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Macrophages-regulating nanomedicines for sepsis therapy

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ABSTRACT

Sepsis is the leading cause of death in intensive care unit (ICU), which is caused by deregulated immune responses to pathogens infection. Clinically, sepsis treatment is limited to antibiotics and supportive care, while there still lacks of specific molecular therapy. As a type of immune dysfunction disease, macrophages have been recognized as the key immune cells precipitating in the whole process of sepsis, which is activated into M1-like to trigger various inflammatory responses at early stage whereas polarized into M2-like to cause immunosuppression in later stage. Therefore, great attention has been paid on the design of nanomedicines to regulate the functions of macrophages for etiological treatment of sepsis, by virtue of the unique advantages of nano-drug delivery systems, such as enhanced drug bioavailability, targetability, reduced side-effects. This critical review aims to summarize the recent progress of macrophages-regulating nanoparticles for sepsis therapy. First, the essential roles of macrophages in the development and progression of sepsis have been introduced, including the positive roles of macrophages to combat infections and dysfunction of macrophages to cause body damages. We then focus our main attention to discuss the nanomedicines with different therapeutic mechanisms corresponding to each stage of sepsis, such as infection blockage, inflammation inhibition, immune functions recovery, as well as multifunctional nanomedicines. Finally, a few limitations of current nanomedicines are highlighted, and future perspective are speculated for potential clinical translation, which might pave the way for the development of macrophages-centered nanomedicines for more effective sepsis therapy.

1. Introduction

According to the WHO COVID-19 Dashboard, as of 14 April 2022, the COVID-19 that broke out at the end of 2019 has infected up to 500 million people and caused approximately 6.2 million deaths. In fact, the high mortality rate of COVID-19 patients is closely related to viral sepsis, and most patients with severe illness will eventually progress to typical septic shock, accompanied by inflammatory storms, oxidative stress damage, respiratory and microcirculation disorders [1,2]. Therefore, the development of effective methods to manage sepsis is of great importance for improving the survival rate of patients with COVID-19 infection. Sepsis has been newly defined as life-threatening organ dysfunction resulting from dysregulated host responses to infection, by the Third International Consensus Definition for Sepsis and Septic Shock (Sepsis-3) in 2016 [3]. There are about 50 million sepsis cases and 11 million deaths each year [4]. It often manifests as inflammation, tachycardia and rapid breathing in the early stage of sepsis, while it could progress to hypovolemia, septic shock and multiple organ failure in the later stage. It is also usually accompanied with several complications such as acute lung injury, acute respiratory distress syndrome (ARDS), metabolic acidosis and diffuse intravascular coagulation (DIC) [4,5]. Even if the patients survive, the prognosis will become a huge challenge, for the fact that one in six patients experience severe cognitive impairment [6]. Sepsis damages patients' health both in body and mentality, and make them suffer from heavy financial burdens. As a major public health concern in the world, it is unfortunately that there is still lack of specific method to treat sepsis.

The conventional treatments mainly focus on organ support such as fluid resuscitation, mechanical ventilation and hemodynamics maintenance [7,8], while drug therapy is still an adjunct. Depend on the stage of the pathophysiological process of sepsis, various symptomatic drugs are often used. However, none of these treatments can fundamentally cure sepsis, which stimulates the search of treatments based on pathogenesis. The mechanisms of sepsis are complex and related to a variety of immune cells and

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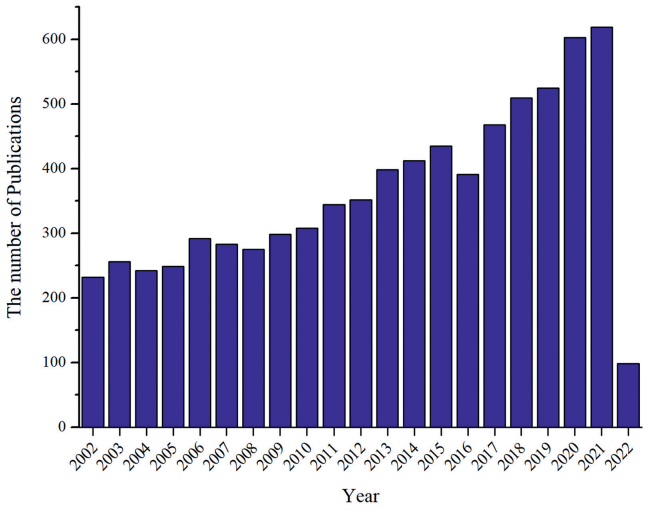

Fig. 1. The publications involving both macrophage and sepsis have increased over past 20 years. The data were obtained by Web of Science.

cytokines [5]. In the early stage, the pathogen infection triggers excessive inflammation, leading to a cytokine storm with coagulation dysfunction, organ damage and so on. In the later stage, on the contrary, the dysfunction of immune cells causes immunosuppression, which leaves patients being vulnerable to secondary infections, leading to septic shock and multiple organ dysfunction [5]. According to the current research findings, macrophages play crucial roles during the process of sepsis, and more and more relevant studies have been reported in the last two decades (Fig. 1). Macrophages are widely distributed in various tissues and play multiple roles in all the stages of sepsis, such as recognition, phagocytosis, bactericidal, antigen presentation, cytokine secretion [9]. In the early stage of sepsis, macrophages differentiate into M1-like and secrete a large number of pro-inflammatory factors and chemokines, aggravating the inflammatory response. In the later stage, on the contrary, macrophages are excessively apoptotic or polarize into the M2 phenotype to produce a mass of anti-inflammatory factors, leading to immune dysfunction and organ damage [9,10]. Therefore, regulating the function of macrophages holds a great promise for etiological treatment of sepsis.

The regulation of macrophages mainly relies on the induction or hindrance of many receptors, metabolites and pathways involved in sepsis. Therefore, how to perform precise drug delivery in the treatment of sepsis is worth thinking. Targeting strategy based on the nanosystem is a very good choice. Through rational structure design, nanoparticles (NPs) with desired particle size, shape, surface modifications can be fabricated, making it possible to deliver drugs to target site with reduced side-effects [11]. What is more, they can also able to deliver multiple drugs for combinatorial therapy. Recently, the use of nanomedicine to treat sepsis has attracted great attention [12–14]. For example, Yang *et al.* designed a $\gamma 3$ modified PLGA nanosystem to target the site of inflammation for co-delivery of a broad-spectrum antibiotics (spafloxacin) and anti-inflammatory agent (tacrolimus). Such multi-modal therapy has been applied on a mouse model of sepsis caused by acute lung infection, achieving a significant improvement of the survival rate [14].

Given the unique advantages of nanoparticles for drug delivery, recent years have witnessed a surge in the development nanomedicine for sepsis therapy, and several of them are promising for clinical translation. With significant advancement in the field, it is right time to summarize progress being made, which would definitely help to direct future research. While general reviews of nanomedicine for sepsis therapy have been reported previous [7,15-17], we focused our attention on macrophages-centered designs of nanosystems, given the critical roles of macrophages in the progression of sepsis. We first discussed the pathological roles of macrophages during sepsis in detail, and highlighted the mechanisms of regulating macrophages at different disease stages for sepsis therapy. Then, various design strategies of nanomedicine have been reviewed systematically, including the blockage of pathogens infection, inhibiting macrophage-mediated inflammatory response, restoring the immune function of macrophage, as well as multifunctional nanomedicines to regulate macrophages in multiple aspects. Finally, the problem and future perspective of the field were speculated to pave the clinical translation of macrophages-centered nanomedicine for sepsis therapy.

2. The roles of macrophages in the progression of sepsis

It is generally believed that the activation of the innate and adaptive immunity of the host by infection or injury is involved in the initiation of sepsis [18,19]. Among the microorganisms that induce sepsis, gram-negative bacteria are the most common, followed by gram-positive bacteria and fungi [20]. Macrophages, which are widely distributed in various tissues, are the core elements for the body to identify and eliminate pathogens and play an important role in both innate immunity and adaptive immunity. The precursors of macrophages are monocytes which have a short life span in the blood and undergo apoptosis in the absence of stimulation. However, when the host is invaded by pathogens, monocytes migrate to the infected site and differentiate into macrophages [21,22]. Macrophages are highly plastic because they can switch to different phenotypes according to environmental stimuli to perform corresponding functions [23]. M1-like macrophages, also known as classical activated macrophages, exert anti-infective and pro-inflammatory effects, which is due to their high expression of pattern recognition receptor (PRR) that can interact with pathogen/damage-associated molecular pattern (PAMP/DAMP). The PRRs that mediate the inflammatory response of sepsis are mainly Toll-like receptors (TLRs) and NOD-like receptors (NLRs) [24,25].

Taking gram-negative bacteria infection as an example, lipopolysaccharide (LPS) is the PAMP of Gram-negative bacteria, which activates myeloid differentiation proteins 88 (MyD88) by binding to TLR4 on the surface of macrophage. The MyD88 signaling pathway involves multiple kinases including interleukin 1 (IL-1) receptor-associated kinase 4 (IRAK4), tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) and the inhibitor kappa B kinase β (IKKβ). The activation of above-mentioned kinases subsequently activates nuclear transcription factor (NF-κB) and promotes M1-like polarization [24,26]. NLRs, as another important PRRs, can assemble into inflammasomes under the stimulation of LPS. Upon activation of the NLRP3 inflammasome, caspase-1 induces the maturation and release of IL-1β and IL-18, which causes cell pyroptosis and the production of reactive oxygen and nitrogen species (RONS) [27,28]. It is worth mentioning that excessive RONS can also induce the assembly and activation of NLRP3 inflammasome. M1-like macrophages not only secrete chemokines to recruit other cells such as natural killer cells, neutrophils, and activated T cells to infection site, but also highly express major histocompatibility complex (MHC) and present antigens to T cells to trigger specific immunity [29]. In addition, M1-like macrophages secrete large amounts of pro-inflammatory factors including IL-1β, IL-12, IL-6, TNF-α and RONS, which are critical to the elimination of pathogens [30].

In the early stages of sepsis, the metabolism of M1-like macrophages changes from oxidative phosphorylation to glycolysis and pentose phosphate pathway, called metabolic reprogramming,

which is similar to the metabolic pathway of tumor cells proliferation. There are only two ATP produced by glycolysis of a glucose molecule, but the process is very fast, so M1-like macrophages increase glucose uptake to meet the requirement of rapid energy supply for pathogens killing [31,32]. Importantly, hypoxia inducible factor 1α (HIF-1α) and mammalian target of rapamycin complex 1 (mTORC1) are confirmed to be agonists for the above-mentioned metabolic shift [33,34]. Macrophage autophagy also plays an important role in protecting the host from foreign pathogens and danger factors through many ways such as dying directly, presenting antigens, modulating inflammatory processes and releasing RONS [35]. It has been proven that LPS can promote macrophage autophagy through the TLR and heme oxygenase-1-dependent pathway [36]. On balance, macrophages act as an indispensable role in pathogen identification and elimination. For patients, eliminating infection as soon as possible is beneficial to survival and prognosis.

However, the killing effect of macrophages on pathogens in innate immunity is non-specific, and the released pro-inflammatory factors and RONS can also damage normal tissues and cells like endothelial cells, resulting in the production of numerous DAMPs like cell-free DNA (cfDNA) [37]. Similar to PAMPs, DAMPs also need to be disposed by macrophages, which continues to promote the activation of PRR and subsequent inflammatory pathway, leading to the formation of more inflammatory mediators. In this case, the body is in a state of excessive and persistent inflammation, eventually causing a cytokine storm. Macrophage-mediated hyperinflammation has harmful effects on multiple tissues and organs, as described in detail as follows: Pulmonary hyperinflammation may cause ARDS, a life-threatening disease characterized by unexplained respiratory failure due to hypoxemia [38]. Excessive inflammation of the brain might lead to headaches, apathy, lethargy, or delirium [39]. Other organs frequently affected by inflammation include the kidneys (acute kidney injury) and the liver (liver dysfunction, with increased levels of bilirubin or transaminases) [40,41]. In addition, the pro-inflammatory response of macrophages is closely linked and mutually regulated with the coagulation system and endothelial system of the body. More concretely, it has been demonstrated that inflammasomes can induce the release of coagulation factor 3 (F3), and F3 is the main initiator of coagulopathy in sepsis [42]. A major pathological syndrome of coagulopathy is DIC, which is a serious common complication of sepsis characterized by widespread microvascular thrombosis and massive platelet depletion [43]. Furthermore, the release of extreme inflammatory factors like RONS from macrophages leads to endothelial cell dysfunction and increased vascular permeability and leakage [44]. Endothelial dysfunction has extensive harmful impact on cardiovascular system, such as hypotension, tachycardia, or even septic shock, resulting in more tissue damage and eventually systemic inflammatory response syndrome [45]. Therefore, during the overwhelming inflammatory phase of sepsis, targeted inhibition of M1-like macrophages is expected to reduce organ damage and patient mortality. Besides, the promotion of macrophage autophagy can negatively regulate inflammatory response and protect the body, on account of restraining the activation of NLRP3 inflammasome and the release of inflammatory cytokines [46].

From the 1970s to the beginning of this century, it was generally believed that deaths from sepsis were almost caused by the above-mentioned excessive pro-inflammatory reactions. However, with the many intensive researches on sepsis pathophysiology, more and more evidences have demonstrated that for many patients, it is not excessive immune activation but immune suppression, also known as immune paralysis, is the major immune dysfunction associated with high mortality [47,48]. The role of macrophages in the immunosuppressive phase of sepsis is specifically described as follows. Due to the persistent inflammatory state and cytokine storm, a substantial apoptosis-inducing factors including TNF-α, high-mobility group box-1 protein (HMGB1) are released. The rapid increase of apoptosis of numerous immune cells including macrophages, is the main reason for sepsis entering immunosuppression stage [49]. Unlike necrosis, which normally stimulates the immune system to boost its defenses against microbes, apoptosis leads to cell immunological incompetence [50]. Under the stimulation of IL-13, IL-4, glucocorticoid, immune complex and other factors, most of the macrophages that escape from apoptosis are polarized into M2 phenotype, also known as alternative activated macrophages. M2-like macrophages are characterized by the secretion of transforming growth factor β (TGF-β) and IL-10 and other anti-inflammatory cytokines, diminished antigen presentation ability and up-regulated expression of programmed cell death 1 (PD-1) ligand 1 (PD-L1) [29,30]. Importantly, the interaction between PD-L1 of macrophages and PD-1 on the surface of T cells can inhibit the activation and proliferation of T cells, thereby suppressing the body's adaptive immunity [51,52]. In addition, the metabolic feature of M2-like macrophages is also different from that of M1-like because of its enhanced fatty acid metabolism and increased oxidative phosphorylation efficiency [33,53,54]. Immunosuppression period generally lasts for several months or years, during which patients are prone to serious complications and death due to secondary infections. Therefore, it is necessary to restore the immune function of patients as far as possible. In light of the versatility and high plasticity of macrophages in immune homeostasis, many therapeutic strategies targeting macrophages may become effective to combat sepsis induced immunosuppression, such as antagonizing apoptosis and inducing trained immunity [55,56].

3. The design of macrophages-centered nanomedicines for sepsis treatment

Sepsis is characterized by complex pathology, rapid disease progression and high treatment cost [4,57]. Even after successful rescue, there are still many lifelong complications, which seriously affect the quality of life of patients. Although anti-infection therapy and organ function support technology have made great progress, it is faced with the dilemma of microbial resistance and large consumption of medical resources [58]. The use of other traditional drugs such as glucocorticoids also could not reduce the mortality of sepsis [59]. Therefore, seeking for more effective treatment to deal with sepsis is still urgently desired.

In recent decades, the application of nano-drugs in disease therapy has been a compelling research field and the considerable advance in related technologies has been accomplished. Since Doxil was approved by FDA as the first nano-drug in 1995, lots of nano-drugs have been listed or entered clinical trials globally for kinds of indications, including cancer, fungal infections, iron deficiency anemia and so on [60–62]. Nano-drugs, also known as nanoparticles, are prepared by nanotechnology with diameters between 1 nm and 1000 nm. Compared with traditional drugs, they have a series of advantages such as enhanced solubility and bioavailability, targetability, pro-longed circulation half-life, as well as decreased side-effects. Moreover, codelivery of multiple drugs with the aid of nanocarriers can exert multifunctional and multitarget therapy, which is particularly attractive in sepsis therapy [63–65].

As aforementioned, macrophages are not only involved in the clearance of pathogens in sepsis, but also in the balance of pro-inflammatory and anti-inflammatory responses, changes in immune status and so on, which provides many potential targets for the treatment of sepsis [66]. Therefore, macrophages have been used as a preclinical research model in lots of studies. It is worth mentioning that the increase of vascular permeability during sep-

sis makes the selective enrichment of NPs in damaged tissues and organs, similar to the EPR effect in tumors, so as to enhance the curative effect to a certain extent [67]. Overall, NPs directing to macrophages are very promising in sepsis management. Herein we illustrate the development of the corresponding NPs and categorize them *via* their respective functions, hoping to provide a reference for future drug design and promote transformation of NPs from bench to bedside.

3.1. Blocking pathogens infection of macrophages

Pathogen infection is the origin of sepsis and can lead to immune disorders finally. Patients are usually treated with antibiotics quickly after diagnosis, and the survival rate of patients would be reduced by 7.6% every 30 min of delayed treatment [7,68,69], which shows the importance of controlling the source of infection in time. Even in the immunosuppressive stage, the clearance of pathogens is also the key therapy, because it can reduce the signal transmission between pathogenic bacteria and immune cells, alleviate the progress of inflammation, and reduce the risk of secondary infection. However, the efficacy of antibiotics can be limited to poor targeting, short half-life, and the existence of various drug-resistant bacteria. In fact, there is also a bactericidal system dominated by immune cells in the host. Macrophages are one of the most effective pathogen scavengers during infection [70], killing invading microorganisms by direct phagocytosis, lysis and initiating inflammatory signals. Recently, many nano-formulations based on macrophages have been used as new bactericidal strategies and indicate excellent preclinical results. Next, these nanomedicines with anti-infection capabilities are discussed in detail.

3.1.1. Removing extracellular endotoxins and pathogens

In sepsis, some bacteria release endotoxins (also known as LPS) when they divide, die, or are treated with antibiotics. Clinical studies have shown that higher levels of endotoxin correlate to worsened clinical outcomes [71,72]. Removal or neutralization of endotoxins has been treated as a potential strategy for sepsis treatment, but there are still some challenges [73]. For example, polymyxin can effectively neutralize endotoxin, but its clinical application is limited due to its strong nephrotoxicity and neurotoxicity [71,74,75]. Removing LPS from blood using polymyxin B (PMX-B) column or activated charcoal hemoperfusion is another approach to improve the survival rate of endotoxic mice [76,77]. However, the selectivity and removal efficiency are not satisfied [73].

Considering the intrinsic and great LPS-binding capacity of macrophages, biomimetic formulations of macrophage prepared by nanotechnology have been developed [78]. Shen *et al.* constructed disguised NPs by wrapping iron oxide nanoclusters with macrophage membranes (Fe_3O_4@MMs). Macrophage membrane acted as a bait to capture LPS selectively through the high affinity between membrane receptors (CD14, TLR-4) and LPS. The inner iron oxide cores with positive charge could stabilize the membranes shell and neutralize negative charged LPS. *In vitro*, it showed that the Fe_3O_4@MMs could adsorb LPS effectively with a clearance rate of 81.1%, while the PMX-B group was only 7.8% as control. In endotoxic mice, the Fe_3O_4@MMs could significantly improve the survival rate over 5 days as compared to the PMX-B group with all mice died within 48 h [73]. Similarly, Thamphiwatana *et al.* developed macrophage-mimetic NPs (denoted as MΦ-NPs) through wrapping biodegradable PLGA cores with the natural macrophage membranes, which significantly lowered bacteria burden in the blood and spleen and inhibited inflammation in septic mice [79].

Sepsis can be caused by different gram-positive and gram-negative strains, including all multidrug-resistant members of ESKAPE panel pathogens that is considered most threatening to human health, so how to withstand drug-resistant bacteria has always been a difficult problem in sepsis treatment. Recently, Liu *et al.* designed a microfluidic device equipped with a silicon capture surface with a highly periodic nanowired structure (SiNWs), which wrapped by bacteria-activated macrophage membrane (Fig. 2). Different from other methods *via* covalent bonding, physically adsorbed membrane coatings on SiNWs maintained fluidity to allow spatial rearrangement of adsorbed TLRs and other membrane components, enabling the wide spectrum identification and high capture efficiency of pathogens. The results showed that the SiNWs could fight against all ESKAPE member pathogens. This broad-spectrum capture efficiency demonstrated the potential application of the membrane-coated nanowired surfaces in a clinical scenario [69].

3.1.2. Clearing intracellular pathogens

During infection, microorganisms can be phagocytosed in vacuoles called phagosomes by macrophages and then be destroyed by the contents of lysosomes such as lysozyme. However, many bacteria such as *S. aureus* and *E. coli* have evolved immune escape mechanisms to avoid being killed [70,80]. In addition, the decline of host immunity will also limit the bactericidal ability, resulting in bacteria survival in the cytoplasm. Some surviving bacteria can even penetrate the cell membranes and leave host cells, resulting in secondary infections. Therefore, it is crucial to remove intracellular microorganisms.

S. aureus is generally an extracellular pathogen, but it is also capable of invading and surviving inside phagocytic cells [81]. Hussain *et al.* developed a cyclic 9-amino acid peptide CARGGLKSC (CARG) targeting intracellular pathogens by *in vivo* phage screening in a *S. aureus*-induced pneumonia model. CARG could specifically recognize *S. aureus*, along with biocompatibility, safety and high loading ability when combined with nanosystems. After coating CARG on silver NPs (AgNPs), *S. aureus* and CARG-AgNPs were absorbed by macrophages together *in vitro*, and the absorption rate was higher than that of AgNPs; Combining CARG with vancomycin-carrying porous silicon nanoparticles (pSiNPs), the survival rate was 100% for CARG-pSiNP-vancomycin (1 mg/kg), while a higher dose of free vancomycin (9 mg/kg) only achieved survival rate of 70%-80% in a mouse lung infection model [5,82].

However, the above nanoformulations only exerted specific effects on *S. aureus*, whereas in reality, septic hosts are often exposed to mixed bacterial infections, which is a great challenge in sepsis treatment [82]. Hou *et al.* designed and constructed the AMP-CatB mRNA encoding an antimicrobial peptide IB367 (AMP-IB367), a cathepsin B (CatB) and a CatB-sensitive linker (MACs), which delivered to macrophages by encapsulated in vitamin C liposomal nanoparticles (VCLNP). In the cytoplasm, the mRNA was translated to functional proteins and the proteins were further translocated into lysosomes. When phagosomes encapsulating bacteria fused with lysosomes, the ingested bacteria were exposed to prestored AMP-IB367 to be killed. The MAC-BMDMs (Bone marrow-derived macrophages) could significantly reduce the bacterial burden in blood compared to PBS or PBS-BMDMs. The therapeutic efficacy of MAC-BMDMs was also reflected in the survival rate (83%), which was much higher than that of the PBS group and PBS-BMDM group. This work opened up the possibility of developing nanoparticle-cell therapies for infectious diseases [5,70].

3.2. Inhibiting macrophages-mediated inflammatory response

The inflammatory response is an important means for hosts to handle with pathogens and endogenous danger signals. However, uncontrollable and persistent inflammation constitutes the central stage of sepsis progression, which is closely associated with the

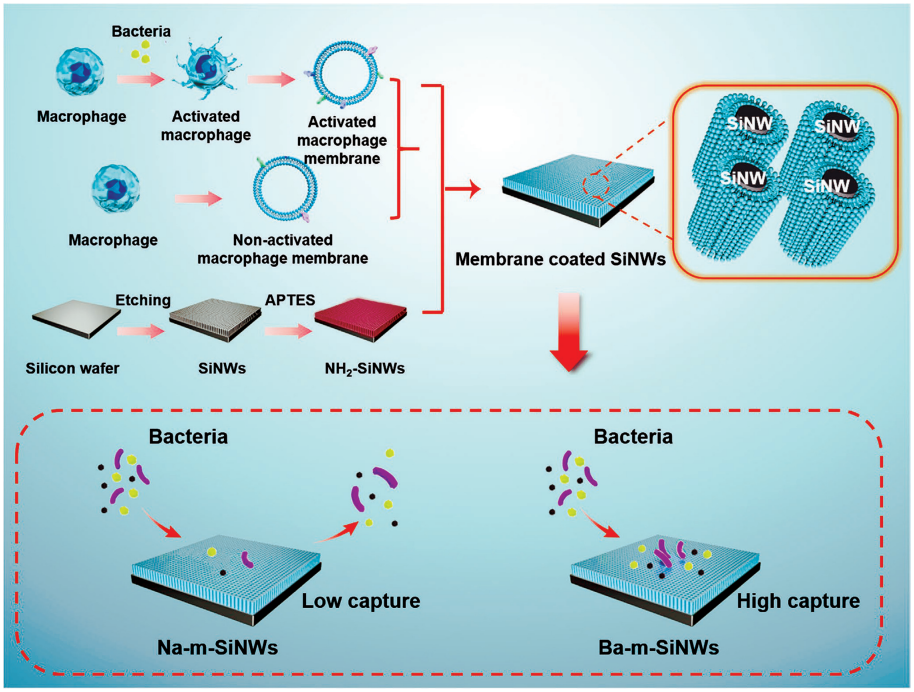

Fig. 2. Schematic presentation of the preparation of non-activated (Na) and bacterially activated (Ba) macrophage membranes on NH_2-Si nanowired surfaces. Nanowires (NWs) coated with Ba macrophage membranes (Ba-m-SiNWs) had higher pathogen capture than that of Na-m-SiNWs. Copied with permission [69]. Copyright 2021, John Wiley and Sons.

early peak of mortality. The balance between pro-inflammatory and anti-inflammatory is completely broken at this stage. Specifically, macrophages are continuously activated by PAMPs and DAMPs and inflammatory mediators like ROS accumulate in large quantities. Together with the mutual promotion of other pathological events such as complement activation, blood coagulation and endothelial dysfunction, all the factors lead to extensive tissue damage and significantly increase in the risk of multiple organ failure [83]. Therefore, effectively inhibiting the pro-inflammatory response of macrophages is a key measure to improve immune function and prognosis in septic patients.

3.2.1. Interfering the interaction of PAMPs/DAMPs and macrophages

There is a general acceptance that PAMPs/DAMPs binding to PRRs is the trigger that activates the continuous loop of excessive inflammation, hence limiting the initiation of this process can serve as a potential strategy to control the severity of inflammation and prevent its progression throughout the body. Upon PAMPs/DAMPs recognition by PRRs of macrophages, a series of inflammatory responses are triggered, including the overproduction of cytokines and bioactive mediators of inflammation and the induction of a vicious circle between inflammatory responses and RONS. This cycle starts with the generation of RONS, and the RONS subsequently leads to an increase in endothelial permeability, which makes PAMPs/DAMPs leak through the vascular wall to other non-infected parts of the body and damage normal cells and tissues. This results in a lot of DAMPs being generated, which in turn interact with the corresponding PRRs to set off a new round of inflammation [1]. Therefore, it has great therapeutic potential to use nano-drugs to inhibit the interaction between PAMPs/DAMPs and PRRs of macrophages, because it can reduce the activation of inflammatory pathway from the source. There are two main strategies to block inflammation before it starts, *i.e.*, (1) the use of PRRs antagonists and (2) clearance of inflammatory circulating PAMPs/DAMPs [1,84,85]. Although several nano-based antagonists of PRRs have been developed and proved effective in preclinical studies, several limitations have been noticed, such as potential damage to normal immune function and the occurrence of immunosuppression. Therefore, we focused our attention to the second strategy.

LPS, also called endotoxin, is an essential pathogenic ingredient of gram-negative bacteria cytoderm and the most potent PAMPs found in nature which is responsible for sepsis. Interactions between LPS and macrophages involve a membrane receptor TLR4, and cytosolic receptors caspase-11 in murine and the homologous caspases-4/-5 in human, initiating several signal cascades which could cause the release of pro-inflammatory mediators like cytokines, the assembly of inflammasome and pyroptosis [86]. Based on this mechanism, neutralization of LPS is supposed to treat gram-negative bacterial sepsis, and such method has been reviewed above (section 3.1.1). However, the aforementioned type of core-based biomimetic NPs always required intact membranes, which seems unnecessary from an economic perspective since the binding of toxins are dependent on specific proteins located in small regions of membrane. Besides, abundant polymeric cores may lead to some underlying health problems, while the limited reaction sites of natural membrane hinder the chemical modification to avoid NPs from rapid clearance in the body. To address this limitation, Jiang *et al.* combined PEGylated lipids with macrophage membranes to produce macrophage-mimetic hybrid liposome (M-Lipo) by co-incubating and extrusion [87]. M-Lipo possessed the advantages of both natural membrane and artificial lipid, which maintained the LPS-binding capacity and the navigation in the bloodstream for a long time. In addition, a greater number of NPs could be prepared by the same amount of macrophage membranes compared with traditional membrane-coated NPs. The outstanding therapeutic potency of M-Lipo was validated in LPS-challenged macrophages and D-galactosamine hydrochloride (800 mg/kg) and LPS (5 μg/kg) induced endotoxic shock mice [87].

Cell-free DNA (cfDNA) is another circulating danger signal, which derives from pathogens and damaged cells. It not only plays an important role in increasing the severity and duration of in-

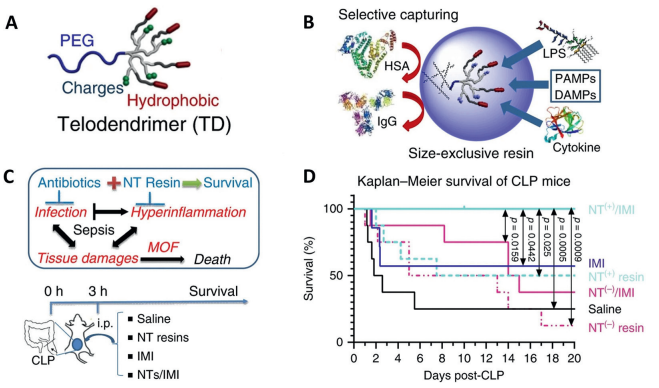

Fig. 3. (A) The schematic diagram of telodendrimer (TD) nanotrap (NT) composition. (B) The selective capturing PAMPs/DAMPs ability of NT. (C) The sepsis therapy by combining antibiotics and NT resin to control both infection and hyperinflammation. (D) The survival of CLP-induced septic mice with different treatments. Copied with permission [94]. Copyright 2020, Springer Nature.

flammation through TLR activation, but also has prognostic utility in patients with severe sepsis [88]. Therefore, scavenging cfDNA may modulate the overwhelming inflammatory response in sepsis progression. Inspired by this principle, Dawulieti *et al.* designed two kinds of polyethylenimine (PEI)-coated mesoporous silica NPs (MSN-PEI) with different average molecular weight (25 kDa and 800 Da) of PEI, named MSN-PEI 25K and MSN-PEI 800 respectively [89]. Since MSN-PEI 25K contained stronger charge density of PEI, it possessed higher cfDNA binding capacity and stronger capability to attenuate cfDNA-driven pro-inflammatory effects *in vitro*. Furthermore, repetitive intraperitoneal administration of MSN-PEI 25K resulted in a greater protection against cecal ligation and puncture (CLP)-induced multiple organ injury as compared to MSN-PEI 800. Similarly, Liu *et al.* used a simple one-pot process to synthesize cfDNA-scavenging NPs, which consisted of zeolitic imidazolate framework-8 (ZIF-8) grafted by PEI (600 Da, 1800 Da or 25 kDa) (PEI-*g*-ZIF) [90]. Such system exhibited excellent DNA binding affinity, long retention time, providing a promising strategy for treating sepsis.

Apart from LPS-binding biomimetic NPs and cfDNA-scavenging NPs mentioned above, lots of nanoformulations which sequestrate other different inflammation initiators (like α-hemolysin, aerolysin and histones) have also been reported [91–93]. For instance, a flexible linear-dendritic telodendrimer (TD) nanotrap (NT) was developed with the ability to selectively absorb broad range of PAMPs/DAMPs through multivalent electrostatic and hydrophobic interactions (Figs. 3A and B) [94]. The administration of NT could attenuate hyperinflammation and reduce tissue damages in the CLP-induced sepsis models. Notably, the combination of NT with traditional antibiotics yielded a 100% survival rate of the severe septic mice (Figs. 3C and D).

3.2.2. Scavenging RONS

During sepsis, the final killing and clearance of the engulfed microbes and necrotic debris by macrophages demands highly on the microbicidal activity driven by RONS and lysosomal enzymes. However, the excessive production of RONS has been alternatively considered as a major cause of multiorgan dysfunction. Specifically, RONS, including superoxide anion radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), hypochlorite anion (OCl_2^-), nitric oxide (NO) and peroxynitrite anion ($ONOO^-$), have a profound effect on sepsis progression which manifest in damaging cellular proteins, lipids, and nucleic acids, leading to cytopathic hypoxia, upregulating inflammatory cytokines production, and enhanced endothelial permeability [25,95]. RONS participate in the vicious circle with inflammation triggered by PAMPs/DAMPs [1]. Pathophysiological events associated with dysregulate RONS are responsible for multiple organ failure and poor sepsis outcomes. Therefore, the control of RONS level is also attractive for sepsis treatment. Small molecular antioxidants such as *N*-acetyl cysteine and vitamin C have been employed for the treatment of sepsis [96,97]. However, these conventional antioxidants had difficulty in reducing multiple RONS because of poor stability and low activity under physiological conditions, thus limiting the therapeutic efficacy. Nanoparticles have showed potential to scavenge abroad range of RONS [98,99].

Ceria NPs are known to function as potential antioxidants by shuttling between Ce^{3+} and Ce^{4+}, which partially attenuate pro-inflammatory responses in mice with sepsis. Nevertheless, the low rate of regeneration from Ce^{4+} to Ce^{3+} compromises their efficacy and enhances the toxicity because of the large doses used [100]. To overcome this issue, Soh *et al.* incorporated Zr^{4+} ions into ceria NPs to heighten Ce^{3+}/Ce^{4+} ratio and improve the reprocessing of Ce^{3+} [101]. They synthesized a series of ceria-zirconia NPs (CZ NPs; $Ce_xZr_{1-x}O_2$, where x = 0.2, 0.4, 0.7 and 1; termed 2CZ, 4CZ, 7CZ and 10CZ NPs, respectively). It was found that 7CZ NPs exerted the most powerful antioxidant effect among all the CZ NPs in aqueous media and can scavenge NO in LPS-stimulated macrophages whereas ceria NPs cannot. Furthermore, in both LPS-induced endotoxemia and CLP-induced bacteremia sepsis model, a single dose of 7CZ NPs markedly attenuated the vicious cycle of inflammation, reduced organ injury and significantly improved the survival rate, suggesting the capability of 7CZ NPs to manage complex sepsis.

Other metal-based nanoparticles consisting of transition metals such as manganese (Mn), cobalt (Co), wolfram (Wo) and molybdenum (Mo) have also been developed as RONS scavengers and exhibit outstanding therapeutic efficacy in sepsis [102–104]. For example, Yim *et al.* designed three kinds of ultrathin nanosheets (WS_2, $MoSe_2$, and WSe_2 nanosheets) functionalized with an amphiphilic diblock copolymer (PCL-*b*-PEG) [104]. In inflammatory BMDMs, these three nanosheets effectively obliterated mitochondrial and intracellular H_2O_2, $^{\bullet}OH$, $O_2^{\bullet-}$, and NO along with suppressing the excessive secretion of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-12p40). Specifically, WS_2 nanosheets exhibited the most excellent performance compared with the other two. Moreover, administration of WS_2 nanosheets greatly increased the survival rate of CLP-induced bacteremia mice up to 90%, accompanied by the decrease of pro-inflammatory cytokine levels in serum and bacterial burden in blood and peritoneal fluid. Additionally, the WS_2 nanosheets indicated good biocompatibility since they could be completely excreted from the murine organs 3 days after intravenous injection. Consequently, these nanosheets with robust RONS clearance capability might provide favorable protection of multiorgan dysfunction for sepsis management.

3.2.3. Reducing the amount of overactivated M1-like macrophages

M1-like macrophages is not only the host's primary effector cell for pathogen eradication, but also responsible for persistent excessive inflammation. However, the continuous activation of M1-like macrophages can lead to the release of a large number of inflammatory mediators, which damage normal tissues and promote the onset of cytokine storms. Moreover, the inflammatory response mediated by M1-like macrophages is interconnected and cross-regulated with the coagulation system and endothelial system, which results in multiple organ dysfunction and increases the risk of death [10]. Therefore, reducing the amount of overactivated M1-like macrophages is expected to curb hyperinflammation in the early phase for effective sepsis treatment.

Chen *et al.* constructed a novel intracellular modulatory nanoplatform which could specifically attack the inflammatory macrophages to stimulate and control their apoptosis [105]. The targeting capability of the proposed nanoplatform derived from

its cationic co-polypeptides (PLL-*b*-PLT) nanogels, which bound to the LPS-infected macrophages membranes *via* electrostatic interactions. Once the overactivated macrophages phagocytized the nanoplatform, the encapsulated TNF-related apoptosis-inducing ligands (TRAIL) were released and induced caspase-dependent apoptosis in a dose-dependent manner. Noteworthy, except LPS-induced sepsis model, another important sepsis-inducing nosocomial infection bacteria—*K. pneumoniae* was used to test the therapeutic feasibility of this drug. The results showed that intraperitoneal injections of TRAIL-encapsulated nanogels reduced LPS-induced lung and renal pathology and inflammatory cytokines production, prolonged survival and lowered bacterial numbers of mice infected with *K. pneumoniae* while preventing significant weight loss, indicating promoting M1-like macrophages apoptosis could be a promising therapy for sepsis caused by bacterial infections.

As previously discussed, macrophages are highly plastic and can polarize into different phenotypes under varying stimuli. During the overwhelming inflammation phase of sepsis, M1-like macrophages prevail and M2-like have a meagre quantity, resulting in the balance between pro- and anti-inflammation being tipped. Thus, targeted regulation of macrophage polarization theoretically can significantly inhibit inflammatory response. Capitalized on the fact, Taratummarat *et al.* reported spherical AuNPs that can promote the polarization of M1-like macrophages into M2-like macrophages and alter the expression of pro- and anti- inflammatory cytokines [106]. AuNPs induced low biomarker level of M1-like macrophages polarization and high biomarker level of M2-like. As such, the production of TNF-α, IL-1β and IL-6 were reduced but IL-10 was increased *in vitro*. In an *E. coli* and CLP surgery induced mouse sepsis model, it was demonstrated by flow-cytometric analysis that AuNPs treatment reduced the percentage of M1-like macrophages ($F4/80^+$ and $CD86^+$) and increased the percentage of M2-like ($F4/80^+$ and $CD206^+$). Moreover, septic mouse mortality, kidney and liver injury, and blood bacterial burdens were attenuated. Although these results supported the role of AuNPs manipulating macrophages polarization against bacterial sepsis, the mechanisms details of AuNPs-induced M2-like polarization lacked, hence further studies are needed on this topic.

3.2.4. Controlling the production of cytokines

The overproduction of typical pro-inflammatory cytokines (*i.e.*, IL-1β, IL-6 and TNF-α) secreted by activated macrophages induces the systemic inflammation response that is associated with the development of sepsis. IL-1β and TNF-α are essential for leukocyte recruitment by promoting endothelial adhesion and diapedesis, and the level of IL-6 is negatively correlated with the survival time of patients. The anti-inflammatory cytokines (*i.e.*, IL-10 and TGF-β) secreted by macrophages, on the other hand, are involved in angiogenesis, tissue remodeling and immune regulation [26,107,108]. Given pro-inflammatory cytokines mediated inflammation could be counterbalanced by anti-inflammatory responses, regulating cytokines would be a good choice for sepsis management.

To avoid the adverse effects on immune and high cost caused by cytokine monoclonal antibodies, He *et al.* designed TNF-α siRNA-loaded polypeptide hybrid NPs (HNPs) to inhibit TNF-α (Fig. 4A) [109]. In LPS/D-GalN-induced hepatic sepsis, the administration of HNPs could knockdown TNF-α to significantly alleviate pro-inflammatory responses *via* efficient delivery of TNF-α siRNA into macrophage. Because TNF-α contributes to the induction of IL-1β and IL-6 during the LPS-triggered inflammatory cascade, HNPs-mediated TNF-α depletion also reduced serum IL-1β and IL-6 levels by 76% and 81%. In addition, the biocompatibility such as cytotoxicity, hemolysis, acute toxicity and immune toxicity of HNPs were evaluated both *in vitro* and *in vivo*, and all results indicated the low toxicity for biomedical applications.

Several phytomedicines, such as ginsenoside derivatives and hesperidin, have been demonstrated with inherent anti-inflammatory activity, while their *in vivo* applications have been limited by low aqueous solubility and poor bioavailability. Recently, with the aid of nanotechnology, their pharmacokinetic limitations have been addressed, showing benefits for reducing inflammatory cytokines *in vivo* [110,111]. For example, curcumin (Cur), a natural biphenolic compound isolated from *Curcuma longa* L., has poor solubility and is prone to degradation at alkaline pH. Wang *et al.* used solid lipid NPs (SLNs) as the drug carrier to prepare Cur-loaded SLNs (Cur-SLNs) by emulsification and low-temperature solidification (Fig. 4B) [13]. The therapeutic efficacy of Cur-SLNs and its underlying mechanisms were studied by using a firefly luciferase transgenic mouse. Compared to free Cur, Cur-SLNs notably reduced levels of IL-1β expression especially at 3 h post LPS injection. Also, Cur-SLNs could remarkably decrease serum IL-6, TNF-α and IL-1β but increase anti-inflammatory cytokine IL-10. The sepsis-induced damage to multiple organs (kidney, liver, and heart) was also alleviated. Western blot analysis illustrated that anti-inflammatory mechanisms can be contributed to IκBα degradation and suppression of NF-κB activation. This work suggests that the appropriate nanomaterials might fill the shortage of free agents to improve the therapeutic efficacy.

3.2.5. Inducing autophagy in macrophages

Macrophage autophagy is activated in sepsis and plays a role in inflammation and immunity. Autophagy is the process in which cytoplasmic substances or pathogens are phagocytized by a double-membrane-bound vesicles, called autophagosomes, and then fused with lysosomes to degrade and recycle the sequestered substrates. It has been demonstrated that autophagy could negatively regulate the inflammatory response and have a protective effect on tissue and organ damage, including brain injury and cardiac dysfunction, in mouse sepsis model [112,113]. In addition, the level of autophagy is related to the classification, and death of macrophages, the inflammasome activation and cytokines induction [46,114-116]. Thus, the specific regulation of macrophage autophagy is a good direction for sepsis therapy.

Xu *et al.* have recently reported superparamagnetic iron oxide NPs (SPIONs) of γ-Fe_2O_3 NPs could induce autophagy in macrophages, which inhibited the systemic inflammatory response in LPS-induced sepsis model and enhanced the expression of anti-inflammatory factor IL-10 [117]. Concretely, SPIONs attenuated the level of serum IL-6 and TNF-α while promoted IL-10, and relieved liver injury as shown by decreased liver damage signs. Such effects were related to macrophage autophagy triggered by SPIONs treatment in septic mice. Of note, both *in vitro* and *in vivo* illustrated that SPIONs-induced autophagy was attributed to the activation of Cav1-Notch1/HES1 signaling.

3.2.6. Inhibiting macrophages pyroptosis

Pyroptosis, a type of violent and programmed lysis of inflammatory cells death, is usually accompanied by cell rupture and the release of pro-inflammatory factors. The main factors that induce pyroptosis are PAMPs, especially LPS. As previously mentioned, in addition to interacting with TLR4 extracellularly, LPS can also enter the cytoplasm of macrophages *via* OMVs, HMGB1 and LPS binding protein. After internalization, LPS binds to human caspases-4/5 and caspase-11 of mouse. The caspase-4/5/11 then activates several pyroptosis effectors including pannexin-1, NLRP3 inflammasome, and gasdermin D (GSDMD) [86,118]. Specifically, P2$\times$7 activation results in ATP and potassium efflux, both of which are cytotoxic. Once NLRP3 inflammasome is assembled, it cleaves pro-caspase-1 to generate caspase-1. Caspase-1 then cleaves pro-IL-1β and pro-IL-18 into their mature form: IL-1β, IL-18. As for GSDMD, it is cleaved to release the N-terminal active domain (GSDMD-NT), and

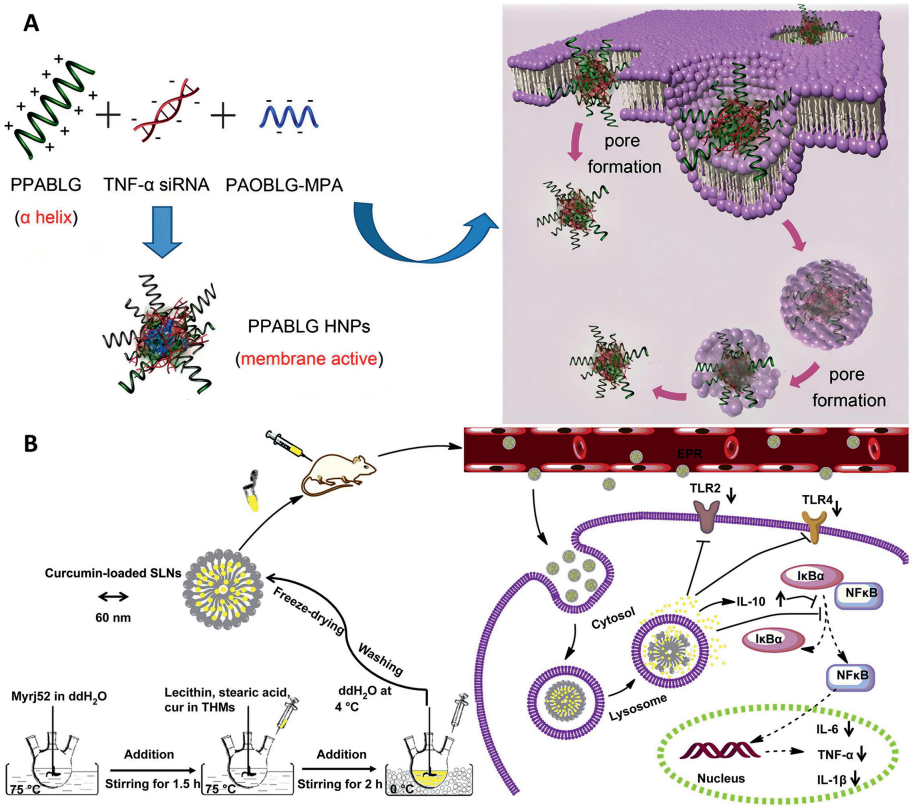

Fig. 4. (A) The illustration of HNPs for the efficient delivery of TNF-α siRNA, indicating the effective cellular internalization and endosomal escape. Copied with permission [109]. Copyright 2016, American Chemical Society. (B) The schematic diagram of Cur-SLNs preparation and their mechanisms of treating the LPS-induced sepsis. Copied with permission [13]. Copyright 2015, Elsevier.

GSDMD-NT then accumulates in the cell membrane and oligomerizes to form pores, leading to cell rupture and enabling the release of a large number of pro-inflammatory factors [86,119]. There is no doubt that pyroptosis in macrophages increases the level of inflammation during sepsis. Excessive pyroptosis may promote the occurrence of inflammatory storm and immune dysregulation, leading to immune cells depletion, multi-organ failure or septic shock, which is life-threatening. Hence, the inhibition of pyroptosis is also a potent target for sepsis.

Disulfiram (DSF), an old drug for anti-alcoholism, was found to suppress macrophage pyroptosis by inhibiting NLRP3 inflammasome activation and blocking GSDMD-induced pore formation [120]. Whereas, DSF is almost insoluble in water and owns a very short half-life. Ou *et al.* designed a nano-biomimetic DSF delivery system based on lactoferrin (DSF-LF NPs) [121], with surface decoration of soy lecithin and DSPE-mPEG$_{2000}$ to prolong systemic circulation of DSF. DSF-LF NPs particle size was around 160 nm with the ζ potential about +10 mV. The DSF showed a sustained-release pattern, and thus exerted a slightly weaker activity to inhibit pyroptosis and IL-1β release as compared to free DSF *in vitro*. However, a strong efficacy was observed *in vivo*, with 100% survival for all LPS-induced septic mice.

3.3. Restoring the immune functions of macrophages

Patients who survive excessive inflammation (approximately 60%) tend to experience compensatory immunosuppression at a later stage, a process that has gained attention following repeated failures of anti-inflammatory approaches in clinical sepsis treatment [7,122]. Immunosuppression caused by sepsis can make patients suffer from repeated and persistent secondary infections, leading to multiple organ dysfunction and becoming a high-risk factor of death. Sepsis related immunosuppression is characterized by impaired innate and adaptive immune responses, mainly including uncontrolled apoptosis, inactivation and dysfunction of various immune cells, among which damaged macrophages play a major role in antibacterial defense defects [66,123]. Apoptosis inducing factors, which are produced and released in cytokine storm, including TNF-α and HMGB1, can induce and promote apoptosis of macrophages. For some macrophages that escape apoptosis, their polarization direction can be changed under the stimulation of certain cytokines such as IL-13 and IL-4 [10]. The transformation of macrophage phenotype to M2-like results in lots of anti-inflammatory mediators such as IL-10 and TGF-β being secreted [9,10]. Meanwhile, related inflammatory pathways such as TLR4 receptor and NF-κB become inactivated, and pro-inflammatory factors are reduced, leading to host immune paralysis and accelerating the apoptosis of T cells. In addition, the function of macrophages as antigen-presenting cells is also inhibited, and epigenetic modification changes lead to decreased MHC expression on the membrane surface and worse presentation ability, resulting in insufficient elimination of pathogens [123]. Therefore, restoring the immune function of macrophages in the suppression stage is of great significance for the sepsis treatment [7].

3.3.1. Inhibiting apoptosis of macrophages

Apoptosis is one of the main causes of immunosuppression, as a form of programmed cell death [47,124]. Preventing the apoptosis of immune cells, especially macrophages, is an important way to improve immune function of patients with sepsis. Apoptosis of immune cells mainly includes mitochondria-mediated endogenous way and death receptor-mediated exogenous way, both of which

occur in sepsis [7,124]. The mitochondria-triggered intrinsic apoptosis pathway is achieved by altering the permeability of the mitochondrial outer membrane, which Bcl-2 family proteins such as Bcl-2, Bax, and Bid can control by regulating the membrane potential [47]. It has been reported that apoptosis is one of the pathways in oxidative stress-induced cell death [125]. For example, *t*-BHP, one of the most commonly used initiator of free radical reactions, can induce macrophages apoptosis by activating the mitochondria-mediated way [125,126]. Han *et al.* constructed a facile and efficient chitosan-hydroxyethyl NPs encapsulated by trolox as a model antioxidant (trolox-CS NPs) to resist *t*-BHP-induced apoptosis. They modified chitosan with ethylene oxide to form self-assembled NPs in an aqueous environment, without using any toxic solvent [125]. Delivery of trolox through CS nanosystems improved its stability and cellular absorption, increasing delivery efficiency. The results showed that compared with free trolox, trolox-CS NPs exhibited better inhibitory effect on apoptosis of macrophages induced by *t*-BHP. The free trolox pretreated cells demonstrated 19.98% of cell in apoptosis stage and 65.80% of cell in alive stage, while trolox-CS NPs treated cells showed 12.54% in apoptosis and 79.55% in alive stage. Trolox-CS NPs showed significantly better efficacy than free trolox to up-regulate the anti-apoptotic protein Bcl-2 and down-regulate the pro-apoptotic protein Bax. These results suggested that trolox-CS NPs could effectively inhibit *t*-BHP-induced apoptosis through mitochondria-involved apoptotic pathway [125].

The extrinsic pathway of apoptosis is achieved by the specific binding of death receptors to death ligands, including Fas-FasL, TNFR1-TNF, TRAILR1-TRAIL and PD-1-PD-L1 [47,123]. Among which, PD-1-PD-L1 plays a crucial role in immunosuppression and becomes a hot target for sepsis immunotherapy [127]. Although using anti-PD-1/PD-L1 antibodies has shown good prospects in both animal experiments and clinical trials reflected by increased host resistance to infection and improved survival rate [128], it may cause immune-related adverse events (irAEs) due to persistent blockade of the PD-1 pathway, leading to excessive activation of the immune response [122]. Peptide-based therapy is an alternative drug modality that can realize a rapid pharmacokinetic profile, reducing the incidence of precipitating irAEs. Meanwhile, it can also provide more formulation and delivery options with better tissue penetration to improve treatment efficiency [129,130]. Phares *et al.* reported that peptide-based PD-1 antagonist LD01 could improve the phagocytosis activity of macrophages, reduce bacterial burden, and improve the survival rate significantly compared with α-PD-1 monoclonal antibody [122]. Unfortunately, we have found that there are very few studies on the treatment of sepsis based on death receptor-ligand nano-formulations. We expect it to be a promising option for sepsis immunotherapy in the future.

3.3.2. Immune stimulation to macrophages

In the immunosuppressive stage of sepsis, in addition to apoptosis, macrophages also show immune dysfunction, which is characterized by downregulation of pro-inflammatory factors, impaired antigen presentation ability, and endotoxin tolerance [131]. The inhibition of immune functions results in a paralyzed immune system that is unable to eradicate pathogens, leaving the host highly vulnerable to secondary infection [131]. At present, it has been confirmed in clinical trials that immunostimulatory drugs can enhance the clearance of pathogenic bacteria, which is beneficial to the recovery of immune function. Therefore, providing immune stimulation to immunocompromised macrophages will bring a new treatment option to patients with sepsis [132,133].

We have mentioned the interactions of PAMPs and PRRs as the key to initiating host inflammatory response and activating immune defense [132]. Among them, TLR-4 is a very important PRR, which can recruit macrophages to the infection site to promote the rapid clearance of pathogens after recognizing PAMPs [134]. However, during immunosuppression in sepsis, TLR4 signaling can be inactivated in the host, that aggravates the progression of secondary infection [135]. Some researchers found that the anti-infection ability of patients was improved after pretreatment with TLR4-related immunomodulators [132]. Monophosphoryl lipid A (MPLA) is a synthetic TLR agonists used in clinic, which can enhance the bactericidal effect of macrophages *in vivo* with no toxicity. However, it is a hydrophobic molecule [136,137]. Zhao *et al.* loaded MPLA into PLGA nanoparticles, which improved its solubility. In the *E. coli*-induced sepsis, these nanoparticles could significantly improve host survival by upregulating the percentage of macrophages, promoting the levels of various functional cytokines, and accelerating bacterial clearance. Surprisingly, surviving mice pretreated with MPLA@PLGA NPs after the first septic infection could establish acquired immunity to against the second infection [132].

In addition to TLRs, NLRs such as NOD1 and NOD2 also play key roles in identifying pathogens and resisting viral or parasitic infections [138,139]. It is found that multiple activation of TLR and NOD signaling could synergistically improve the body's defense against infection [42,140]. Zhao *et al.* developed a type of two-phase releasing immune-stimulating composite by mixing alginate (ALG) and NOD2 agonist muramyl dipeptide (MDP) with MPLA@PLGA NPs (P-M) [140]. In the *E. coli*-induced sepsis mice model, MDP + P-M@ALG could be rapidly gelated *in vivo* post subcutaneous injection owing to the binding of ALG with endogenous Ca^{2+}, and the small molecule MDP was rapidly released, which provided protection against infection by activating innate immune cells. Meanwhile, MPLA in the P-M NPs formulation showed largely sustained release profile to allow continuous modulation of the immune system and long-term broad protections against various infections (Fig. 5). The results showed that mice treated with the two-phase releasing nanoformulation had a survival advantage over the single agonist group [140]. This multi-agonist nanoformulation has offered a very promising treatment option for sepsis patients in the immunosuppressive stage.

After being invaded by pathogens, the innate immune system can non-specifically defend against infection by building immune memory, which is called trained immunity [141]. The induction and maintenance of trained immunity is mediated through epigenetic reprogramming, among which β-glucan is a typical agonist. Studies have shown that β-glucan can induce trained immunity and restore epigenetic, transcriptional, and functional programs in monocytes during LPS tolerance [141]. Pan *et al.* synthesized novel NPs (BSNPs) by coupling β-glucan and Ferumoxytol, a superparamagnetic iron oxide (SPIO) with low cytotoxicity, at a mass ratio of 3:20, which could prevent sepsis caused by *E. coli* and CLP and protect mice from secondary infection [56]. BSNPs trained macrophages into a more active state, enhanced phagocytosis and digestion of bacteria, and promoted macrophages to produce pro-inflammatory factors, killing bacteria in a mTOR dependent manner. In conclusion, this research has provided a basis for the application of nanomaterials in training septic patients immunity [56].

3.3.3. Repolarizing M2-like macrophages

Immunosuppression in sepsis is associated with the unbalanced process of substantially reduced pro-inflammatory M1-like macrophages and substantially increased anti-inflammatory M2-like macrophages. The polarization of macrophages is a complex procedure influenced by multiple signaling molecules, transcription factors, epigenetic modifications, and metabolic reprogramming [142]. Based on the fact that inflammation can be suppressed by inducing macrophages polarize to M2-like, we can speculate that regulating the polarization of macrophages to M1-like during the period of immunosuppression may be an effective method to restore immunity. It has been shown that blocking the dif-

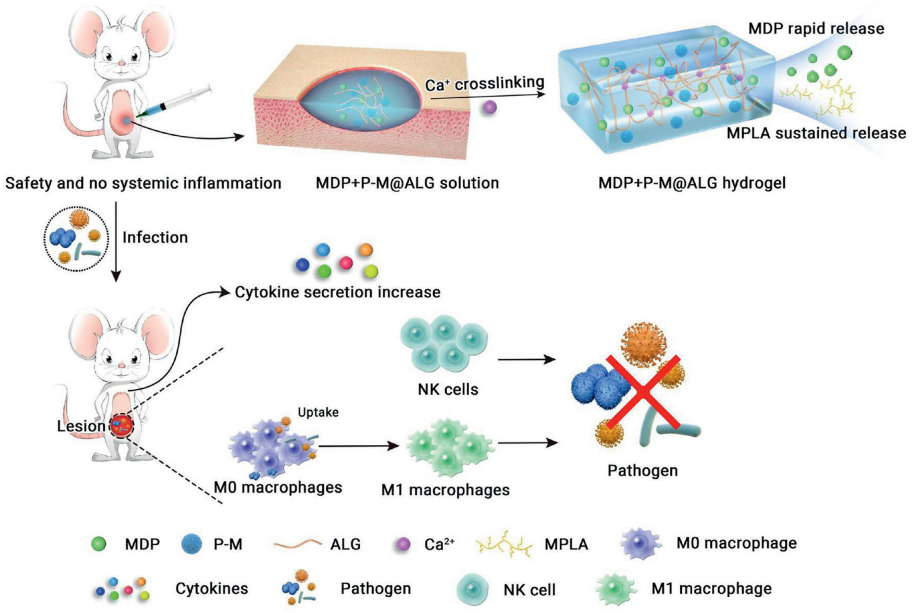

Fig. 5. The scheme illustration of the mechanism of the two-phase releasing immune-stimulating composite (MDP + P-M@ALG) to treat sepsis. Copied with permission [140]. Copyright 2021, Elsevier.

ferentiation signaling of M2-like macrophages, or making M2-like macrophages exposed to M1-like signaling, can induce repolarization of M2-like macrophages.

Colony stimulating factor (CSF-1) can promote the differentiation and proliferation of M2-like macrophages by binding to the receptor CSF-1R. Some researchers found that the inhibition of CSF-1R realized the repolarization of M2-like macrophages to M1-like [143–147]. For instance, Leber *et al.* constructed an α-mannosyl functionalized cationic nanohydrogel particle (ManNP) loaded with anti-CSF-1R siRNA *via* amphiphilic precursor block copolymers bearing one single α-mannosyl moiety at their chain end. It not only protected sensitive substances from being degraded by nucleases to increase cellular absorption, but also realized M2-like macrophages specific delivery *via* surface modification mannose to bind highly expressed $CD206^+$ on M2-like macrophages, resulting in reduced non-specific uptake of non-targeted phenotypes. The results showed that ManNPs loaded with anti-CSF-1R siRNA could significantly knockdown CSF-1R transcription in M2-like macrophages, but had no significant effect on M1-like. In contrast, anti-CSF-1R-loaded NonNPs (without α-mannose functionalization on the NPs surface) did not produce any significant inhibition of CSF-1R transcription in macrophages of either phenotype, suggesting that anti-CSF-1R siRNA ManNPs could modulate the polarizing function of M2-like macrophages by specific cell-targeting way [143].

IFN-γ is a major inducer of polarization to M1-like macrophages and can promote the increase of pro-inflammatory cytokines, the production of ROS and the activation of inducible nitric oxide synthase (NOS2) in macrophages by cooperating with LPS [142,148,149]. IFN-γ therapy has been proven beneficial for reversing this immunosuppressive stage of macrophages during sepsis [47]. Castro *et al.* used chitosan/poly(γ-glutamic acid) NPs, which have shown an immunostimulatory effect, as carriers for IFN-γ to improve its half-life and stability, as well as regulating the repolarization of M2-like macrophages towards to M1-like. The results showed that M2-like macrophages treated with IFN-γ-NPs increased the secretion of pro-inflammatory cytokines such as IL-6, IL-12p40 and TNF-α. Besides the direct effects on macrophage polarization, IFN-γ-NPs also induced immune stimulation profiles on other cell types, enhancing the host's immune capacity [150,151]. Although many methods based on nanoformulations to induce repolarization of M2-like macrophages have achieved success, such studies mainly focused on immunotherapy in cancer, and the efficacy on sepsis still remains to be explored. It is hoped that similarities between sepsis-induced immunosuppression and immunodeficiency in cancer may inspire new therapeutic strategies to stimulate immune function of septic patients.

3.4. Multifunctional nanomedicines

As described above, the uncontrollable inflammation caused by macrophages in sepsis provides the most diverse targets for disease intervention, and nano-drugs targeting any of them have made noticeable improvement. Furthermore, it would be not surprising that therapeutic benefits could be increased distinctly when drugs interfere with more than one pathological process. The most common strategy for the preparation of multifunctional nano-drugs to treat sepsis is using nanomaterials to delivery multiple agents with different action mechanisms. For instance, Dormont *et al.* proposed a novel squalene (SQ)-based nanoformulation that effectively deliverd two anti-inflammatory agents of adenosine (Ad) and tocopherol (VitE), termed as SQAd/VitE NPs [152], which succeeded in inhibiting pro-inflammatory cytokines production and scavenging RONS simultaneously.

The drug loading of Ad and VitE in final multidrug NPs was as high as 18.6% and 50%, respectively. *In vivo* biodistribution studies confirmed that SQAd/VitE NPs not only prolonged circulation time of the encapsulated two agents, but also directed them to inflamed sites. Compared to free-drug or single-drug controls, SQAd/VitE NPs was significantly efficient at suppressing intracellular H_2O_2 generation and nitrite accumulation. Meanwhile, the concentration of TNF-α in supernatant of LPS-challenged macrophages treated with the multidrug NPs was lowest. Different from the time point of drugs injection in most anti-sepsis efficacy tests, the NPs were administered 30 min after LPS injection, which could better fit with the clinical conditions. The results in septic mice showed the same tendency with that in cells, revealing that SQAd/VitE NPs made profound impact on some classical inflammatory cytokines

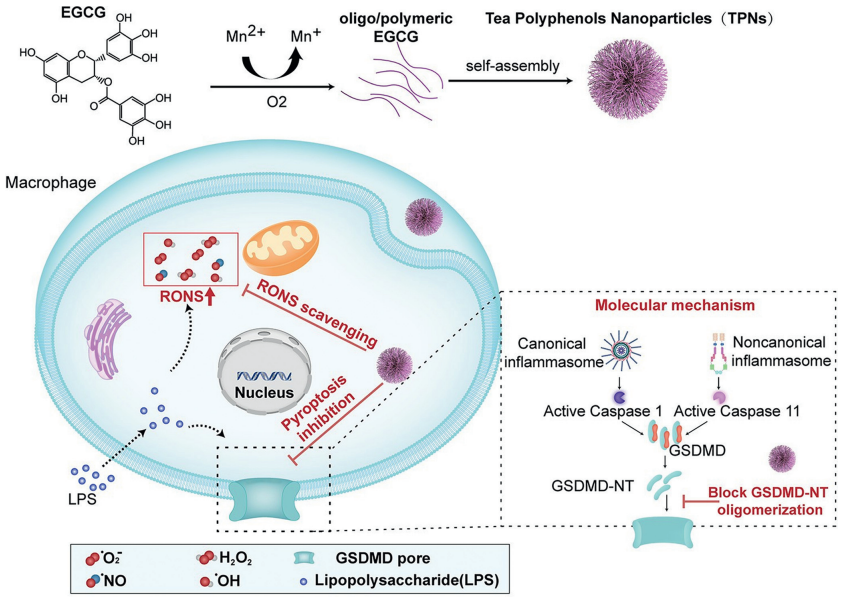

Fig. 6. The schematic illustration of TPNs preparation and their mechanisms of sepsis treatment through scavenging RONS and inhibiting pyroptosis. Copied with permission [153]. Copyright 2022, American Chemical Society.

and malondialdehyde, which is an indicator of oxidative stress *in vivo*. Moreover, it was observed that a significant drop in blood pressure related to free drugs Ad/VitE, but employing nanoformulation helped to minimize this severe side effect.

However, the translation of multidrug NPs from lab to clinic is usually faced with some key problems, such as the complex preparation process hindering scale-up production, the interaction of the loaded cargoes. Recently, we reported that a nanomaterial has realized the excellent treatment of sepsis by relying on its intrinsic pharmacological activities without carrying any drugs [153], showing superior advantages and prospects for application (Fig. 6). In this study, the multifunctional NPs, named tea polyphenols NPs (TPNs), were generated by the polymerization of epigallocatechin-3-gallate (EGCG) with Mn^{2+} catalysis under a rather mild condition. The Mn content of TPNs was quantified only 0.8% by ICP-MS analysis, verifying EGCG polymerization dominated TPNs preparation rather than Mn^{2+} coordinating with polyphenols, thus greatly reducing the potential toxicity induced by transition metals. The array of experiments in solution, cells and animals have systematically confirmed that TPNs exerted robust anti-sepsis effects, which was attributed to their capacity for clearing extensive RONS and blocking macrophages pyroptosis. The former activity originated from the polyphenols-derived structure and the latter was due to the inhibition of oligomerization of GSDMD-NT. In summary, the drug-free TPNs present a promising candidate for sepsis management and deserve more in-depth investigations.

4. Conclusion

Sepsis is a disease with complicated pathophysiology and high mortality. However, there is no effective and specific drugs approved for sepsis treatment. In 2017, sepsis has been regarded by the WHO as a global health priority, which aims to reduce its negative impact on individuals and social economy [154]. It should be noted that the outbreak and pandemic of COVID-19 also contributes to sepsis occurrence, leading to another heavy burden on healthcare systems worldwide. Increasing evidences have demonstrated that macrophages are involved in the whole progression of sepsis, which attracted great attention to research the therapeutic potential of nano-drugs targeting macrophages. In this review, we give a general description of sepsis and highlight the multiple roles of macrophages in the disease. Then, a comprehensive review was made on nanoparticles to address each stage of sepsis by regulating macrophage's function. These researches on NPs-based therapies have yielded favorable results of sepsis treatment, profiting by improved bioavailability, targeted drug delivery, good biocompatibility and so on, of which the mechanisms cover anti-infection, anti-inflammation, and immunomodulation.

Despite the recent advancements mentioned above, there exists several obvious limitations and challenges in terms of clinical translation of these drugs. First, most raw materials used in NPs are not certified as pharmaceutical excipients and lack of critical data on the long-term safety. Second, only few investigations try to target macrophages actively through modifying ligands on NPs. Indeed, passive targeting alone may reduce efficacy and damage other cells. Third, in cell-based assays, researchers generally select immortalized mouse macrophages, which is likely to be less reliable than primary cell culture or human macrophages. Fourth, the clinical correlation of animal models is weak, due to the majority of septic patients are aged and have underlying diseases while young and healthy mice are employed in the laboratory. Fifth, researches to explore multifunctional preparations with the ability of address different hazards of sepsis remain scare. Last but not least, to date, numerous NPs are centered on dealing with the infection and overwhelming inflammation in sepsis while rarely involved in immune suppression. To conclude, there is still a wide gap between preclinical findings with clinical implementation for developing macrophage-targeting NPs to treat sepsis. But fortunately, more emerging examples of commercially available nano-drugs of other diseases prompt an optimistic foreground among investigators devoted to seeking robust nanotherapeutics for sepsis remedy. It is hoped that this review can serve as a reference for future optimization of drug design.

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.

Acknowledgments

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