The Prospect of Biomimetic Immune Cell Membrane-Coated Nanomedicines for Treatment of Serious Bacterial Infections and Sepsis

Alexandria Hoffman\textsuperscript{a} and Victor Nizet\textsuperscript{a,b}

\textsuperscript{a}Division of Host-Microbe Systems and Therapeutics, Department of Pediatrics, UC San Diego School of Medicine, La Jolla, California, USA; \textsuperscript{b}Skaggs School of Pharmacy and Pharmaceutical Sciences, UC San Diego, La Jolla, California, USA

Address correspondence to Victor Nizet, vnizet@health.ucsd.edu

Running Title: Membrane-Coated Nanosponges for Serious Bacterial Infections

Manuscript pages: 35
Figures: 4
Word Count: 12,493
Abstract: 204
Introduction: 472
Discussion: 138
Non-standard abbreviations

ADAM10, a disintegrin and metalloproteinase domain-containing protein 10
CD47, Integrin Associated Protein
CD59, membrane attack complex (MAC)-inhibitory protein
CD64, Fc receptor
DIC, disseminated intravascular coagulation
EPIC II, Extended Prevalence of Infection in Intensive Care
EPR, enhanced permeability and retention
HIV- human immunodeficiency virus
ICON, Intensive Care over Nations
IFN-γ, interferon gamma
IL, interleukin
LPS, lipopolysaccharide
MAb, monoclonal antibody
M-NP, macrophage membrane-coated nanoparticle
NET-, neutrophil extracellular trap
N-NP, neutrophil membrane-coated nanoparticle
PEG, poly(ethylene glycol)
PEG-DA, polyethylene glycol diacrylate
PLGA, poly(lactic-co-glycolic acid)
PMP, platelet microbicidal proteins
P-NP, platelet membrane-coated nanoparticle
RBC, red blood cell
RBC-NP, red blood cell membrane-coated nanoparticle
ROS, reactive oxygen species
SIRPα, signal regulatory protein alpha
SIRS, systemic Inflammatory Response Syndrome
SLO, streptolysin O
SOAP, sepsis Occurrence in Acutely Ill Patients
T3SS, type III secretion system
TC-NP, T cell membrane-coated nanoparticle
TiO$_2$, Titanium dioxide
TLR4, Toll-like receptor 4
TNF-α, Tumor necrosis factor alpha
UV, Ultraviolet
WHO, World Health Organization
Abstract

Invasive bacterial infections and sepsis are persistent global health concerns, complicated further by the escalating threat of antibiotic resistance. Over the past 40 years, collaborative endeavors to improve the diagnosis and critical care of septic patients have improved outcomes, yet grappling with the intricate immune dysfunction underlying the septic condition remains a formidable challenge. Anti-inflammatory interventions that exhibited promise in murine models failed to manifest consistent survival benefits in clinical studies through recent decades. Novel therapeutic approaches that target bacterial virulence factors, for example with monoclonal antibodies, aim to thwart pathogen-driven damage and restore an advantage to the immune system. A pioneering technology addressing this challenge is biomimetic nanoparticles—a therapeutic platform featuring nanoscale particles enveloped in natural cell membranes. Borne from the quest for a durable drug delivery system, the original red blood cell-coated nanoparticles showcased a broad capacity to absorb bacterial and environmental toxins from serum. Tailoring the membrane coating to immune cell sources imparts unique characteristics to the nanoparticles suitable for broader application in infectious disease. Their capacity to bind both inflammatory signals and virulence factors assembles the most promising sepsis therapies into a singular, pathogen-agnostic therapeutic. This review explores the ongoing work on immune cell-coated nanoparticle therapeutics for infection and sepsis.

Significance Statement

Invasive bacterial infections and sepsis are a major global health problem made worse by expanding antibiotic resistance, meaning better treatment options are urgently needed. Biomimetic cell-membrane coated nanoparticles are an innovative therapeutic platform that deploys a multifaceted mechanism to action to neutralize microbial virulence factors, capture endotoxins, and bind excessive host proinflammatory cytokines, seeking to reduce host tissue injury, aid in microbial clearance, and improve patient outcomes.
Introduction

Accurately assessing the global burden of sepsis is challenging due to frequent changes in its definition (Bone et al., 1992; Levy et al., 2003; Singer et al., 2016). Nevertheless, a meta-analysis of studies from high-income countries estimates that there are over 50 million cases of sepsis worldwide each year (Fleischmann et al., 2016). While sepsis manifests as a highly heterogeneous disease, it typically originates from an inciting infection. Findings from the SOAP, EPIC II, and ICON studies consistently indicate that a substantial number of these infections originate in the lung or abdomen (Vincent et al., 2006, 2009; Sakr et al., 2018). Furthermore, consensus across these studies highlights the prevalence of specific pathogens: *Staphylococcus aureus* emerges as the most common Gram-positive organism, while *Escherichia coli* and *Pseudomonas aeruginosa* stand out as the most prevalent Gram-negative isolates. A seemingly straightforward infection can progress to sepsis when the host's immune response fails to control the infection, leading to a self-destructive hyperinflammatory state. Even with effective antibiotic administration, this hyperinflammatory state can culminate in organ failure and death in the most severe cases. The ongoing rise of antibiotic-resistant strains has only heightened the urgency to discover effective management strategies for these perilous medical conditions (Singer et al., 2016).

Despite advances in supportive care, the occurrence of organ failure continues to serve as a robust predictor of sepsis mortality (Metnitz et al., 2001; Blanco et al., 2008). In a recent multicenter study, respiratory and cardiovascular failure emerged as the most common types of organ failure, and their combination detrimentally impacts systemic oxygenation, playing a pivotal role in determining sepsis outcomes (Blanco et al., 2008). Severe sepsis poses a particularly life-threatening scenario for pediatric and geriatric populations, with predicted inhospital mortality rates of 25% and 48%, respectively (Weiss et al., 2015; Martin-Löeches et al., 2019). Even among survivors, severe sepsis can lead to persistent cognitive and physical limitations that endure for years after hospital discharge (Iwashyna et al., 2010). Initiatives by organizations such as the World Health Organization (WHO) and the Surviving Sepsis Campaign have significantly enhanced sepsis diagnosis and supportive care (Rhodes et al., 2015; World Health Organization, 2020; Evans et al., 2021). Current supportive therapies encompass fluid resuscitation, vasoactive medications, mechanical ventilation, as well as blood products and corticosteroids in specific situations (Evans et al., 2021). Beyond supportive therapy, sepsis treatment revolves around antimicrobial agents. The guidelines from the
Surviving Sepsis Campaign underscore the critical importance of promptly administering antibiotics and removing any identifiable sources of infection, such as catheters (Evans et al., 2021). Specific sepsis therapies targeting the dysregulated immune response are currently lacking. Despite the impressive progress made in supportive therapies, sepsis mortality and disability rates remain high, underscoring the urgent need for innovative treatments. A shift towards therapeutic strategies addressing the dysregulated immune response could present promising avenues for improving outcomes in sepsis.

**Septic inflammation: A double-edged sword**

The initial definition of sepsis classified it as part of a broader condition known as systemic inflammatory response syndrome (SIRS) (Bone et al., 1992). Despite multiple updates to its definition, the close association between sepsis and systemic inflammation remains paramount. In patients with sepsis, elevated inflammatory cytokines are closely linked to organ dysfunction and mortality (Damas et al., 1989, 1992; Pinsky et al., 1993; Bozza et al., 2007). In mouse models, these cytokines have been implicated in acute kidney and lung injuries (Cunningham et al., 2002; Nechemia-Arbely et al., 2008; Ahuja et al., 2012; Bhargava et al., 2013; Xu et al., 2014). Notably, mice lacking inflammatory cytokines or certain inflammatory pathways exhibit resistance to endotoxemia models of sepsis (Pfeffer et al., 1993; Böhrer et al., 1997; Cunningham et al., 2002). However, when confronted with live bacterial or viral infections, mice deficient in the cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), two key inflammatory cytokines, show poorer control of the infection (Pfeffer et al., 1993; Kopf et al., 1994). This underscores the dual role of inflammatory cytokines in coordinating the immune response—they are crucial for infection control, but prolonged excessive inflammation has detrimental effects on organ function. Consequently, anti-inflammatory therapy in sepsis must strike a delicate balance to preserve an effective immune response while limiting organ damage.

The early conceptualization of sepsis as an inflammatory disease led to the emergence of a first wave of immunomodulatory sepsis therapies that aimed to mitigate inflammation by obstructing inflammatory cytokines. Despite encouraging preclinical outcomes, all of these therapies faced disappointment in clinical trials. Anti-TNF-α monoclonal antibodies (MAbs), (Fisher et al., 1993; Abraham et al., 1995, 1998; Dhainaut et al., 1995; Cohen and Carlet, 1996; Clark et al., 1998) antibody fragments, (Reinhart et al., 1996, 2001) and soluble TNF-α receptors (Fisher et al.,
1996) did not demonstrate improved survival in human studies. Similarly, interleukin 1 (IL-1) receptor antagonists, while initially displaying promise, proved ineffective in improving 28-day mortality in larger clinical trials (Fisher, Dhainaut, et al., 1994; Fisher, Slotman, et al., 1994; Opal et al., 1997). Furthermore, efforts targeting other specific sources of heightened inflammation, such as oxidative stress and eicosanoids, yielded no significant improvement in the survival of sepsis patients (Bernard et al., 1997; Patel et al., 2012; Sakr et al., 2014).

**Anti-toxin therapy**

Pathogenic bacteria exhibit a spectrum of virulence factors that empower them to evade or neutralize the host immune system (Johnson, 1991; Zeconci and Scali, 2013). When targeted against these virulence factors, monoclonal antibody (MAb) therapy holds the potential to augment the immune system’s capacity to clear the infection while simultaneously mitigating damage to non-immune cells. Encouragingly, human trials involving MAbs against *Clostridioides difficile* toxins have already demonstrated a reduction in the recurrence of colitis caused by this pathogen (Lowy et al., 2010). This success has spurred ongoing endeavors to develop MAb anti-toxin therapies for other pathogens responsible for the majority of sepsis cases, including *S. aureus*, *E. coli*, and *P. aeruginosa*.

In *S. aureus* infection, α-toxin, also known as α-hemolysin, plays a significant role in inducing host cell death and immune dysfunction (Tkaczyk et al., 2013; Scherr et al., 2015). This potent virulence factor targets host cells by binding to a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) expressed on the host cell surface. Studies have revealed that mice deficient in ADAM10 are protected against lethal *S. aureus* infections, underscoring the importance of α-toxin’s interaction with ADAM10 in the pathogenesis of the infection (Inoshima et al., 2011). Researchers have been exploring the use of MAbs targeting α-toxin as a potential therapeutic approach with promising results in preclinical models. In a pneumonia model, anti-α-toxin MAbs improved survival, and in a dermonecrosis model, these MAbs reduced the size of skin lesions (Ragle Brook E. and Bubeck Wardenburg Juliane, 2009; Tkaczyk C. et al., 2012). Early human trials of two distinct monoclonal anti-α-toxin therapies have demonstrated good tolerability, paving the way for ongoing analysis in larger randomized controlled trials (François et al., 2018, 2021).
In contrast to the free release of α-toxin by *S. aureus*, the opportunistic pathogen *P. aeruginosa* employs a type III secretion system (T3SS) to directly inject toxins into host cells (Hauser, 2009). Strains of *P. aeruginosa* possessing the T3SS are more likely to infect humans and are associated with poor clinical outcomes (Hauser *et al.*, 2002; Ledizet *et al.*, 2012). In mouse models, the attenuation of *P. aeruginosa* virulence has been observed upon knocking out components of the T3SS (Shaver and Hauser, 2004). Despite the direct injection of effector proteins into host cells by the T3SS, targeting the structural proteins with MAbs has shown promising results. Preclinical studies indicate that MAbs against these structural proteins effectively reduce lung injury and edema caused by *P. aeruginosa* infections (Faure *et al.*, 2003). A notable example is a bivalent MAb therapy targeting the T3SS structural proteins PcrV and Psl, which has demonstrated good tolerability in humans and shown potential for efficacy in patients with less systemic inflammation (Chastre *et al.*, 2020). MAbs targeting bacterial virulence factors exhibit promise as adjunctive therapies for bacterial infections. However, their efficacy in sepsis is constrained by the time required for a positive culture to identify the pathogen. In the context of sepsis, where time is crucial, a faster and more versatile method of neutralizing bacterial toxins would be highly advantageous compared to a delayed and pathogen-specific approach.

As outlined above, sepsis is a multifaceted condition involving an invasive pathogen alongside a protracted heightened immune response. Although MAbs hold promise as therapies against the initiating infection, their scope does not extend to alleviating the overactive immune response. Conversely, while anti-inflammatory therapy may effectively curtail the immune response, it lacks the capacity to assist the immune system in managing the infection. In this review, we will scrutinize the existing literature on immune biomimetic nanoparticles in the context of infection and sepsis, exploring their potential to address both aspects of this intricate clinical scenario (Figure 1).

**DEVELOPMENT OF BIOMIMETIC NANOPARTICLES**

**Nanoparticles in medicine: origins and advantages**

The most basic form of membranous nanoparticle is the liposome. Initially developed in the 1960s, liposomes were under investigation as delivery systems for therapies as early as 1971 (Bangham *et al.*, 1965; Gregoriadis and Ryman, 1971). By 1995, a liposomal drug formulation of doxorubicin (Doxil®) had gained approval in the United States (Barenholz, 2012). When drugs
are enclosed within a liposome, their bioavailability is not immediate. Instead, they become locally available when liposomes accumulate in clearance organs, such as the liver, or in specific tissues through the enhanced permeability and retention (EPR) effect (Gregoriadis and Ryman, 1971). This localized availability is particularly advantageous for chemotherapeutic drugs, enabling them to target tumors and diminish overall toxicity (Meunier et al., 1991; Gabizon, 1995). Additionally, liposomes offer the benefit of transporting usually insoluble drugs by manipulating the pH of the nanomedicine or incorporating the drugs into the liposomal bilayer (Mayer et al., 1986; Haran et al., 1993). The use of liposomal drug delivery systems extends the retention time of small molecule therapies in the bloodstream compared to their "free" counterparts (Gill et al., 1995). Nevertheless, the limited nanoparticle retention time in the blood is a continuing challenge.

**Nanoparticle retention: a recurring challenge**

The loading of drugs into liposomes enhances their retention in the bloodstream compared to their "free" counterparts (Gill et al., 1995). However, the mononuclear phagocyte system (MPS) in the liver and spleen can still rapidly clear them from the body (Senior et al., 1985). This clearance is somewhat size-dependent, with smaller liposomes exhibiting longer retention times (Allen et al., 1995). To surmount this limitation and enhance retention while evading the MPS system, researchers have devised strategies such as coating liposomes with negatively charged sugars to create Stealth® liposomes (Allen and Hansen, 1991). A significant advancement in liposomal retention occurred with the introduction of poly(ethylene glycol) (PEG) liposomes, substantially reducing clearance (Klibanov et al., 1990; Allen et al., 1991). Nevertheless, the presence of naturally occurring anti-PEG antibodies in some patients poses a potential challenge to the universal effectiveness of PEGylated liposomes (Knop et al., 2010). Furthermore, after an initial dose of PEGylated liposomes, subsequent doses of the same nanoparticles are cleared at an accelerated rate, (Ishida et al., 2003) prompting concerns about the use of PEGylated liposomes for drugs requiring prolonged regimens.

**Nanoparticle Retention: Biomimicry for immune evasion**

In an innovative approach to enhance nanoparticle retention, researchers took inspiration from the natural characteristics of circulating red blood cells (RBCs). RBCs exhibit an impressive lifespan of approximately 120 days and present multiple signals that deter their opsonization or phagocytosis by the systems responsible for clearing nanoparticles. One such signal found on healthy RBCs is integrin-associated protein (CD47), serving as a "don't eat me" signal. CD47 on
RBCs binds to signal regulatory protein alpha (SIRPα) on macrophages, effectively inhibiting phagocytosis (Oldenborg et al., 2000). Additionally, RBCs express various complement inactivating proteins including C8 binding protein, (Schönermark et al., 1986) homologous restriction protein, (Zalman et al., 1986) decay-accelerating factor, complement receptor 1, (Kim et al., 2008) and CD59 (Babiker et al., 2002). These proteins prevent the complement aggregation on the surface of RBCs, a process that could lead to the rapid clearance of liposomes (Szebeni et al., 2011). The objective of researchers was to integrate these “don’t eat me” signals onto engineered nanoparticles, aiming to confer upon them some of the retention advantages observed in circulating RBCs.

Rather than opting for a single "don't eat me" signal, researchers repurposed intact RBC membranes containing the complete array of signals to craft RBC biomimetic nanoparticles (RBC-NP). These RBC-NP can be engineered either as liposomes or with solid biodegradable cores. While the retention time of RBC-liposome hybrids is not explicitly reported, RBC-NP with PLGA cores exhibit prolonged retention in the bloodstream. At 24 hours, their retention increases from 11% to 29%, and at 48 hours, it rises from 2% to 11% compared to PEGylated PLGA cores (Hu et al., 2011). Similar to liposomes, the clearance time of RBC biomimetic nanoparticles is size-dependent, with larger nanoparticles (200 nm) being cleared more rapidly than smaller ones (80 nm) (Li et al., 2019). Importantly, subsequent doses of RBC biomimetic nanoparticles do not exhibit accelerated blood clearance, suggesting that these nanoparticles can circumvent the clearance issues observed with PEGylated nanoparticles (Rao et al., 2015). In summary, the utilization of RBC membranes to cloak nanoparticles allows them to elude endogenous clearance mechanisms, significantly enhancing retention times in the bloodstream.

BIOMIMETIC NANOPARTICLES AND THEIR USE IN INFECTIOUS DISEASE

Biomimetic nanoparticles have potential beyond drug delivery. The suite of plasma membrane proteins on these nanoparticles enables them to function as effective decoys for endogenous cells, mimicking their receptor profile and serving as reservoirs for soluble mediators of infection and sepsis (Figure 2). This decoy effect has the capacity to protect host cells from damage and reinforce the immune response, thereby enhancing the host's ability to combat infections.

Red Blood Cells
Experimental evidence indicates that RBC biomimetic nanoparticles (RBC-NPs) serve as highly effective decoys for bloodborne toxins that typically target host RBCs and innate immune cells. In vitro studies demonstrate that RBC-NPs can effectively mitgate hemolysis induced by S. aureus α-toxin (Hu et al., 2013; Zhang et al., 2017; He et al., 2019). These protective effects extend beyond specific pathogens, with RBC-NPs exhibiting similar efficacy against streptolysin O (SLO) from Streptococcus pyogenes, listeriolysin O from Listeria monocytogenes, and the bee venom component melittin (Escajadillo et al., 2017; Chen et al., 2018). In addition to RBCs, bacterial toxins also target immune cells to compromise their function or lead to their lysis. In this context, the RBC-NPs decoy function protects the integrity of immune cells, preventing SLO-induced macrophage death and enhancing neutrophil killing of S. pyogenes (Escajadillo et al., 2017). In a more complex system involving S. aureus supernatant, wherein additional secreted leukocidins beyond α-toxin are present, administration of RBC-NPs demonstrated a significant reduction in toxicity and cell death (Chen et al., 2019). In vivo studies highlight the capacity of RBC-NPs to enhance survival in mouse models of intravenous toxemia caused by a broad range of toxins, surpassing the efficacy of both free RBC membranes and PEGylated PLGA cores (Hu et al., 2013; Chen et al., 2018, 2019; He et al., 2019). Additionally, RBC biomimetic nanoparticles exhibit a noteworthy scavenging of human-made chemical pollutants. Specifically, they effectively bind to free dichlorvos, a common pesticide ingredient that impairs acetylcholinesterase (AChE), preserving systemic AChE activity. Administration of RBC-NPs to mice challenged with intravenous and oral dichlorvos showed significant improvement in survival in both models (Pang et al., 2015). These cumulative findings underscore the role of biomimetic nanoparticles as efficient scavengers for a diverse array of naturally occurring and manufactured toxins.

**Platelets**

While not traditionally classified as immune cells, circulating platelets play a crucial role in host defense against bacterial pathogens. Their rapid adherence to the vascular endothelium and aggregation with one another enable effective collaboration with the innate immune system, forming bacteria-immobilizing immuno-thrombi (Wong et al., 2013; Prasad et al., 2015; McDonald et al., 2017). Upon activation, platelets release stored platelet microbicidal proteins (PMPs) that exhibit dual microbicidal and chemokine properties (Nicolai et al., 2019). In bacterial infections and sepsis, platelet dysfunction and pathology are common. Notably, platelets are targeted by well-known virulence factors such as S. aureus alpha toxin and clumping factor A (Bhakdi et al., 1988; Siboo et al., 2001). The widespread thrombosis induced...
by these factors can lead to disseminated intravascular coagulation (DIC) and severe organ damage while concurrently depleting the body of platelets necessary to maintain vascular integrity (Levi et al., 1999). Consequently, low platelet counts, referred to as thrombocytopenia, are prevalent in human sepsis, and this condition is associated with kidney injury and prolonged stays in intensive care units (Venkata et al., 2013).

Platelet membrane-coated nanoparticles (P-NPs) inherit functional properties from platelets, including the capacity to bind to areas of damaged or inflamed vasculature. Upon intravenous injection of fluorescent P-NPs, they selectively localize to aortic atherosclerotic lesions (Hu et al., 2015; Dehaini et al., 2017). In contrast, RBC-NPs do not exhibit such localization, and blended nanoparticles containing both platelet and RBC membranes show reduced degrees of localization, suggesting localization to the lesion is contingent on the NP membrane's identity (Dehaini et al., 2017). Furthermore, P-NPs can directly bind to bacteria, such as *P. aeruginosa* and *S. aureus*, through various cell membrane receptors (Hu et al., 2015; Peng et al., 2021). This bacterial binding is also influenced by the membrane identity, with P-NPs demonstrating significantly increased binding to *S. aureus* compared to RBC-NPs (Hu et al., 2015).

While bacterial adhesion in isolation has limited therapeutic value, when coupled with a functionalized core, it can enhance microbicidal activity. P-NPs loaded with vancomycin demonstrate improved killing of *S. aureus* compared to vancomycin-loaded RBC-NPs (Hu et al., 2015). Similarly, when a copper silicate microsphere with inherent antimicrobial action under near-infrared light is coated with a platelet membrane, it exhibits enhanced killing of *P. aeruginosa* (Peng et al., 2021). In mouse models, coating these functionalized cores with platelet membrane led to a reduction in bacterial burden compared to antibiotics alone, cores alone, and RBC membrane-coated controls (Hu et al., 2015; Peng et al., 2021).

Beyond their adhesive properties, platelets are targeted by bacterial virulence factors. P-NPs can function as decoys for these products, effectively shielding host platelets and other immune targets from damage or activation, promoting a more effective host response. Notably, P-NPs demonstrate a dose-responsive adsorption of lipopolysaccharide (LPS), preventing macrophage activation and inflammation (Peng et al., 2021). This decoy function also reduces the cytotoxicity of *S. aureus* supernatant against platelets and macrophages (Kim et al., 2021). Even without the addition of a functionalized core, P-NPs exhibit potent effects *in vivo*. In a mouse model of systemic *S. aureus* infection, P-NPs improve survival, reduce bacterial burden,
and decrease serum IL-6 levels, underscoring their therapeutic potential in combating bacterial infections and mitigating the associated inflammatory response (Kim et al., 2021).

**IMMUNE CELL BIOMIMETIC NANOPARTICLES IN INFECTION AND SEPSIS**

The studies mentioned above demonstrate the effectiveness of RBC-NPs as decoys for bloodborne toxins, highlighting the advantage of inhibiting these toxins to enhance the immune system’s response during an infection. However, bacterial toxins represent only one facet of sepsis. Cytokines produced by the host immune system play a significant role in causing damage and organ dysfunction during sepsis. Intercepting both pathogen-derived toxins and host-derived cytokines necessitates a nanoparticle coated with the membrane of a cell that has evolved to detect and respond to pathogens, cellular damage, and inflammation. In the following discussion, we will explore recent advancements in the development of biomimetic nanoparticles derived from innate and adaptive immune cells.

**Neutrophils**

Neutrophils, as a critical component of the immune response, play a vital role in combating infections during sepsis. They represent the first and most abundant immune cells to arrive at the site of infection, enabling them to initiate an immediate microbicidal response (Page and Good, 1958). Neutrophils accomplish this through the production of antimicrobial peptides, reactive oxygen species, and neutrophil extracellular traps (NETs), contributing to the containment and neutralization of invading pathogens (Kolaczkowska and Kubes, 2013). Additionally, neutrophils contribute to the activation and recruitment of professional antigen-presenting cells, engaging the entire immune system in the defense against the infection (Chertov et al., 1997; Bennouna et al., 2003). During sepsis, neutrophils can display dysfunctional behaviors, including impaired apoptosis and migration, as well as deleterious overproduction of NETs, which may contribute to tissue damage (Shen et al., 2017). In clinical settings, markers associated with neutrophil activity, such as CD64 expression and serum interleukin-8 (IL8), a neutrophil chemokine, have been linked to the severity of sepsis and the development of organ failure (Livaditi et al., 2006). Additionally, ratios of mature neutrophils to
immature granulocytes or leukocytes have been identified as potential predictors of sepsis mortality (Ahn et al., 2018; Ni et al., 2019).

The potential of neutrophil membrane-coated nanoparticles (N-NPs) in infectious disease models is relatively unexplored, yet they encompass several valuable functions that could be advantageous for managing infections and sepsis. One of their key abilities is to sequester soluble mediators of sepsis. In models of inflammatory arthritis and spinal cord injury, N-NPs have demonstrated the capability to adsorb inflammatory cytokines like IL-1β and TNF-α in a dose-dependent manner, effectively reducing the inflammatory activation of macrophages (Zhang et al., 2018; Bi et al., 2021). Moreover, in the inflammatory arthritis model, N-NPs exhibited significant efficacy in reducing serum inflammatory cytokines and preserving cartilage content (Zhang et al., 2018).

N-NPs also inherit the ability to accumulate at sites of injury and inflammation from neutrophils. In various disease models, N-NPs have demonstrated superior penetration into specific tissues compared to control nanoparticles, such as RBC-NPs. For instance, in a model of inflammatory arthritis, N-NPs exhibited greater accumulation in cartilage, showcasing their ability to target and deliver therapeutic agents effectively to affected areas (Zhang et al., 2018). In acute pancreatitis models, N-NPs displayed a dramatic ability to accumulate in the pancreas, making them a promising candidate for targeted drug delivery to this specific organ during inflammatory conditions (Zhou et al., 2019; Hassanzadeh et al., 2021). Additionally, N-NPs have shown temporary accumulation in injured spinal and brain tissue, indicating their potential application in delivering therapies to these sensitive regions (Dong et al., 2019; Bi et al., 2021). When N-NPs are combined with anti-inflammatory cores, they can significantly enhance the anti-inflammatory activity of these cores, effectively reducing local inflammation and tissue damage (Dong et al., 2019; Zhou et al., 2019; Bi et al., 2021; Hassanzadeh et al., 2021). Importantly, it is not yet determined how much of this anti-inflammatory effect is due to the adsorption of cytokines, extended nanoparticle clearance time, or improved proximity to the inflamed tissue.

Macrophages

Macrophages play a crucial role in the recognition and clearance of infections, contributing to both the early innate response and the activation of adaptive immune cells (Unanue, 1984; Gordon and Plüddemann, 2017). During infections, they secrete inflammatory cytokines such as IL-1β, IL-6, and TNF-α in response to bacterial components and host-derived cytokines (Sagy et
In sepsis, macrophages do not undergo apoptosis during the immunosuppressive phase; however, their response to bacterial stimuli becomes blunted (Munoz et al., 1991). Researchers have explored strategies to reprogram macrophages into an anti-inflammatory phenotype, utilizing signals like prostaglandin-E2, which has demonstrated improved survival in mouse models of sepsis (Németh et al., 2008). Additionally, supplementing endogenous macrophages with reprogrammed macrophages has also shown potential benefits (Anderson et al., 2013). Another innovative approach to modulating macrophage behavior is through the use of macrophage membrane-coated nanoparticles (M-NPs). By harnessing the repertoire of plasma membrane proteins from macrophages, these nanoparticles have the ability to intercept both pathogen-derived and host-derived inflammatory signals (Figure 3).

Of all the immune nanoparticles discussed in this review, M-NPs have the greatest diversity in their fabrication. In their simplest form, M-NPs are created by isolating macrophage membranes and incorporating them into either empty liposomes or onto a PLGA core (Thamphiwatana et al., 2017; Ou et al., 2020; Q Zhang et al., 2020). Hybrid M-NPs can be formed by blending macrophage membranes with PEGylated lipids, resulting in M-NP/PEGylated liposomes. This combination leverages the benefits of both components, potentially enhancing the retention time of the nanoparticles in the bloodstream and improving their stability (Jiang et al., 2019). Alternatively, investigators can isolate macrophage plasma membrane proteins and insert them into a synthetic membrane, creating a pseudo-macrophage liposome. This approach allows for more precise control over the nanoparticle composition and properties (Molinaro et al., 2019). Whole cell methods can also introduce polyethylene glycol diacrylate (PEG-DA) into the live cells, followed by the application of ultraviolet (UV) light to form a stable gel in the cytosolic compartment with an intact and stable plasma membrane (Gao et al., 2023). More complex M-NPs incorporate antibiotic cores to enhance bacterial killing. By loading antibiotics within the nanoparticle, these M-NPs can directly target pathogens and improve the efficiency of antimicrobial treatment. In some cases, researchers have explored the use of near-infrared-responsive or microwave-responsive cores for M-NPs (Wang et al., 2018; Fu et al., 2021). When activated, these cores produce hyper-localized heat or reactive oxygen species (ROS), further enhancing the therapeutic effects of the nanoparticles in targeted regions. Differences in M-NP preparation methods can significantly impact their effectiveness in killing bacteria. For instance, in a comparison between the widely used co-extrusion method and the gentler electroporation method, electroporated M-NPs exhibited significantly greater bactericidal activity than coextruded M-NPs (Shi et al., 2021). This suggests that the coextrusion process may
deform or inactivate the membrane proteins responsible for bacterial attachment, leading to less effective killing of bacteria.

The capability of M-NPs to bind lipopolysaccharide (LPS) underscores their potential as potent immune regulators. Numerous studies have shown that M-NPs can effectively sequester LPS, preventing its activation of macrophages and the subsequent propagation of inflammatory responses (Thamphiwatana et al., 2017; Jiang et al., 2019; Molinaro et al., 2019; Shen et al., 2019; Ou et al., 2020; Shi et al., 2021). The binding of LPS by M-NPs is facilitated by specific cell surface proteins rather than non-specific interactions. This specificity is evident from the fact that coincubation with either anti-TLR4 or anti-CD14 antibodies significantly hampers the sequestration of LPS by M-NPs (Thamphiwatana et al., 2017). Furthermore, in a hybrid M-NP/liposome composed of a combination of macrophage membrane and PEGylated lipids, the capacity for LPS binding increased proportionally with the higher percentage of macrophage membrane incorporated into the liposome (Jiang et al., 2019). LPS binding capacity was further enhanced when the source macrophages were genetically modified to overexpress toll-like receptor 4 (TLR4) (Ou et al., 2020). This demonstrates the active involvement of specific cell surface proteins in the effective binding of LPS by M-NPs. In mouse endotoxemia models, M-NPs demonstrated a significant improvement in survival when administered either before, during, or 30 minutes after LPS injection (Jiang et al., 2019; Molinaro et al., 2019; Shen et al., 2019). These beneficial effects were specific to M-NPs and were not observed with RBC-NPs when compared side-by-side (Thamphiwatana et al., 2017).

In addition to intercepting bacterial products, the array of cytokine receptors on macrophages enables M-NPs to intercept host inflammatory signals as well. In experiments involving purified cytokines, M-NPs specifically sequestered IL-6, TNF-α, and IFN-γ in a dose-dependent manner (Thamphiwatana et al., 2017; Fu et al., 2021). In various in vivo mouse models of E. coli peritonitis, osteomyelitis, endotoxemia, and acute pancreatitis, M-NP treatment resulted in decreased levels of inflammatory cytokines in the serum (Thamphiwatana et al., 2017; Shen et al., 2019; Fu et al., 2021; Q Zhang et al., 2021). However, it is essential to acknowledge that determining whether this decrease is solely due to cytokine sequestration is challenging. Nevertheless, these findings underscore the potential of M-NPs as promising candidates for the treatment of sepsis and inflammatory conditions.
Macrophages display a remarkable ability to adapt and alter their plasma membrane protein composition in response to external stimuli. When exposed to bacterial stimuli, macrophages shift towards a pathogen-killing phenotype (Ma et al., 2003). Studies have demonstrated that by exposing live macrophages to either *E. coli* or *S. aureus* before isolating their membranes, the resulting M-NPs exhibit an enhanced ability to trap the specific bacteria to which they were previously exposed (Wang et al., 2018; Shen et al., 2019; Gao et al., 2023). Importantly, these studies did not directly compare the LPS- or cytokine-binding capacity of unactivated M-NPs. In contrast, another group conducted experiments using a transgenic RAW cell line with constitutive TLR4 overexpression. They found that M-NPs derived from these cells exhibited a higher efficiency in binding LPS compared to M-NPs derived from wild-type RAW macrophages (Ou et al., 2020). In gelated macrophages, pre-activation against any bacteria improved survival and bacterial clearance in both *E. coli* and *S. aureus* infections (Gao et al., 2023). This suggests that the level of receptor expression on the macrophage membrane at the time of isolation directly corresponds to the amount of that receptor's ligand that the resulting M-NP can bind. Therefore, there is potential for fine-tuning the binding capacity of M-NPs through pre-activation, which could open up new possibilities for targeted therapeutic interventions in the future.

M-NPs exhibit enhanced efficacy in combating infections when paired with functionalized cores. Cores containing titanium dioxide (TiO₂) generate bactericidal reactive oxygen species (ROS) in response to UV light (Shi et al., 2021). Fe₃O₄/Au nanoparticles can produce ROS and heat in response to microwaves (Fu et al., 2021), while gold nanorods or gold/silver nanocages can generate bactericidal heat in response to near infrared light (Wang et al., 2018; Li et al., 2021). Furthermore, drugs can be loaded into the core of the M-NP or directly into the macrophage membranes, capitalizing on the membranes' ability to evade immune cell clearance and adhere to pathogenic bacteria (Li et al., 2020; Q Zhang et al., 2021). In laboratory studies, the bactericidal effects of the functionalized cores and/or drugs were consistently beneficial. However, the advantages of membrane coating over the naked core were not always straightforward. Membrane coating appeared to be particularly advantageous when macrophages were first exposed to specific bacteria before harvesting their membranes. For instance, mixing the nanogel containing gold rods with membranes from macrophages exposed to *S. aureus* resulted in significantly greater *S. aureus* killing. Similarly, mixing *E. coli*-exposed macrophage membranes with the same nanogel increased the killing of *E. coli*. Interestingly, this effect seemed to be species-specific, as the nanogel containing *S. aureus* membrane had poorer performance against *E. coli* and vice versa (Li et al., 2021). In animal models of *S.*
*aureus* skin infection, osteomyelitis, and peritonitis, macrophage membrane-coated nanoparticles demonstrated a significant decrease in bacterial loads compared to core-only controls (Wang et al., 2018; Li et al., 2020; Shi et al., 2021). Additionally, these models exhibited improved healing outcomes, as indicated by reduced lesion size or increased bone deposition (Wang et al., 2018; Shi et al., 2021). Like N-NPs, M-NPs and gelated macrophages can accumulate at sites of inflammation including infection, atherosclerotic lesions, and tumors (Wang et al., 2021; Yue et al., 2021; Gao et al., 2023, 2024). This allows them to deliver an anti-inflammatory or antibiotic payload efficiently. While some of these effects may be attributed to the increased retention time of the M-NPs *in vivo*, comparisons between pre-activated and unactivated membrane-coated nanoparticles suggest that the membrane identity also contributes to these improvements (Wang et al., 2018). Taken together, the preparation method and the *in vivo* context both play essential roles in determining the effectiveness of M-NPs in combating infections.

**T cells**

T cells play a pivotal role in the immune response, and during sepsis, they undergo extensive apoptosis and transition towards an anti-inflammatory regulatory T cell phenotype. This shift contributes to a secondary immunosuppressive phase, making patients susceptible to opportunistic pathogens (Monneret et al., 2003; Wesche et al., 2005). Despite their crucial role in sepsis and post-septic immunosuppression, the application of T cell membrane-coated nanoparticles (TC-NPs) in infectious diseases has been limited to viral infection models. TC-NPs have shown promising results in neutralizing multiple strains of the human immunodeficiency virus (HIV). The specific suite of membrane proteins on T cells allows TC-NPs to effectively target and neutralize the virus (Wei et al., 2018; G Zhang et al., 2020; Campbell et al., 2021). Furthermore, when loaded with apoptosis-inducing drugs, TC-NPs have exhibited a preferential ability to induce apoptotic cell death in HIV-infected T cells and macrophages, while sparing uninfected cells (Campbell et al., 2021). This targeted approach underscores the potential of TC-NPs as a specific and effective therapeutic strategy in combating viral infections. The exploration of TC-NPs in bacterial sepsis and other infectious diseases represents an area that requires further investigation.

**Manufacturing Challenges and Improvements**

Immune biomimetic nanoparticles can protect the host from pathogenic insult and detrimental endogenous inflammation during sepsis. However, the novel nature of biomimetic nanoparticle
therapy presents novel challenges in their production. First, biomimetic nanoparticle fabrication is a complex multi-step process and can suffer from batch-to-batch variation. To detect and limit this variation, advances in computer modeling can predict nanoparticle-cell interactions based on standard measurements such as shape, size, and zeta potential (Singh et al., 2021; Zhang et al., 2021). By applying these machine-learning based quality control steps, functional batch-to-batch variation may be minimized. The second major manufacturing challenge is in parent-cell production. Acquisition of large numbers of RBCs or platelets only requires access to donated human blood, but immune cells are much more time- and labor-intensive to collect. Rather than collecting directly from donors, the M-NPs and TC-NPs discussed in this review almost exclusively use immortalized cell lines. Sterile mass production of these cells is expensive, but achievable with bioreactors (Wang et al., 2005). Finally, the extraction of the membrane from the parent cell is labor and time intensive (Chugh et al., 2021). Recently, intracellular gelation has been used to separate intracellular contents from their plasma membrane for rapid membrane isolation (Lin et al., 2021, 2024). By understanding and overcoming the unique technical and practical challenges of biomimetic nanoparticle production, the future hope is that they can be safely translated into life-saving therapies for infection and sepsis.

**Conclusion**

Cell membrane-coated nanoparticles present a promising strategy for tackling bacterial infections and sepsis. By mimicking the functions of immune cells, these nanoparticles can adeptly intercept soluble virulence factors and host-derived inflammatory signals, potentially mitigating the detrimental effects of sepsis (Figure 4). Additionally, their capacity to adhere to damaged tissues or microbes enables precise and targeted antibiotic delivery, thereby enhancing the effectiveness of infection treatment.

The complex nature of immune biomimetic nanoparticles positions them well to address the complexity of sepsis. By incorporating multiple functionalities into a single therapeutic system, these nanoparticles offer a novel and innovative approach to combat infectious diseases and sepsis, providing optimism for enhanced patient outcomes and the potential to revolutionize immunomodulatory therapies. Further research is essential to fully unlock their potential across various infectious scenarios and optimize their properties for successful translation into human clinical medicine.
Acknowledgements
A.H. has been supported by the NIH/NIGMS San Diego IRACDA Program (K12GM068524) and NIAID T32AI007469. Our laboratory work in nanotherapeutics is supported by NIH R01 grant AI176554 and the CARB-X Accelerator.

Conflicts of Interest
A.H. declares no conflicts of interest. V.N. serves on the Scientific Advisory Board of Cellics Therapeutics, which is developing membrane-coated nanotherapeutics for medical applications; the company had no role in the content of this manuscript.

Authorship Contributions
Wrote the manuscript: Hoffman, A. and Nizet, V.

Data Availability Statement
This article contains no datasets generated or analyzed during the current study.

References


Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RMH, and Sibbald WJ


Nicolai L, Gaertner F, and Massberg S (2019) Platelets in host defense: experimental and


**FIGURE LEGENDS**

**Figure 1.** Potential for multifactorial mechanism of action of biomimetic nanoparticles compared to targeted sepsis therapeutics. The pathophysiological disturbance to cell and organ system function in sepsis is precipitated and driven by both microbial and host factors. Microbial virulence factors, such as toxins, can directly induce host cell death or dysfunction, while an excessive proinflammatory "cytokine storm" can drive hypotension, ventilation-perfusion mismatch, coagulopathy, and multi-organ system failure. A number of investigational sepsis therapeutics, including monoclonal antibodies, have been targeted to inactivate specific microbial virulence factors, such as a pore-forming toxin. Others, for example, soluble cytokine receptors, seek to neutralize a key individual pro-inflammatory host factor. Because a host...
immune cell membrane-coated nanoparticle mimics the natural surface of the cell from which it was derived, it has the potential to bind and sequester diverse bacterial and host cell factors in a pathogen-agnostic or toxin-agnostic manner, to reduce diverse harmful stimuli in the infected host experiencing sepsis.

**Figure 2. Biomimetic membrane-coated nanoparticles retain the surface architecture of the cell from which they were derived.** Following self-assembly on a precision engineered nanocore of a specified size, for ~100 nM, and chosen composition, such as the biodegradable polymer poly(lactic-co-glycolic acid), host cell membranes retain their native, properly-oriented lipid bilayer architecture. Surface expressed structures including proteins and glycoconjugates reflect the parent cell of origin, such as CD47 present on red-blood cell membranes, which serves as a "don't eat me signal" to prolong circulating half-life of the nanoparticle, or Toll-like receptor-4 (TLR4) present on macrophage membranes, which can capture and neutralize bacterial lipopolysaccharide.

**Figure 3. Shared and unique properties of immune cell membrane-coated biomimetic nanoparticles.** (A) By presenting an intact host cell-derived lipid bilayer on their nanoscale surface, both red blood cell-derived and immune cell-derived nanoparticles can absorb and neutralize harmful bacterial pore-forming toxins, thus serving as a "nanosponge" to limit cell death and toxicity. (B) Immune cell membrane-derived nanoparticles, for example macrophage membrane biomimetic nanoparticles, by virtue of their specific array of surface receptors, provide additional immunomodulatory mechanisms of action beyond toxin neutralization, as they can bind and inactivate bacterial endotoxins (e.g. lipopolysaccharide) and pro-inflammatory cytokines to mitigate the initiation and propagation of cytokine storm.
Figure 4. Mechanisms of action of biomimetic cell membrane-coated nanoparticles.

Potential therapeutic benefits in the setting of severe infection and sepsis reflect the particular surface properties and receptors of the parent cell from which the membranes were derived.
Figure 1
Figure 2
Figure 3
<table>
<thead>
<tr>
<th>Red Blood Cell Nanoparticle</th>
<th>Platelet Nanoparticle</th>
<th>Neutrophil Nanoparticle</th>
<th>Macrophage Nanoparticle</th>
<th>T cell Nanoparticle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sequester bacterial toxins</strong></td>
<td><strong>Sequester bacterial toxins</strong></td>
<td><strong>Sequester bacterial toxins</strong></td>
<td><strong>Sequester bacterial toxins</strong></td>
<td><strong>Sequester bacterial toxins</strong>*</td>
</tr>
<tr>
<td><strong>Sequester endotoxin</strong></td>
<td><strong>Sequestrer endotoxin</strong>*</td>
<td><strong>Sequester inflammatory cytokines</strong></td>
<td><strong>Sequester inflammatory cytokines</strong></td>
<td><strong>Sequester inflammatory cytokines</strong></td>
</tr>
<tr>
<td><strong>Localize to inflammed tissue</strong></td>
<td><strong>Localize to inflammed tissue</strong></td>
<td><strong>Localize to inflammed tissue</strong></td>
<td><strong>Localize to inflammed tissue</strong></td>
<td><strong>Sequester inflammatory cytokines</strong>*</td>
</tr>
<tr>
<td><strong>Sequester inflammatory cytokines</strong></td>
<td><strong>Sequester inflammatory cytokines</strong></td>
<td><strong>Sequester inflammatory cytokines</strong></td>
<td><strong>Sequester inflammatory cytokines</strong></td>
<td><strong>Sequester inflammatory cytokines</strong></td>
</tr>
</tbody>
</table>

*Italic font: predicted mechanism of action based on receptor expression

**Bold font: experimentally proven mechanism of action.**

**Figure 4**