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# Nanomedicine approaches to reduce cytokine storms in severe infections

Mohd Ahmar Rauf<sup>a,b</sup>, Maryam Nisar<sup>c</sup>, Hosam Abdelhady<sup>d</sup>, Navnath Gavande<sup>b</sup>,  
Arun K. Iyer<sup>b,\*</sup>

<sup>a</sup> Department of Surgery, Miller School of Medicine, Sylvester Comprehensive Cancer Centre, University of Miami, FL 33136, USA

<sup>b</sup> Department of Pharmaceutical Sciences, Wayne State University, Detroit, MI 48201, USA

<sup>c</sup> Department of Biochemistry, Aligarh Muslim University, Aligarh, UP 202002, India

<sup>d</sup> Department of Physiology & Pharmacology, College of Osteopathic Medicine, Sam Houston State University, Conroe, TX 77341, USA

During a cytokine storm, dysregulated proinflammatory cytokines are produced in excess. Cytokine storms occur in multiple infectious diseases, including Coronavirus 2019 (COVID-19). Thus, eliminating cytokine storms to enhance patient outcomes is crucial. Given the numerous cytokines involved, individual therapies might have little effect. Traditional cytokines might be less effective than medicines that target malfunctioning macrophages. Nanomedicine-based therapeutics reduce cytokine production in animal models of proinflammatory illnesses. The unique physicochemical features and controlled nano–bio interactions of nanotechnology show promise in healthcare and could be used to treat several stages of this virus-induced sickness, including cytokine storm mortality. Macrophage-oriented nanomedicines can minimize cytokine storms and associated harmful effects, enhancing patient outcomes. Here, we also discuss engineering possibilities for enhancing macrophage efficacy with nanodrug carriers.

**Keywords:** Cytokine storm; COVID-19; Nanomedicine; Infectious diseases; ARDS; SARS

## Introduction

According to the WHO, infectious diseases are a primary cause of death worldwide, especially in low-income countries. Small proteins (~40 kDa) called cytokines are produced and released to signal cell infections. The term ‘cytokine storm’ was used in 1993 for the first time to describe graft-versus-host disease (GVHD) resulting from an activation cascade involving auto-amplifying cytokines.<sup>1–2</sup> In 2003, the cytokine storm was linked to influenza reactions,<sup>3</sup> and various diseases caused by viruses, bacteria, and fungi. Excessive cytokine production is a hallmark of COVID-19, which can aggravate lung disease and cause organ dysfunction and failure.<sup>4</sup> Cytokine secretion is an intrinsic component of the activation and regulation of innate and adaptive immune responses stimulated by pathogen exposure.

Therefore, treating the cytokine storm would effectively avoid deterioration in patients with COVID-19 infections and save lives.<sup>4–5</sup> Strategies include targeting macrophages and associated monocyte-linear immune cells, which dwell in most bodily tissues and regulate cytokine-mediated inflammatory reactions and inborn and adaptive immune reactions. Monocytes produce chemicals that encourage leukocyte infiltration and activity. Macrophage proinflammatory reactions to injury or infection are self-limiting, facilitating pathogen clearance while minimizing tissue damage and remodeling. Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes enhanced proinflammatory responses that add to the severity of the disease. Cytokine profiles in severe cases of COVID-19 are comparable to those in macrophage activation syndrome

\* Corresponding author. Iyer, A.K. ([arun.iyer@wayne.edu](mailto:arun.iyer@wayne.edu))

(MALS), indicating macrophage dysfunction.<sup>6</sup> COVID-19 also affects the lungs, decreasing alveolar macrophages, increasing inflammatory macrophage infiltration, and increasing interleukin (IL)-6 levels, which create monocytes/macrophages.<sup>2,6</sup> Thus, macrophage dysfunction is a possible therapeutic target for cytokine storm syndrome. Currently available small-molecule inhibitors cannot target specific cell types or cell lines required to achieve therapeutic medication concentrations without creating significant off-target effects.

The physicochemical features of nanomaterials and controlled nano-bio interactions make nanotechnology relevant in healthcare. COVID-19 could be treated by using upstream and downstream methods. Nanotechnology could also offer treatments at different stages of this viral disease, including a strategy to combat a late-stage cytokine storm. Nanomedicine approaches that use nanoparticles (NPs) to identify cell-specific components on target cells help reduce cytokine storms caused by overactive immune responses.<sup>7-8</sup> In this review, we highlight the clinical and pathological features of the coronavirus-related cytokine storm. Possible medication efficacy, safety, and molecular processes are discussed. We also detail the biology of cytokine storms in severe infections, the role of macrophage malfunction in this process, and how to improve patient outcomes with macrophage-based nanomedicine therapy.

### Cytokine storms in infectious disease

#### The pathology of cytokine storms

Numerous cytokines generated during proinflammatory immune responses can activate distinct populations of leukocytes and recruit them via chemotaxis in response to a concentration gradient.<sup>2,9-10</sup> Many proinflammatory reactions involve cytokines, including ILs, chemokines, and interferons (IFNs), with the TNF $\alpha$ , IL6, IL8, and IL1 families particularly well studied. Cytokines have a crucial role in inflammatory processes by promoting the detection of pathogens, the recruitment and removal of threats, and the homeostasis of immune cells.<sup>9,11</sup> TNF $\alpha$  and IL1 $\beta$  can promote vasodilation and vascular permeation to increase the infiltration of leukocytes. At the same time, IL6 can promote the expression of complementary proteins that have a crucial role in innate immune reactions.<sup>10,12-15</sup> Studying individual cytokines and their receptors can be difficult even though these have consequences for therapeutic efforts because several chemokine receptors have been shown to bind multiple chemokine receptors, resulting in significant redundancy in the chemokine network.<sup>9</sup>

Nevertheless, cytokine storms are usually only triggered by a few pathogens, such as Gram-negative bacteria and SARS-CoV-2.<sup>16-17</sup> Cytokine initiation and progression is a complex process involving many factors and immune cells. For instance, SARS-CoV cause rapid virus replication, which results in delayed IFN release during the early stages of infection. As a result, TNF $\alpha$  stimulates the release of cytokines, including IL6, IL1 $\beta$ , and IL6, into the lungs, accompanied by macrophages.<sup>18-19</sup> When activated by IFN signaling, these macrophages secrete chemokines (e.g., CCL2 and CCL3) that promote the local accumulation of other proinflammatory immune cells, such as neutrophils, monocytes, and dendritic cells, all of which can produce additional cytokines

and reactive oxygen species (ROS), thereby increasing the severity of the infection (Fig. 1).

SARS-CoV2 is highly contagious. Advanced age (>60 years), smoking, poor diet, and past immunological reactions, such as obesity, diabetes, hypertension, cardiovascular disease, and cancer can result in significant symptoms.<sup>12,20-22</sup> Although cytokine storms, disease pathologies, and mortality have established links, no therapeutic strategies have been developed to mitigate the harmful effects of cytokine storms.

#### Cytokine storm and its consequences in severe infections

COVID-19 models cytokine storm toxicity and mortality. As-yet unknown processes cause the pathology of COVID-19. Multiple clinical experiments have shown a considerable increase in the blood circulation of proinflammatory cytokines, including IL1, IL6, IL8, IL21, TNF-5-007, and MCP1, in many patients with severe disease.<sup>2,17,23</sup> Cytokine storms induce higher mortality in patients with severe COVID-19 by causing respiratory distress syndrome (RDS), shock, and organ damage and failure. Acute (A)RDS is the leading cause of death in patients with SARS-CoV-2, SARS-CoV, or Middle East respiratory syndrome (MERS)-CoV.

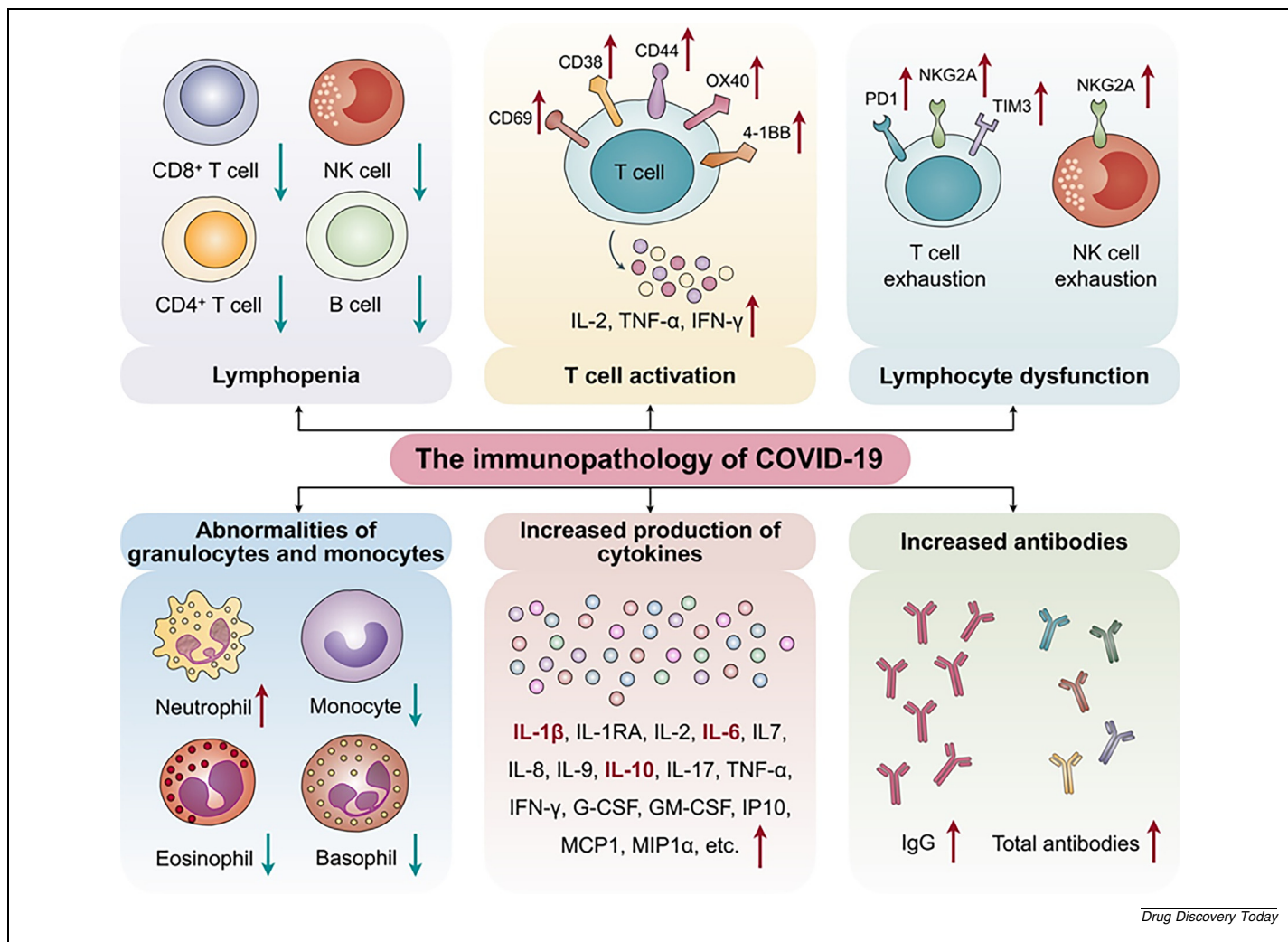
In septic diseases, pneumonia, and other infections, cytokine storms induce morbidity and mortality (Fig. 2). ARDS is an acute inflammatory reaction that damages lung endothelial and epithelial barriers. Cytokines trigger and increase inflammation during ARDS. In extreme cases, cytokine storms can be used to overcome cytokines by using immunosuppressive drugs (methylprednisolone, hydroxychloroquine, chloroquine, and leflunomide), proinflammatory cytokine inhibitors (IFN-5-007, IL1 $\beta$ , IL6, IL17A, M-CSF and TNF-5-007), and factor modulators that regulate innate and adaptive immune responses (e.g., C55 $\beta$ ).<sup>2,24-26</sup> Immunomodulating cytokines are unable to prevent cytokine storms and associated illnesses. In patients with severe COVID-19, treatment options targeting immune cells that mediate cytokine storms might be more effective.

#### Role of macrophage dysfunction in the genesis of cytokine storms

##### Role of macrophages in infection control

Macrophages are innate immune cells that absorb or phagocytose foreign bodies, including viruses, in most tissues. They mediate inborn immunity, adaptive immune regulation, and proinflammatory effects. However, uncontrolled macrophage activity in infectious pathogens and metabolic disorders causes pathology.<sup>27-28</sup> Macrophages and macrophages from tissue-resident recruited from circulating in response to infections migrate to wounded or infected sites to exert regulatory effects that result in immune responses.<sup>28-29</sup>

Mature macrophages can shift phenotypic and functional polarization in response to signals from the local milieu that lead naive or relaxed 'M0 monocytes' to adopt classically activated 'M1' or 'M2-like' phenotypes or to facilitate interconversion of 'M1 and M2' macrophages. M1-like macrophages generate proinflammatory cytokines, such as IL1 $\beta$ , IL6, and TNF $\alpha$ , to modulate local tissue and pathogen-free immune responses.<sup>29-31</sup> M2-like macrophages or 'alternatively activated' macrophages show more diverse phenotypes, mainly involved in tissue repair and

**FIGURE 1**

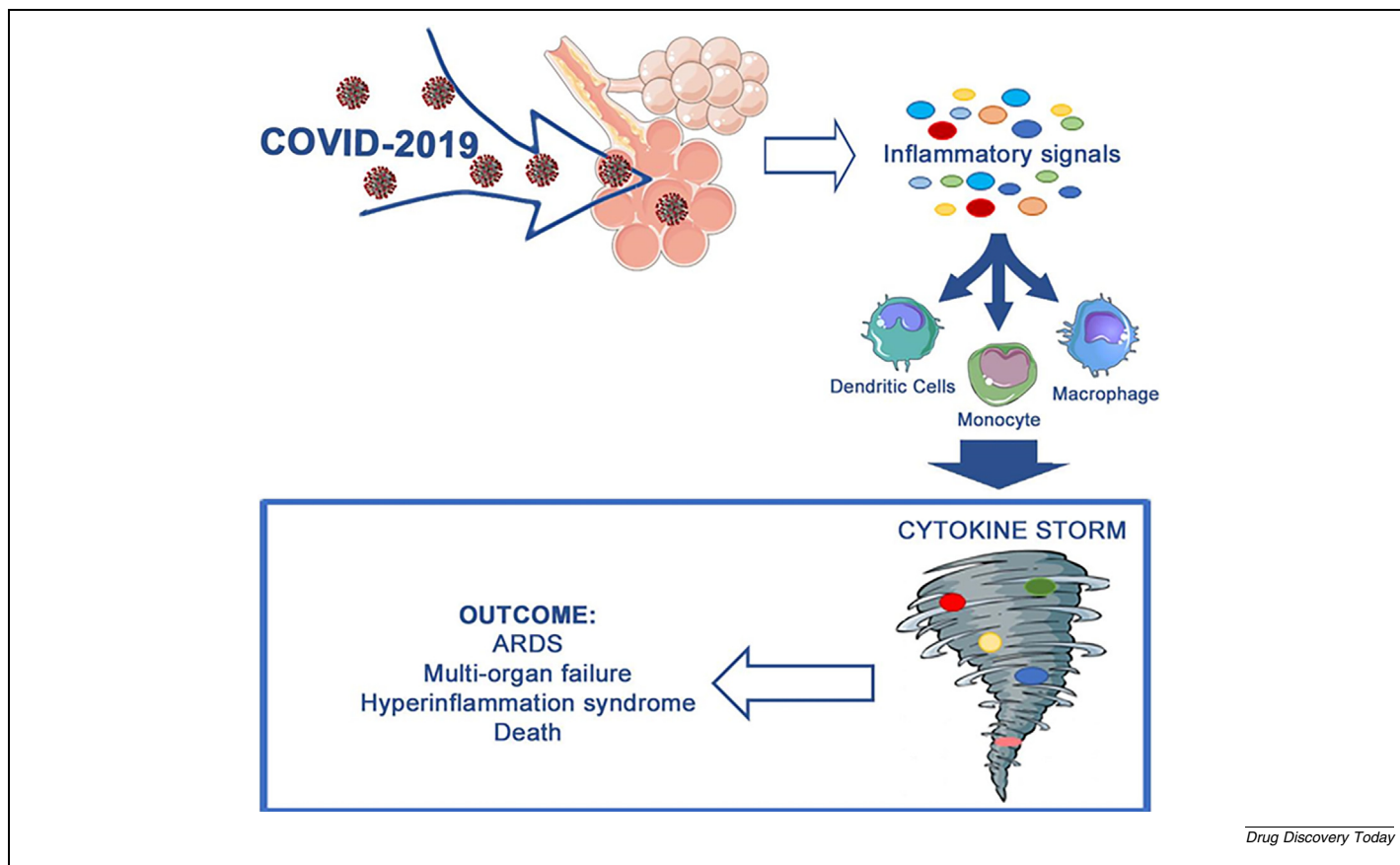
Coronavirus 2019 (COVID-19) immunopathology. COVID-19 causes lymphopenia, lymphocyte activation and malfunction, granulocyte and monocyte abnormalities, increased cytokine production, and increased antibody production. Lymphopenia is a common symptom of COVID-19 in patients, particularly in severe cases. CD4<sup>+</sup> and CD8<sup>+</sup> T cells of patients display high amounts of CD69, CD38, and CD44, and virus-specific T cells have a central memory pattern, with elevated levels of interferon (IFN)- $\gamma$ , tumor necrosis factor (TNF)- $\alpha$ , and interleukin (IL)-2. By contrast, lymphocytes exhibit an exhaustion phenotype because of increased expression of programmed cell death protein 1 (PD1), T cell immunoglobulin domain and mucin domain 3 (TIM3), and killer cell lectin-like receptor subfamily C member 1 (NKG2A). In severe cases, neutrophil levels are higher, whereas eosinophils, basophils, and monocytes are decreased. Another crucial feature of severe COVID-19 is increased cytokine production, particularly of IL1, IL6, and IL10. Abbreviation: NK, natural killer.

inflammatory response resolution. Given local microenvironments, macrophages can develop mixed phenotypes instead of adopting extreme M1-like or M2-like phenotypes defined by *in vitro* characterization studies.<sup>33–34</sup> During infection, macrophages also exhibit large histocompatibility complexes (MHC I and MHC II) of Type I and Type II proteolytic peptides derived from engulfed pathogens to enable T lymphocytes to recognize these peptides and cause a pathogen-specific adaptive immune response.

#### Cytokine response in extreme viral infection

The initial line of defence against infection is an inborn immunological response that is quick and well coordinated. Dysregulation and excessive responses can simultaneously induce local and systemic tissue malfunction and injury. By contrast, weak immune reactions might result in uncontrolled microbial

or viral multiplication, leading to systemic disease. Pathogen phagocytosis by macrophages can trigger an IFN response, increase adaptive immunity,<sup>2,6,24,33</sup> encourage differentiation of M1-like inflammatory phenotypes, and stimulate various pro-inflammatory cytokines, such as TNF $\alpha$ , IL1, IL6, IL8, and IL12 (Fig. 2). These cytokines have local and systemic effects, promoting vascular permeability and lymphocyte recruitment. Although this reaction is helpful when cytokines are released in appropriate amounts, it can be dangerous when cytokine secretion is unregulated or excessive. For example, increased serum IL6 is related to ARDS, respiratory failure, and adverse clinical responses in patients with COVID-19,<sup>4–5</sup> and is indicative of severe MERS-CoV and SARS-CoV-2 infections. IL6 is produced primarily by monocytes and macrophages, and its activation activates Janus kinase (JAK) and signal transducer and transcrip-



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**FIGURE 2**

During Coronavirus 2019 (COVID-19) infection, the immune system of the host responds abnormally. Adapted, with permission, from.<sup>34</sup> Abbreviation: ARDS, acute respiratory distress syndrome.

tion activator 3 (STAT3), which induce cytokine production in lymphocytes and neutrophils.

Failure of macrophages can result in uncontrollable cytokine release, causing cytokine-related storms in various severe viral infections, including SARS and COVID-19 (Fig. 1).<sup>2,17,23</sup> After SARS-CoV infection, autopsy and necropsy studies revealed a build-up of inflammatory monocyte macrophages (IMMs) in the lungs of humans and animals. In patients who died with SARS, the degree of penetration of macrophages and neutrophils and the distribution of these cells in the periphery have been recorded. However, in patients with MERS, the severity of lung lesions has been linked to macrophages and peripheral circulation. According to recent research, the alveolar lumen of patients who died with COVID-19 contained numerous macrophages.<sup>2,34</sup> These findings suggest macrophages as a significant source of cytokines and chemokines linked to fatal viral infections. Macrophages can also be used as viral targets and reservoirs, allowing viruses to replicate and spread quickly.<sup>34</sup> In addition, macrophages can improve their potential to generate proinflammatory phenotypes and react to the important receptor for SARS-CoV-2 infection, Angiotensin conversion enzyme 2 (ACE2).<sup>4</sup> In macrophages and lymphocytes, SARS-CoV virus particles and RNA have been identified,<sup>32</sup> and macrophages infected with SARS-CoV have a delayed but strong

expression of IFN and proinflammatory cytokines.<sup>36</sup> In the marginal spleen and lymph node sinuses of patients with COVID-19, SARS-CoV-2 nuclear macrophages ACE2+ were found, showing upregulation of IL6.

Macrophage dysfunction is also an essential part of the pathology of other viral and microbial pathogen infections, especially as these diseases progress to sepsis as a result of contradictory monocyte/macrophage reactions to these pathogens. Macrophages are usually activated as a response to risk-related stimuli that occur when different pathogen-associated molecular patterns (PAMPs) are detected by one of several receptors (pattern recognition receptors; PRRs) on or within the macrophage cell surface or their cytosols or endosomes, including several Toll-like receptor families (TLRs).<sup>35</sup> TLRs can cause macrophages to adopt proinflammatory M1-like phenotypes and generate components that increase innate and adaptive immune responses to important pathogens. Overactive macrophages induce local or systemic damage. Sepsis, the most significant cause of death in hospitals, is caused by systemic inflammation from bacteria or viral infections, which can result in tissue damage and organ failure. Macrophage dysfunction and cytokine storm syndrome cause increased mortality in septic illness.<sup>6</sup> Together, the dysfunction of macrophages contributes significantly to excessive inflammation and increased mortality in severe infections and sepsis.



## Modulating macrophage dysfunction: Therapeutic approaches

Clinical and experimental evidence shows that macrophage malfunction causes inflammatory cytokines in severe infections. Targeting macrophages can minimize cytokine storms, which can cause pathology and death in extreme conditions. Traditional drug delivery systems were used in preclinical models of inflammatory diseases and cancer to stimulate macrophage responses.<sup>35–36</sup> These proposed treatments can be broken down into three categories: (i) macrophage elimination using macrophage-specific or selectively toxic therapies to minimize dysregulated macrophage activity; (ii) macrophage invasion inhibition at disease locations by blocking chemotactic monocyte surface receptors (e.g., CCR2) to restrict inflammatory reactions; and (iii) macrophage-depleting drugs can reduce tumor macrophage invasions, such as CSF1R inhibitors and clodronate or CCR2–CCL2 pathway inhibitors.

By contrast, these approaches might not be optimal as treatments in the clinic. Many surface indicators targeted by these techniques are not specific to monocytes and macrophages, such as CCR2 and CSF1R, which are found in various cell types. As a result, systemic administration of medications that target these parameters could have unintended consequences. This is also a significant challenge for approaches to macrophage reprogramming, because their specificity is dependent on the specificity of the receptors or pathways activated by anti-inflammatory agents or cytokine inhibitors in the use of standard systemic medications in monocytes/macrophages.

### Nanomedicine approaches to targeting macrophages

COVID-19 vaccine research is focusing on discovering techniques that activate T cells and B cells against this virus. It is also crucial to expedite the development of specific next-generation vaccines that can target specific demographic groups or individuals with compromised immunity. Currently, mRNA-based COVID-19 vaccines using lipid NPs (LNPs) as carriers are in clinical trials. mRNA is susceptible to extracellular RNase destruction, making the formulation of its delivery vehicle essential. LNPs are

virus-sized particles (80–200 nm) that self-assemble from cationic lipids that can be ionizable. Several studies have demonstrated that they can successfully transfer mRNA into the cytoplasm. Using intramuscular and intradermal routes allows sustained-release mRNA expression kinetics, which leads to high antibody titers and immunological responses to B and T cells. The nanocarrier distribution spectrum is important because most COVID-19 vaccine candidates are complex biological molecules (DNA, mRNA, recombinant proteins, engineered antigen-presenting cells, etc.).

In recent years, nanomedicine has emerged as a promising platform for treating various medical conditions, including bioavailability, tissue specificity, and toxicity associated with conventional medicines. In recent years, significant progress has been made in developing nanomedicine-based diagnostic and treatment tools for disease, including using NPs to enhance intranasal delivery of therapeutics for respiratory infections. Numerous studies have examined the ability of nanomedicines to reduce macrophage dysfunction in preclinical models of infectious or chronic inflammatory diseases to increase effectiveness and decrease side effects (Table 1). NPs used in macrophage-targeting approaches can vary significantly in origin (i.e., natural versus synthetic) and composition, but their macrophage uptake mechanisms can usually be identified as belonging to one of two distinct pathways: nonspecific phagocytosis (passive targeting) or receptor-mediated endocytosis (active targeting). In passive targeting, the unmodified NPs of medium size (10–300 nm diameter), such as extracellular vehicles (EVs) and liposomes, are taken up by macrophages. These NPs concentrate primarily at infection and inflammatory sites because of phagocytosis and micropinocytosis by monocyte/macrophage lineage cells, which are prevalent at these locations,<sup>36–37</sup> although *in vitro* studies indicated clathrin-mediated endocytosis mechanisms occur in specific NPs.<sup>38</sup> Using various modifying methods that add macrophage-specific molecules to their surfaces, natural and synthetic NPs can be altered to promote their active targeting of macrophages.<sup>39–40</sup> However, additional studies to detect potential macrophage surface markers would help improve the biolog-

TABLE 1

### Examples of methods using a nanomedicine approach to control macrophage dysfunction.

NP	Targeting mechanism	Therapeutic agent	Therapeutic effects	Refs
Liposomes	Passive	Clodronate	Reduced serum IL1/TNF $\alpha$ and hepatic IL1/TNF $\alpha$ IL6/MCP1 expression and STAT3 p38 MAPK/ERK signaling decreased in colon	44–45
Human MSC-EVs	Passive	Endogenous miRNAs/ proteins	Suppressed M1-like macrophages and boosted M2-like macrophages in lungs Increased survival and reduced neutrophil infiltration and cytokine production in lungs Reduced inflammatory cell infiltration and decreased TNF $\alpha$ release	52–54
Tuftsin–alginate NPs	Active	Plasmid DNA encoding IL10	Reduced levels of proinflammatory cytokines IL6, IL1, and TNF $\alpha$ in blood and joints	46–49
TPP–PPM NPs	Active	TNF $\alpha$ siRNA	TNF $\alpha$ expression decreased and colon damage reduced	61–64
Swine MSC-EVs	Passive	Endogenous miRNAs/ proteins	Reduced replication of influenza virus and cytokines in lungs	55–58

ical distribution and therapeutic specificity of macrophage dysfunction. Here, we explore some nanomedicine-based approaches to reduce excessive macrophage activation reactions in viral and chronic disorders.

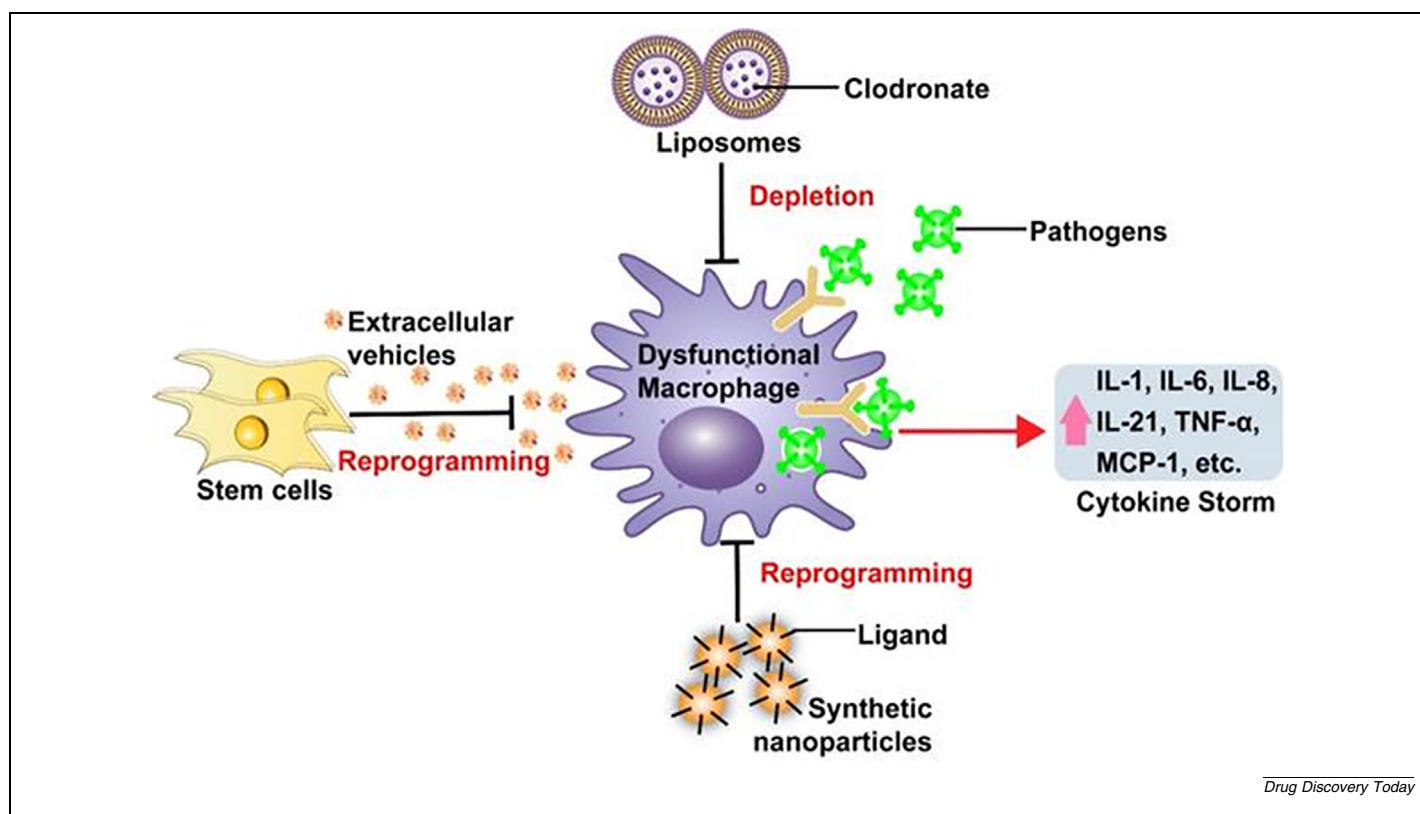
#### Macrophage therapies utilizing target-specific nanoparticles

Macrophage therapeutics aims to modify the activation of macrophages so that the detrimental effects of the cytokine storm, including the worsening effects on infections, are mitigated. This goal is achieved in one of the following three ways: (i) reducing the size of the macrophage population detrimental to infections; (ii) reducing the activity of proinflammatory macrophages; and (iii) inhibiting the cytokine-mediated activation of macrophages in response to inflammatory reactions.<sup>30,39</sup> However, the challenge with any conventional drug delivery strategy is that the drugs used to reprogram macrophages against inflammation are toxic and capable of harmful effects on the host. Therefore, to increase the specificity of these macrophage-specific targeted therapies, macrophage-specific targeted NPs are used to reduce macrophage dysfunction. These strategies have shown modest success in cancer, atherosclerosis, diabetes, and other inflammatory diseases, including arthritis.<sup>36,41–43</sup>

NPs decorated with a macrophage-targeting ligand or relevant peptides have recently been extensively studied to attenuate dysfunctional macrophages.<sup>30</sup> Examples of such NPs are liposomes, polymer NPs, or metallic NPs.<sup>36,42,44</sup> Such specifically designed

NPs target macrophage receptors, including mannose receptors, dectin 1 receptor, tuftsin tetrapeptide receptor, CD44 receptor, FR- $\beta$  receptor, and phosphatidylserine receptor or scavenger receptors of classes A, B, or C. Xiao *et al.* showed a reduction in TNF $\alpha$  when colitis (induced by dextran sodium sulfate) was treated with TNF-siRNA therapy with macrophage-targeted NPs.<sup>6</sup> Similarly, Jain *et al.* showed superior localization at the specific locations and improved the anti-inflammatory effects of alginate NPs acting on the tuftsin receptors in arthritis.<sup>47</sup> Daldrup-Li *et al.* used superparamagnetic iron oxide theragnostic NPs to target tumour-associated macrophages in breast cancer that showed selective accumulation in the macrophages.<sup>47–48</sup> Several other researchers have shown the potential of this approach to reduce proinflammatory reactions that aggravate disease pathologies, selectively accumulate in macrophages, reduce toxic effects, and increase the therapeutic efficacy of conventional delivery systems.<sup>49–50</sup> However, because the receptors targeted are ubiquitous, adverse effects are still possible, and such macrophage therapeutics in clinical trials must be evaluated for their safety profile.

At least two studies have investigated NP-mediated drug delivery to transiently deplete macrophages to minimize dysfunctional macrophage responses (Fig. 3, Table 1). Liposomes, US Food and Drug Administration (FDA)-approved nanocarriers comprising lipid bilayers around a hollow core, were used in this research. Given their physical and chemical properties, macro-



**FIGURE 3**

Effects of macrophage malfunction on the generation of proinflammatory cytokines during severe infection, as well as nanomedicine-based therapy techniques to counteract macrophage dysfunction. Adapted, with permission, from.<sup>35</sup> Abbreviations: IL, interleukin; TNF, tumor necrosis factor.

phages and phagocytic cells gather these particles after systemic distribution (passive targeting). In all investigations, liposomal clodronate disrupted the mitochondrial electron transport chain and released cytochrome C 50 into the cytosol. Liposome-mediated macrophage depletion reduces cytokine production in animals with autoimmune or infectious illnesses, such as rheumatoid arthritis and LPS-induced sepsis. In rats with LPS-induced sepsis, clodronate-containing liposomes depleted macrophages, reducing hepatic IL1 and TNF $\alpha$  expression and blood TNF $\alpha$  levels. In a mouse model of colon cancer treated with azoxymethane and dextran sodium sulfate, clodronate-loaded liposomes reduced IL6 and MCP1 production, as well as STAT3 and MAPK p38/ERK. Recent research on SARS-infected mice using antibody-mediated IMM reduction reduced lung lesions, cytokine levels (CCL2, TNF $\alpha$ , and IL6), and death without changing virus load. These studies show that reducing macrophages with liposomes or other techniques could minimize cytokine production and pathogenesis. Macrophage depletion is only helpful at certain stages of an infection. Improving target specificity does not address potential adverse impacts of systemic macrophage deletion, such as increased infection risk and impaired homeostatic macrophage functions in healthy tissues. Stem cell-derived extracellular vesicles (EVs) or synthetic NPs might reduce proinflammatory cytokine production during an excessive immune response. Several studies evaluated the feasibility of routinely giving anti-inflammatory exosomes to avoid macrophage dysfunction (Fig. 3, Table 1). Exosomes are naturally occurring NPs (50–500 nm) that facilitate cell–cell communication and are rapidly absorbed by circulating monocytes and tissue-resident macrophages in murine models of human disease.<sup>51–53</sup> Macrophage efficacy for native EVs is partially regulated by EV size and the lipid and glycoprotein content of the exterior membrane, which can vary across EVs released by various parental cell types. Endogenous bioactive substances, including proteins, nucleic acids, and lipids, are transported between cells and enable cell–cell communication.<sup>54</sup> In models of acute lung injury (ALI) associated with severe pneumonia and sepsis, mesenchymal stem cell EVs (MSC-EVs) reduced macrophage infiltration and cytokine production drastically.<sup>55–56</sup> In an *ex vivo* scenario in which infused human lungs were infected with *Escherichia coli*, systemic treatment of human MSC-EVs decreased inflammatory influx and expression of TNF $\alpha$ . Exposure to *E. coli* causes severe bacterial pneumonia.<sup>57</sup> Intrathecal treatment of MSC-EVs also inhibited influenza virus replication and the release of proinflammatory cytokines (TNF $\alpha$  and CXCL10) in the lungs of an influenza virus pig model.<sup>57</sup> MSC-EVs reduce lung injury partly because they can alter the respiratory macrophage phenotype.

MSC-EVs have been shown to decrease M1-like proinflammatory morphologies in injured lungs and boost M2-like anti-inflammatory macrophages.<sup>58</sup> This is because endogenous miRNAs (e.g. miR-21 and miR-124) and proteins shuttled through MSC-EVs (e.g. IL10 and TGF $\beta$ ).<sup>57,59</sup> EV-mediated macrophage reprogramming might be superior to liposome-mediated macrophage depletion because of fewer short- and long-term risks. Therapeutic MSC-EV techniques might be safer because they induce fewer immune reactions. EV therapeutic effects are determined by absorption and cargo. EV therapeutic power can be

increased by loading or altering it with macrophage-targeting compounds and therapeutic medicines. Stem cell-derived EVs have been used in early clinical examinations of inflammatory and infectious diseases and the therapeutic benefits of various MSCs against COVID-19.<sup>60–61</sup>

### Therapeutic potential of virus-like particles

Virus-like NPs (VLPs) are a novel strategy that has recently been explored in the theranostics field. They are non-infectious, self-assembled viral coating proteins (capsids), minus the viral genetic material and range in size from 20 nm to 500 nm in diameter. They can be used to encapsulate drugs, small interfering (si)RNAs, RNA aptamers, proteins, and peptides.<sup>44,62–63</sup> They can be easily decorated with macrophage-targeted ligands. They can originate from animal (hepatitis B virus core or surface), plant [cowpea chlorotic mottle virus (CCMV) and cowpea mosaic virus (CPMV)] or bacteriophage (MS2, Q $\beta$ , and *Salmonella typhimurium* P22) sources.<sup>64–65</sup> VLPs loaded with single-strand (ss)-RNA and capsid protein of Papaya Mosaic Virus increased immune responses to infections such as influenza and *Streptococcus pneumoniae*.<sup>44,62</sup> Despite these advantages, VLPs are challenged by a lack of stability, phagocyte-mediated clearance, endosomal escape, accumulation in organs other than the target, low extravasation at the target site, and intrinsic immunogenicity. Thus, further research is needed to overcome these limitations for their use as delivery systems for infections and other diseases.

### Challenges

Nanomaterials have significant optical and electrochemical capabilities and are customizable, biocompatible, and cost-effective. They can be changed and functionalized using different substrates, expanding their application potential. There are still numerous obstacles to overcome despite significant advances in our understanding of COVID-19 treatment. COVID-19 pathophysiology and nano-bio-interface mechanisms are challenging to study because of a lack of information and resources. The multifunctional potential of nanomaterials also requires further exploration. Some nanomaterials might be able to detect or interact with COVID-19, stop it from acting, and modify the immune response to fight against it. More research is needed to understand how NPs work and affect viruses. Understanding such data is crucial for preventing, diagnosing, and treating COVID-19. To understand the long-term impacts of NPs, human *in vivo* toxicokinetics must be studied. To tackle COVID-19, it will also be crucial to mass-produce relevant NPs. Vaccines and therapeutic agents must be made swiftly, accurately, and cost-effectively with perfect size and surface control.

### Concluding remarks

Regulating macrophage-mediated inflammatory responses could be an alternative therapy for proinflammatory disorders in which macrophage dysfunction might cause a cytokine storm, with pathological consequences. The therapeutic potential of macrophage-targeted nanomedicine in animal models of COVID-19, just one disease in which cytokine storms affect disease pathology and mortality, is still being explored. EV and lipo-



some studies using human ACE2 transgenic mice (hACE2) are the best way to establish whether macrophage depletion or activation attenuation is beneficial in severe COVID-19. Nanomedicine can improve the bioavailability of anti-inflammatory small molecules and antibodies explored in COVID-19 clinical trials. These and other nanomedicines will be evaluated using clinically essential indicators, such as survival, lung lesions, and cytokine profiles.

Before beginning such research, there are several variables to consider. To be successful, macrophage-targeted nanomedicines must be provided during infection (e.g., before the hyperinflammatory phase); suppressing the innate immune response during early-stage virus infections is likely to be harmful. Given that cytokine storms associated with severe conditions can be dynamic, periodic cytokine panel screening will be needed to determine the most effective therapeutic time and its consequences. Third, nanomedicines and systemic antiviral medication delivery could synergize. Animal model data are encouraging, but more preclinical research is needed to address

the biosafety, mechanism of action, and delivery methodologies and doses of nanomedicines. Clinical translation requires standard nanomedicine production processes and quality monitoring. Finally, reducing macrophages could raise the risk of immunological disorders and bacterial infections. The continuous collaboration across disciplines and sectors promises an unprecedented rate of development of treatments and possible cures for COVID-19.

### Data availability

No data was used for the research described in the article.

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