAMs and inhibit the progression and metastasis of lung cancer.

4.4. Regulating macrophages via multiple mechanisms

Many pre-clinical studies have proposed technologies for co-delivering multiple drugs for synergistic therapy. Compared with single drug delivery, the co-delivery systems show the following advantages: 1) a wider target range, 2) reduced dose of each individual drug thus minimizing the side effects, and 3) reversing multi-drug resistances to a certain extent. Various nanocarriers have been employed as co-delivery systems, and the drugs can be loaded *via* physical embedding or chemical combination. More and more studies have confirmed the feasibility of co-delivery systems for the treatments of several diseases, such as cancer [154], AS [155], as well as RA [156]. This section will introduce some examples of co-delivery systems to regulate macrophages *via* different mechanisms.

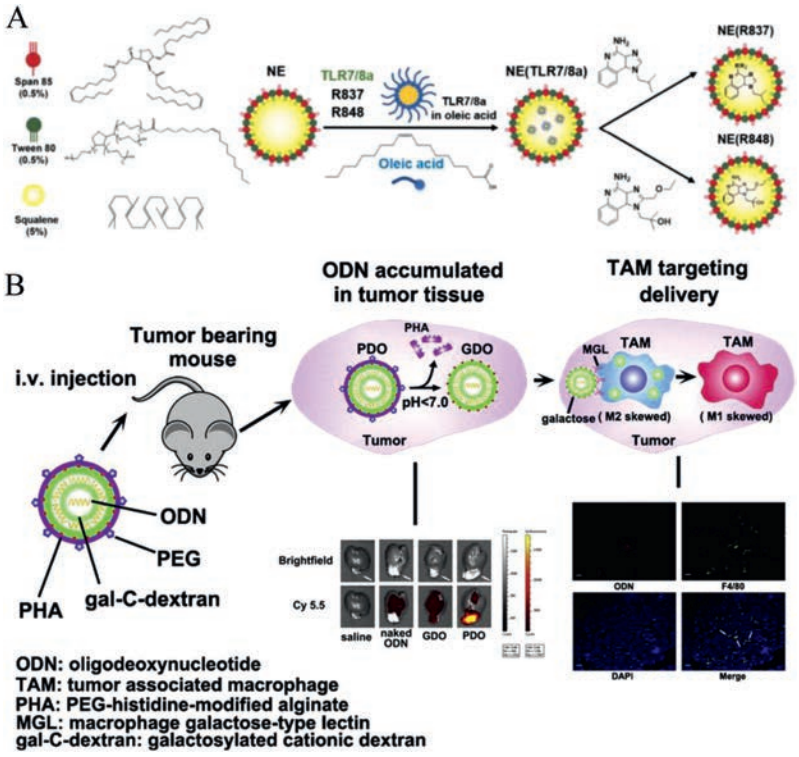

Fig. 9. (A) Schematic illustration of the preparation of NE. The water-insoluble R837 and R848 were dissolved in squalene by oleic acid, and then the mixture was dispersed in a PBS solution containing Span 85 and Tween 80 to obtain NE (R837/R848). Copied with permission [140]. Copyright 2019, American Chemical Society. (B) Schematic illustration of the structure of pH-sensitive galactose modified polymer NPs for TAMs targeting tumor therapy. The pH-sensitive PEG-histidine-modified alginate (PHA) was released in the acidic microenvironment of the tumor, and the NPs actively targeted TAMs through ligand-receptor binding to reverse the polarization of anti-inflammatory TAMs. Copied with permission [125]. Copyright 2012, Elsevier.

The delivery of siRNA and chemical drugs simultaneously *via* nanomedicines resulting in a combination of immunotherapy and chemotherapy has become a promising treatment strategy. The "layer peeling" co-delivery system prepared by Wang *et al.* was able to deliver inhibitor of nuclear NF-κB kinase (IKK) (for immunotherapy) and DOX (for chemotherapy) at the same time [154]. *In vivo*/*in vitro* analysis on tumor-bearing mice indicated that IKKβ-siRNA accumulated in TAMs, while DOX was internalized into neoplasms cells. As a result, TAMs were polarized to M1 macrophage, and the co-delivery system exhibited significantly improved antitumor efficiency.

Macrophages have been recognized as an essential target for AS treatment since they participate in all AS-related processes including initiation, progression, and lesions formation. Recently, a multifunctional nanocarrier composed of poly(ethylene glycol)-*block*-poly(L-aspartic acid) grafted with diethylenetriamine, lysine and cholic acid (PEG-PAsp(DETA)-Lys-CA2) polymer was developed to deliver andrographolide and Notch1-siRNA simultaneously to exert an anti-inflammatory effect (Fig. 10A) [157]. In this design, andrographolide reduced the inflammatory response by preventing the nuclear factor-κB (NF-κB) signaling pathway, while Notch1-siRNA provided a powerful tool to alleviate the development of AS by increasing the population of anti-inflammatory mediators including IL-10 and arginase-1 expression. Such system displayed multiple therapeutic functions, such as NF-κB signaling inhibition, Notch1 gene silencing, MCP-1 up-regulation, and anti-inflammatory cytokines such as IL-10 and arginase-1. Therefore, such combination therapy could simultaneously suppress proinflammatory cytokines expression and increase anti-inflammatory factors generation, which contribute to AS therapy.

In another work, Wang *et al.* proposed to treat arthritis by delivering dexamethasone (Dex) and NF-κB p65-siRNA simultaneously *via* micelles system, which was consisted of two amphiphilic copolymers: polycaprolactone-polyethyleneimine (PCL-PEI) and poly caprolactone-polyethylene glycol (PCL-PEG) (Fig. 10B) [156]. The p65 siRNA was used to target p65, a member of the NF-κB family, and it was expected that M1 macrophages would be repolarized to the M2 state by down-regulating p65. Since the single siRNA was not likely to inhibit the complicated signal transduction of RA, they combined siRNA drugs with Dex, which can inhibit the transcription of NF-κB [158]. Such "combo" nanomedicine could achieve the repolarization of M1 macrophages, and showed superior efficacy in NF-κB signaling suppression compared with single drug Dex or siRNA. In collagen-induced arthritis model, the nanomedicine was delivered in inflamed joints to exert an anti-inflammatory effect, with no noticeable side effects. Therefore, utilizing polymerized hybrid micelle co-delivery system to interfere NF-κB signaling pathway can be regarded as a desirable therapeutic strategy for inflammatory diseases.

Different from the co-delivery systems mentioned above that deliver two or more drugs at the same time, the intrinsic active carriers without any drugs loading can perform multiple functions. For example, we developed folic acid-modified nanoparticles (FA-AgNPs) that could passively accumulate in inflammatory joints and actively target M1 macrophages to exert anti-inflammatory effects (Fig. 11) [159]. The PEGylated AgNPs were modified with folic acid to recognize M1 macrophages *via* specific binding with folate receptor (FR) on cell membrane. After intracellular delivery, the nanoparticles released Ag^+ in response to intracellular stimuli (such as glutathione (GSH)), which concomitantly cause macrophages apoptosis and repolarize M1 macrophage toward M2 phenotype. As a result, such drug-free nanoplatform displayed a multi-regulatory strategy to manage RA.

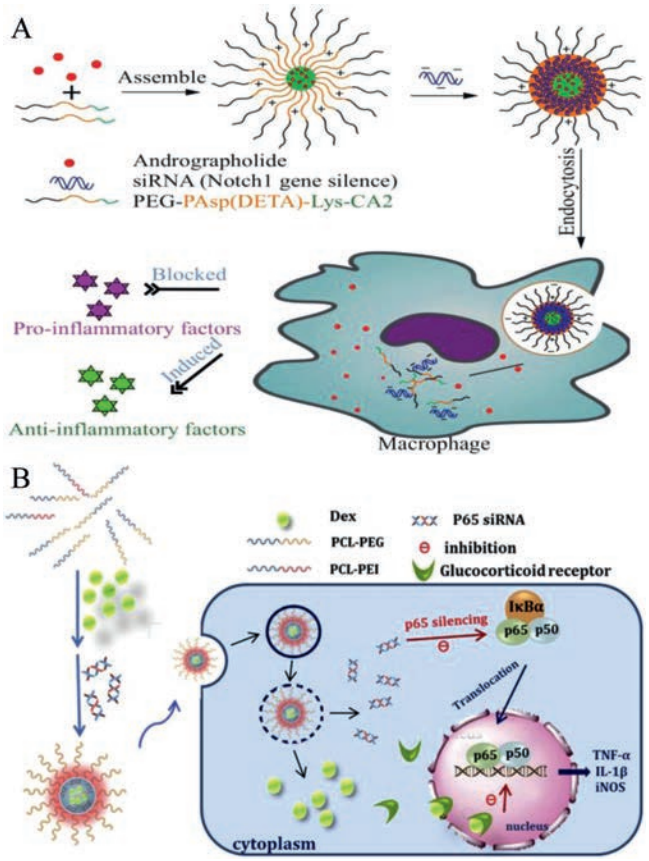

Fig. 10. (A) Schematic illustration of the structure of NPs co-delivering andrographolide and Notch1 siRNA, and the mechanisms for macrophages regulations. Copied with permission [157]. Copyright 2018, Springer. (B) Schematic illustration of the polymerized hybrid micelle co-delivery system loaded dexamethasone (Dex) and NF-κB p65-siRNA for RA therapy. Copied with permission [156]. Copyright 2017, Elsevier.

5. Strategies for macrophages-targeting delivery

Nanomedicine is a promising drug delivery candidate, which gives elegant solutions to address both pharmaceutic and biopharmaceutic issues of various drugs, such as poor solubility, limited bioavailability, rapid *in vivo* clearance, and unwanted side-effects [160]. During *in vivo* circulation, it is desired to deliver therapeutic drugs into diseased tissue and transport into the target cells. Macrophages-targeting delivery can be divided into two strategies: passive targeting and active targeting. The increase of vascular permeability in the lesion site is a favorable condition for passive targeting, which is mainly affected by the particle size of NPs. Active targeting, by contrast, is realized through the specific recognition of the ligand on the surface of NPs and the receptor overexpressed on the surface of macrophage, which is determined by the receptor type and ligand-receptor recognition ability. Next, we will summarize these two strategies individually to clarify the advantages of nanomedicine for drug delivery.

5.1. Passive targeting

Passive targeting is a behavior of NPs to enter specific organelles, cells, tissues or organs independent of the recognition ability of targeted molecules, but is strongly influenced by the size, structure, surface characteristics and other physical and chemical properties of NPs. It is usually achieved through “allowed” biological structures or cellular uptake and some external conditions, such as magnetic fields, electric fields, and special routes of administration. Extensive evidence has shown that loss of endothelial integrity can lead to abnormal angiogenesis with increased vascular permeability in diseases such as cancer [161], RA [162] and AS [163]. This vascular permeability increases the leakage and accumulation of nanomedicine into pathological tissues for enhanced therapeutic efficacy, which will discuss in the follow section.

In 1986, Hiroshi Maeda injected Evans blue solution into the tail vein of tumor-bearing mice, and interestingly, it is found that the Evans blue-plasma protein conjugation showed an obvious tu-

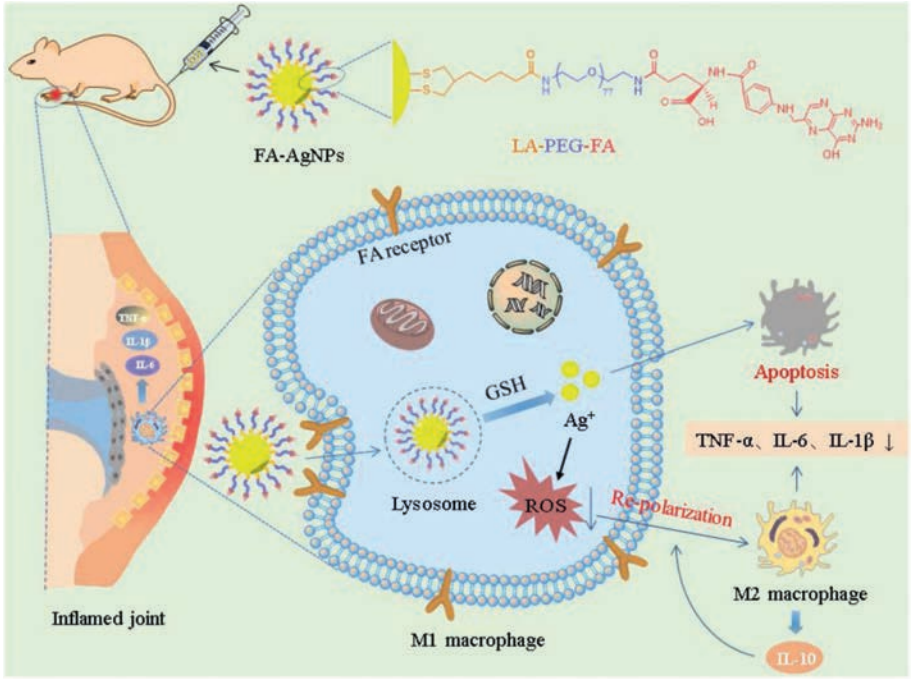

Fig. 11. Schematic illustration the mechanism of FA-AgNPs for RA therapy. FA-AgNPs dissolved and released Ag^+ inside macrophages, which can promote the apoptosis of M1 macrophages and repolarization of M1 macrophages to M2-type. Copied with permission [159]. Copyright 2021, Elsevier.

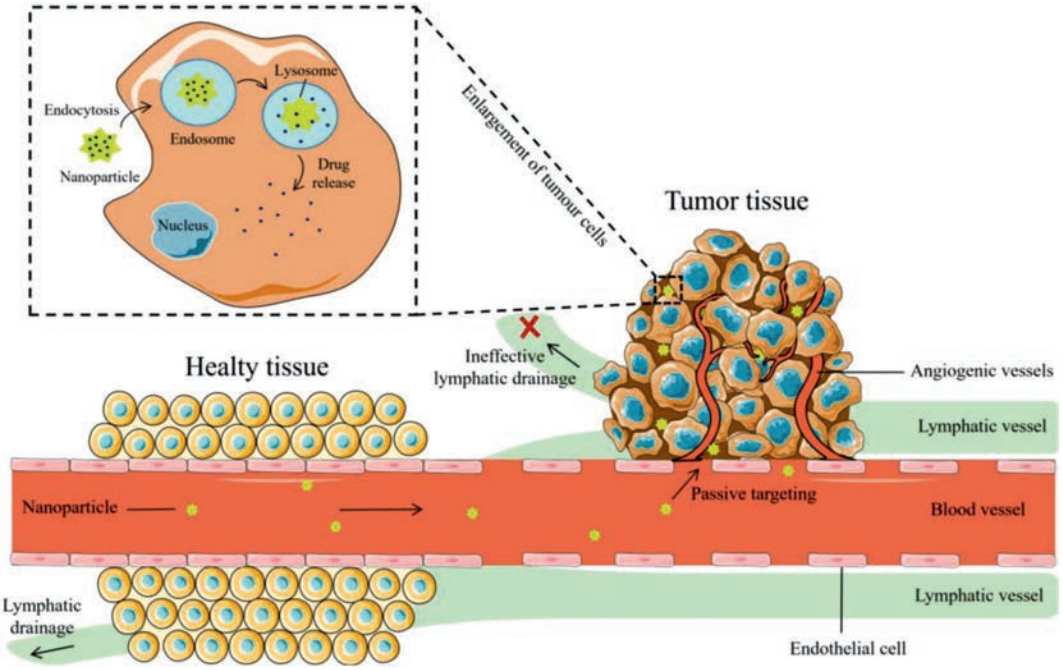

Fig. 12. Schematic illustration of the EPR effect. Increased permeability of tumor angiogenic vessels and ineffective lymphatic drainage allow macromolecular drugs to penetrate and remain in tumor tissues, thereby achieving passive targeting to tumor.

mor accumulation and strong retention, owing to the changes of anatomy and pathophysiology in solid tumors. To meet the rapid growth of tumor, microangiogenesis occurs in tumor tissue, forming new blood supply systems to supply abundant nutrient and oxygen for tumor proliferation. However, the imbalance of angiogenic factors and MMP in tumor tissues leads the mess microvascular structure, missing tube walls, and wide gaps between endothelial cells, which allows passive penetration of macromolecular substances into tumor. In addition, the tumor blood vessels usually lack the smooth muscle layer, which results in increased leakage of neovessels. On the other hand, the accumulated macromolecular drugs can stay in tumor tissue for a long time since the lymphatic clearance system in tumor tissue is insufficient. This phenomenon of leaky microvascular system and impaired lymphatic drainage is so-called "the enhanced penetration and retention effect" (EPR effect) (Fig. 12), which creates great opportunities for targeting delivery of nanomedicines in the tumor tissue [164,165]. Through this mechanism, NPs with a molecular mass greater than 50 kD can selectively accumulate in the tumor stroma. In addition, tumor cells tend to undergo glycolysis for rapid ATP generation, leading to a mild acidic tumor microenvironment [166]. As such, the pH-responsive drug delivery systems can be designed, which is stable under physiological pH conditions but rapidly releases the payload drugs in a slightly acidic environment to exert anti-tumor effects [167–169].

RA is a systemic chronic autoimmune disease characterized by inflamed joint synovium. The abnormal proliferation of blood vessels in the affected joints is one of the hallmarks of RA. Inflammation triggers excessive migration of immune cells to the synovium [162,170]. Meanwhile, hypoxic environment and the emergence of pro-inflammatory mediators promote the expression of VEGF and bFGF, leading to promotion of angiogenesis [162,171]. The width of the synovial membrane increased from 2–3 cell layers to multiple cell layers, and the gap as wide as 700 nm is formed between neovascular endothelial cells to allow increased permeability of diseased joints [172]. Different from the retention effect caused by the lack of tumor lymphatic drainage, the penetrated nano-drugs in the inflamed joint will be endocytosed and retained by the activated synovial cells. This effect is named "the extravasation through leaky vasculature and the subsequent inflammatory cell-mediated sequestration" (ELVIS) effect (Fig. 13). Utilization the characteristics of enhanced synovial vascular permeability to deliver nanomedicine can achieve effective RA targeted therapy [173–178]. Noted that, both particle size and surface chemistry have strong effect on targetability of NPs at arthritic site. For instance, prednisolone-loaded liposomes at 90–100 nm showed significantly better accumulation in inflamed joints than counterpart liposomes of 450–500 nm [179]. The liposomes with surface PEGylation achieved extended circulating half-life and better retention in inflamed joints, giving rise to improved therapeutic effect. Similar effect was also observed in polymeric NPs [180], liposomes [181] and lipid microspheres [182]. Besides, the acidic pH and redox potential in the inflamed synovium can be employed as triggers for responsive drug release [183]. For example, thiolated glycol chitosan NPs were prepared to selectively release TNFα-siRNA in inflamed joints for RA therapy [184].

In the procession of AS, abnormal endothelial function enhances the penetration of lipoproteins and promotes the recruitment of monocytes [185]. Monocytes differentiate into macrophages under the action of pro-inflammatory mediators (*e.g.*, TNF-α, interferon (IFN)-γ, IL-1β) produced by endothelial cells [186,187]. In the initial stage of AS, lipoproteins and immune cells remain in the vascular wall to form plaques. Further development of AS is accompanied by the accumulation of plaques, leading to continuous thickening of the vascular intima. When the thickness surpasses the oxygen diffusion threshold, the local hypoxia induces the expansion of vasa vasorum reaching into the bottom of the plaque, as a compensatory mechanism to maintain the supply of nutrients to the vascular wall (Fig. 14) [188,189]. Such neovessels are leaky, which enables passive targeting delivery of macromolecular drugs to treat AS [190]. Similar to targeting toward tumors and inflamed joints, the size of nanomedicines is the main factor affecting targeting efficiency. Chono *et al.* compared the accumulation capacity of liposomes with different diameters at plaque (*i.e.*, 70 nm, 200 nm and 500 nm) [191]. The results showed that liposomes at 200 nm displayed the best accumulation, attribut-

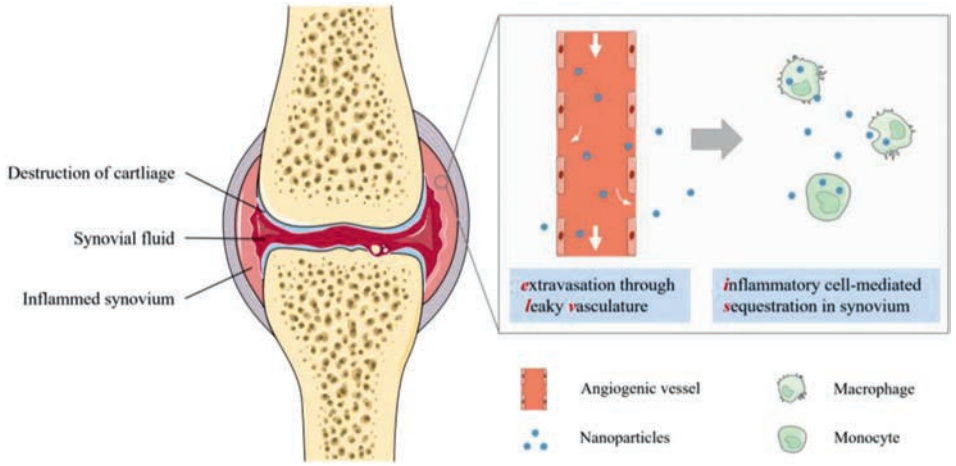

Fig. 13. Schematic illustration of the "ELVIS" effect. Angiogenic vessels are induced by the inflammatory environment. Nanoparticles will extravasate through leaky vasculature and then be swallowed and retained by inflammatory cells (such as macrophage/monocyte) in the inflamed synovium.

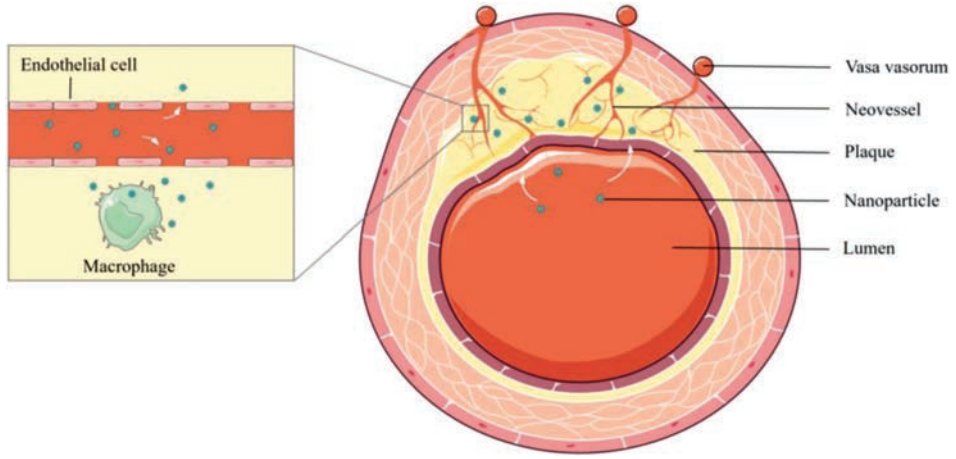

Fig. 14. Schematic illustration of targeting principles in atherosclerotic plaques. In normal blood vessels, oxygen and nutrients are transported to the outside of the vascular wall *via* the vasa vasorum. The local hypoxia caused by accumulation of plaques induces the expansion of vasa vasorum reaching into the bottom of plaques. Increased permeability of neovessels lay the basis for the design of NPs to target AS lesion.

ing to the better extravasation capacity than liposomes of 500 nm and greater uptake efficiency by plaque macrophages than the 70 nm one. Interestingly, the PEG-modified nanomedicine did not significantly improve the therapeutic efficacy, likely due to the lack of internalization by plaque macrophages [192]. However, non-PEGylated nanoparticles are likely cleared by macrophages in liver and spleen. Thus, the balance of NPs PEGylation is worthy of further exploration. In recent years, reconstituted high-density lipoprotein (HDL) NPs have become a hotspot for AS targeting therapy [193]. HDL can be specifically recognized and internalized by plaque macrophages *via* its binding to scavenger receptors on cell membrane, and promote the removal and excretion of cholesterol in plaques [194].

5.2. Active targeting

Different from passive targeting, active targeting is a specific targeting, which mainly targets the over-expressed receptors on the surface of macrophages. As mentioned above, appropriate NPs can be internalized easily by macrophages due to the property of materials or size. Once these NPs are modified with specific molecules, such as the ligands to the surface receptor of macrophages, specific targeting strategy can be achieved. While a variety of specific receptors have been identified on macrophages, only the minority of them have been employed as targeting receptors and such as mannose receptor, galactose receptor. In this section, we will introduce the design principle for active targeting towards macrophages.

5.2.1. Mannose receptor

Mannose receptor (MR), a member of the C-type lectin superfamily, mainly expresses on the surface of macrophages and dendritic cells, and has been employed as target for macrophage specific delivery [195,196]. For example, mannose-modified gene delivery liposomes have been designed to recognize MR for targeting delivery, achieving enhanced immune response [197]. Hattori *et al.* demonstrated the efficacy of mannosylated cationic liposomes, which showed significantly improved antigen presentation to stimulate the immune response as compared to ordinary liposomes [198]. In another work, Locke *et al.* injected ^{64}Cu-loaded mannosylated liposomes (MAN-LIP) into a mouse model of lung cancer, and tracked the distribution through PET imaging [199]. The results indicated that a large number of MAN-LIP accumulated in MR+ TAMs with minimal distribution in other areas of the lung, while unmodified liposomes showed non-specific distribution, demonstrating the targetability of mannosylated NPs.

5.2.2. Galactose receptor

Similar to MR, the macrophage galactose-type lectin (Mgl) is also highly expressed in TAMs, making galactose a suitable ligand for TAM recognition [200]. Capitalized on this fact, Huang *et al*. have established a galactosylated nanoplatform to incorporate and protect an oligonucleotide combination of CpG, anti-IL-10 and anti-IL-10 receptor oligonucleotides for TAM targeting delivery (Fig. 9B). Through intravenous injection into tumor-bearing mice, such nanosystem could obviously accumulate into TAMs, achieving robust tumor growth inhibition effect [125].

5.2.3. Folate receptor

FR is a glycosylphosphatidylinositol linked protein receptor that binds to folic acid-based moieties. FR was initially found to be overexpressed on epithelial cancer, which was utilized to deliver therapeutic drugs toward tumors. It was later found that FR was overexpressed on macrophages as well [201]. Interestingly, folate-modified liposomes showed 10-fold higher accumulation in TAMs than tumor cells, and 50% of the liposomes were internalized *via* FA-mediated delivery [202]. In another study, FR-β antibody conjugated pseudomonas exotoxin A was applied to a nude mice model of glioma and displayed significant TAMs depletion and tumor growth suppression, demonstrating the possibility of glioma treatment *via* directly eliminating macrophages [202].

5.2.4. Scavenger receptor

Scavenger receptor (SR) is structurally unrelated membrane receptor highly expressed by phagocytes (macrophages, dendritic cells and microglia). SR exhibits a variety of properties and promotes endocytosis. Due to the cationic charge of the extracellular collagen domain, SR can be coupled to anionic delivery carriers [203]. At present, various SRs, such as macrophage receptor with collagenous structure (MARCO), CD163, cluster of differentiation 68 (CD68), lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), have been reported. However, their utilization as targeting ligand has been largely unexplored. In one attempt, an anti-CD163 antibody-drug conjugate was developed to deliver Dex to macrophages *via* its binding with CD163 [204]. *In vivo*, such conjugate achieved 50-fold higher potency than that of nonconjugated Dex, and the side effects were also largely minimized.

6. Conclusions and future perspectives

By virtue of the significant progress in elucidating the roles of macrophages in different diseases, macrophages-targeted immunotherapy has attracted increasing attention. In this article, we reviewed the development of macrophages-regulating nanomedicines for chronic diseases therapy. Immunotherapy has been a hot topic in medical field, which has been recognized as a new hope for the treatment of various refractory diseases, such as cancer. As an innate immune effector, macrophages are important bridge to link innate and adaptive immunity *via* antigen presentation and cytokine secretion. In response to external/internal stimuli, macrophages can differentiate and polarized into various phenotypes to fulfill a number of biological functions. However, macrophage disorder is the hallmark of various immunological diseases, in which inappropriately activated macrophages contribute greatly to the progression of the diseases. We summarized the strategies to modulate the diseased macrophages, and nanomedicines showed inherent advantages for targeting drug delivery with improved therapeutic index.

While substantial success has been made in fundamental side in engineering macrophages by using nanomedicines for diseases immunotherapy, the clinical translation examples are still rather limited, indicating formidable challenges remain. Based on the current status of the field, we finally postulate further efforts that may facilitate the development of macrophage-targeting nanomedicine for immunotherapy.

1) Improvement of the safety and efficiency of macrophage-targeting immunotherapy: Biosafety is a trick issue of clinical translation. First, the targeting efficiency is dissatisfactory. For instance, off-target phenomenon, due to the poor recognition effect of target or the non-orientation of passive targeting, has brought great challenges to administration safety and subsequent clinical translation. In order to enhance the efficiency, specific active targeting has attracted more attention recently, but how to reduce the damage to other cells still remains a problem. Except the above-mentioned dissatisfactory targeting efficiency, the toxicity of nanoparticles should also be noticeable. 2) Optimization of nanomaterials: while a number of attractive nanomaterials have been studied recently, most of them did not make real clinical impact. Therefore, in-depth exploration of the interaction between nanomaterials and biological systems and the transport mechanism of nanoparticles will contribute to improving the *in vivo* stability, biodistribution and safety of multifunctional nanocarriers, thereby promoting clinical application. 3) Further exploration of the relationship between macrophages and diseases: Owing to the complexity of the human immune system, our understanding of the role of the macrophage is still in its infancy, only by further understanding the role of macrophages in the occurrence and development of diseases, can we better comprehend the disease mechanism and develop formulations for it, and then can we prevent the occurrence or deterioration of diseases effectively. 4) Feasibility of manufactural technics: As has been all-known, the preparation of nano-formulations is complicated and has many influencing factors, which makes it difficult to control batch-to-batch consistency. Furthermore, the preparation machines are expensive and the preparation processes are complicated, both of which make it difficult to scale-up production. All of these greatly hinder the further development of nanomedicines. We should not only focus on fundamental research, but also strive to development of nanomedicines from a laboratory to a factory. New progresses on this direction in the future will fuel the growth and potential clinical applications of the macrophage-targeting nanomedicines.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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