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Mini-Review

Extracellular Vesicles: A Key Regulator of Macrophage Polarization During Bacterial Infection

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ABSTRACT

Extracellular Vehicles (EVs), including bacterial-derived EVs (bEVs) and host cell-derived exosomes, serve as critical mediators of intercellular communication in bacterial infections. Macrophage polarization into pro-inflammatory M1 or anti-inflammatory M2 subtypes is a central event in the innate immune response. In bacterial infection scenarios, EVs regulate macrophage polarization through molecular mechanisms such as Pattern Recognition Receptors (PRRs) signaling activation and metabolic reprogramming by carrying specific cargo components including proteins and nucleic acids. Pathogen-specific EVs exhibit distinct regulatory patterns, such as *Escherichia coli (E. coli)* EVs modulating polarization via pathogenicity-dependent differentiation effects and *Staphylococcus aureus* EVs regulating non-classical polarization. This mini-review summarizes the mechanisms by which EVs regulate macrophage polarization, pathogen-specific regulatory patterns, and the clinical translational potential.

Key words:

Bacterial infection; extracellular vesicles; macrophage polarization

INTRODUCTION

Macrophages are pivotal innate immune cells that adapt their phenotypes to combat pathogens or promote tissue repair, primarily differentiating into M1 and M2 subtypes. M1 macrophages secrete pro-inflammatory cytokines (e.g., Tumor Necrosis Factor-Alpha (TNF-α) and Interleukin-1 Beta (IL-1β)) to eliminate bacteria, while M2 macrophages produce anti-inflammatory factors (e.g., IL-10, Transforming Growth Factor Beta (TGF-β)) to resolve inflammation and facilitate healing. Extracellular Vesicles (EVs) are lipid bilayer-enclosed nanovesicles secreted by both bacteria and host cells, carrying bioactive molecules such as proteins, nucleic acids, and lipids. Bacterial EVs (bEVs) include Outer Membrane Vesicles (OMVs) from Gram-negative bacteria and Cytoplasmic Membrane Vesicles (CMVs) from gram-positive bacteria, while host-derived exosomes are released by macrophages and other immune cells during infection. EVs transfer their cargo to target cells, thereby modulating macrophage polarization and shaping the immune response [1]. This mini-review focuses on the mechanisms underlying EV-mediated regulation of macrophage polarization and the functional differences among various bacterial EVs.

2. The core mechanisms of EVs regulating macrophage polarization

2.1 Pattern recognition receptor-mediated signal activation mechanism

In the immune regulatory network of bacterial infection, PRRs serve as core molecular hubs, They mediate precise interactions between bacterial-derived EVs and host macrophages, establishing a multi-layered, specific signaling activation network. This framework transcends the traditional M1/M2 binary polarization paradigm, enabling fine-tuned regulation of macrophage polarization and functional phenotypes. PRRs thus serve as pivotal molecular switches linking infection clearance to pathological progression. Toll-Like Receptors (TLRs), PRRs, and NOD-Like Receptors (NLRs)-collaborate synergistically. By recognizing diverse bioactive cargo carried by EVs, they initiate

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differentiated signaling pathways, conferring multifaceted plasticity to the immune response within the infectious microenvironment.

TLR Pathway: TLRs, as the core subtype of the PRR family, regulate macrophage polarization through "subtype-specific signaling diversion." Pathogen-Associated Molecular Patterns (PAMPs) carried by bacterial EVs-such as Lipopolysaccharides (LPS), lipoproteins, peptidoglycans, and nucleic acids [2]-can precisely target different TLR subtypes on the macrophage surface to initiate distinct signaling pathways: LPS on gram-negative bacterial OMVs specifically recognizes TLR4, activating the TRIF-mediated signaling pathway via endocytosis-dependent mechanisms rather than the MyD88 pathway. This triggers Interferon Regulatory Factor 3 (IRF-3) phosphorylation and Nuclear Factor Kappa B (NF-κB) pathway activation, driving type I interferon (IFN-α/β) secretion and upregulating Interferon-Stimulated Genes (ISGs) such as Mx1 and IFIT1 and other ISGs, inducing an antiviral phenotype in macrophages that directly inhibits influenza virus and vesicular stomatitis virus replication. Simultaneously, it releases proinflammatory factors such as C-X-C Motif Chemokine Ligand 8 (CXCL8) and IL-1β [3]. Lipoproteins and peptidoglycans preferentially bind TLR2 (often forming heterodimers with TLR1/TLR6), activating Protein Kinase C (PKC) and Mitogen-Activated Protein Kinase (MAPK) pathways including Extracellular Signal-Regulated Kinase (ERK) and Jun N-Terminal Kinase (JNK). This enhances macrophage M1 polarization and pathogen phagocytic killing capacity [4]. Bacterial nucleic acids encapsulated within EVs can be recognized by TLR7/8 (Ribonuclic Acid (RNA)) and TLR9 (CpG Deoxyiribonuclic Acid (DNA)), further amplifying inflammatory or antiviral signaling cascades. Bacteria can also finely regulate TLR activation efficiency by modifying PAMP structures (e.g., altering the acylation or phosphorylation state of LPS lipopolysaccharide), enabling immune evasion or inflammatory amplification. Concurrently, virulence factors carried by EVs (e.g., Cytotoxin-Associated Gene A Protein (CagA), Peptidylarginine Deiminase (PPAD)) can synergize with TLR signaling, reshaping macrophage function through cross-pathway crosstalk [2].

PRR Pathway: PRRs serve as pivotal molecular hubs regulating macrophage polarization in bacterial-derived EVs, offering a novel paradigm for immune intervention against intractable bacterial infections such as implant-associated infections. Bacterial EVs carry PAMPs like lipopolysaccharides and nucleic acids, or functional proteins such as Cell Division Control Protein 42 (CDC42) delivered by Colorectal Cancer-Derived EVs (CRC-EVs), which bind specifically to surface or intracellular PRRs on macrophages: TLR subtypes activate distinct signaling pathways through "ligand-receptor-signal" branching, driving diverse phenotypic polarization [5]. Generalized PRR-engineered hybrid vesicles mBMSCCXCR4 @OMVs, which accumulate at infection sites via the CXC Chemokine Receptor-4 (CXCR4)-Stromal Cell-Derived Factor-1 (SDF-1) axis. Activation of the TLR/NF-κB pathway promotes M1 polarization, upregulates markers such as CD86 and inducible Nitric Oxide Synthase (iNOS), enhances phagocytic and cytotoxic capabilities, and initiates adaptive immunity. This creates a synergistic "innate-adaptive immune" effect with long-lasting immune memory. Nucleotide-Binding Oligomerization Domain 1 (NOD1) recognizes peptidoglycan derivatives or CDC42 carried by EVs, initiating dual-pathway signaling to regulate metabolic reprogramming or pro-inflammatory factor release [6].

NOD-like receptor: NOD receptors, as the core hub of cytoplasmic PRRs, profoundly regulate macrophage function through specific coupling of "multiple cargo-NOD receptor-downstream signaling" and dual-pathway activation. On one hand, peptidoglycan derivatives (e.g., γ-d-Glutamyl-Meso-Diaminopimelic Acid (iE-DAP)) carried by bacterial EVs directly activate NOD1 upon entering macrophages, downregulating Perilipin 5 (PLIN5) expression *via* the p38/PPARα signaling pathway. This inhibits Fatty Acid Oxidation (FAO) and accumulates palmitic acid, subsequently promoting membrane localization of the co-stimulatory molecule OX40L through protein palmitoylation. This activates $CD8^+$ T cell immunity, forming an "anti-infective regulatory axis" characterized by metabolic reprogramming and immune cascade amplification [7]. On the other hand, functional proteins such as the Rho family small Guanosine Triphosphate (GTPase) CDC42 delivered by EVs switch to the GTP-bound active state within macrophages, specifically activating NOD1. This recruits Receptor-Interacting Protein Kinase 2 (RIP2) to initiate NF-κB and p38-MAPK signaling pathways, inducing the release of proinflammatory factors including IL-6, CCL1, CCL2, driving macrophage polarization toward a proinflammatory phenotype and even contributing to the pathological progression of infection-associated tumors. Both activation pathways operate independently of direct bacterial contact and exhibit highly efficient, sustained signaling characteristics. NOD receptor-mediated polarization exhibits dual properties of "immune defense" and "pathology-oriented" effects: It enhances bacterial clearance during anti-infective immunity while preventing systemic inflammatory storms [5].

2.2 A new mechanism of polarization regulation mediated by metabolic reprogramming

Iron metabolism: In bacterial infections, EVs serve as a central regulatory hub for iron metabolism, precisely modulating macrophage polarization through multiple pathways and participating in both host immune responses and pathogen pathogenicity(as shown in Table 1). EVs from diverse sources-including apoEVs released by host apoptotic cells, bacterial-secreted Outer Membrane Vesicles (OMVs)/MEVs, and engineered EVs-all exert their effects through iron metabolism: apoEVs surface-enrich iron-related receptors like TfR and CD163, enabling efficient capture of serum ferritin. This process restricts bacterial iron acquisition via iron sequestration, reshapes macrophage iron metabolism, and promotes anti-inflammatory polarization [8]. Bacterial OMVs encapsulate iron metabolism-regulating sncRNAs (e.g., E. coli RyhB, Pseudomonas aeruginosa PA2952.1), which are delivered across cells to target host macrophage iron metabolism pathways, thereby regulating the M1/M2 polarization balance [9]. Mycobacterium tuberculosis MEVs carry iron carriers and iron-regulating proteins, disrupting the function of macrophage

membrane iron transporters and leading to intracellular iron accumulation. Simultaneously, they promote M2 polarization through TLR2 agonists and antioxidant proteins, thereby weakening the host's clearance capacity [10]. Engineered EVs (e.g., apoEV@MSN) integrate the membrane of natural apoEVs with microRNA-146a-loaded MSNs, combining iron capture capacity with anti-inflammatory activity. They suppress pro-inflammatory factor release by regulating the NF-κB pathway, promoting macrophage M2 polarization. Further development of the apoEV microRNA (miR)-146a@MSN-Red Blood Cells (RBC) complex enhances circulation stability and iron capture efficiency, ameliorates iron overload in sepsis, mitigates inflammatory injury, and prolongs host survival [8]. Additionally, EVs can indirectly modulate macrophage iron transporter activity by reshaping the microbiota structure-such as enriching Gram-positive bacteria that produce short-chain fatty acids-thereby aiding in polarization regulation [11].

Lipid metabolism: In bacterial infections, EVs precisely regulate macrophage polarization through lipid metabolic reprogramming, exhibiting infection-specific and time-dependent characteristics. Regulatory mechanisms vary among EVs from different sources. Following Salmonella infection, host-derived EVs undergo dynamic remodeling of their glycerophospholipid, sphingolipid, and isoprenoid lipid composition: Early (24h) enrichment of ceramides and phosphatidylethanolamine, followed by later (48h) upregulation of sphingomyelin and coenzyme Q8. These changes influence EV biogenesis, membrane interactions, and metabolic states, activating NF-κB and MAPK pathways to regulate polarization[12]. Apoptotic EVs (ApoEVs) derived from mesenchymal stem cells, in bacterial infection-associated skin injuries, hydrolyze membrane phospholipids via $sPLA_2$-X within the inflammatory environment, releasing Docosahexaenoic Acid (DHA) which is metabolized into Resolvin D5 (RvD5). This inhibits TNF-α secretion, promotes the release of TGF-β and other factors, and drives macrophages toward an anti-inflammatory repair phenotype [13]. Both types of EVs regulate polarization through the core mechanism of "lipid component remodeling-active molecule release-signaling pathway activation". The former focuses on the pro-inflammatory/anti-inflammatory balance within the infectious microenvironment, while the latter emphasizes the resolution of wound inflammation and tissue regeneration.

Table 1: EV-mediated regulation of macrophage polarization *via* metabolic reprogramming

Metabolic dimension	Origin of vesicles	Effect on macrophage	References
Iron metabolism	Host apoEVs	M2 (Anti-inflammatory)	[8]
Iron metabolism	Bacterial OMVs/MEVs	M1/M2 Balance Regulation	[9]
Iron metabolism	Mycobacterium tuberculosis MEVs	M2 (Weakened clearance)	[10]
Iron metabolism	Engineered EVs	M2 (Anti-inflammatory)	[8]
Lipid metabolism	infected host EVs	M1/M2 dynamic regulation	[12]
Lipid metabolism	Apoptotic EVs	M2 (Repair phenotype)	[13]

Specific differences in the regulation of different types of bacterial EVs

EVs derived from different bacterial sources exhibit distinct regulatory characteristics when modulating macrophage polarization, owing to variations in strain virulence, gram-negative classification, and survival strategies(as shown in Table 2). Among Gram-negative bacteria, the regulatory effects of *E. coli* EVs show marked differentiation based on pathogenicity: EVs from common clinical isolates (70-210 nm cup-shaped structures) loaded with LPS and OmpA induce ROS production in a dose-dependent manner after macrophage internalization, mildly favoring M1 polarization, with no cytotoxicity at low concentrations [14]. Highly pathogenic APEC strains' EVs drive potent proinflammatory polarization *via* the TLR4/MyD88/NF-κB pathway and NLRP3 inflammasome, while also inducing macrophage apoptosis and NET formation, exhibiting significantly enhanced pathogenicity. LPS serves as the key regulatory molecule [15]. Probiotic *E. coli* Nissle 1917 (EcN) EVs (50-200 nm) are fundamentally distinct from pathogenic strains. They carry metabolites and outer membrane proteins, exhibit no cytotoxicity, and promote M0-to-M1 polarization through metabolic reprogramming while enhancing macrophage function [16]. Engineered EcN derivatives (ENZC) release bis-nanobodies *via* near-infrared response, targeting immune checkpoint regulation and TDE distribution to drive M2-type TAMs toward M1 polarization, highlighting their mild regulatory advantage [17]. Gram-positive bacteria and intracellular pathogens exhibit more distinctive regulatory patterns: *Staphylococcus aureus* (including Methicillin-Resistant *Staphylococcus aureus* (MRSA)) EVs do not rely on classical M1/M2 polarization. Conventional SAVs (50-300 nm) activate the TLR2-MyD88-p38 MAPK pathway, specifically blocking macrophage phagocytosis [18]. MRSA-induced MM-EVs target TRAF6 *via* TNF-α and miR-146a-5p, inducing necrotic apoptosis in alveolar macrophages, both independent of TLR4 signaling [19]. Brucella EVs (30-120 nm disc-shaped structures) carry 80 bacterial antigens, driving M1 polarization through time-dependent activation of the NF-κB/p65 pathway, enhancing antimicrobial capacity and inducing specific immune responses

[20]. Mycobacterium tuberculosis EVs (123-154 nm) primarily induce differentiation of nave monocytes. They promote cell adhesion and cytoskeletal rearrangement *via* the ERK1/2-MK-2-NF-κB pathway. The differentiated macrophages exhibit full antimicrobial function, with regulation independent of AKT signaling. This mechanism differs markedly from the polarizing or damaging effects observed with EVs from other bacteria [21].

Table 2: Characteristics and regulatory effects of pathogen-derived EVs

Pathogen source	Physical traits	Signaling pathways	Polarization	References
E. coli	70-210nm	ROS production	Mildly biased toward M1	[14]
APEC	-	TLR4/MyD88/NF-κB; NLRP3 inflammasome	Potent pro-inflammatory (M1);NETs	[15]
ECN	50-200nm	Metabolic remodeling	M0→M1	[16]
ENZC	Bacterial derivatives	NIR light-triggered nanobody release	M2→M1	[17]
S. aureus	50-300nm	TLR2-MyD88-p38 MAPK	Impaired phagocytosis	[18]
MRSA	-	TRAF6 targeting (*via* TNF-α/miR-146a-5p)	Necrotic apoptosis	[19]
Brucella	30-120nm	NF-κB/p65 activation	M1 (Tim dependent)	[20]
Mycobacterium tuberculosis	123-154nm	ERK1/2–MK2–NF-κB	Monocyte to Macrophagedifferentiation	[21]

CONCLUSION AND FUTURE PERSPECTIVE

In bacterial infections, EVs derived from different pathogen deliver specific bioactive molecules such as protein and nucleic acids. They specifically regulate macrophage polarization through multiple mechanisms, including TLR, PRR, and NOD receptor-mediated signaling pathways, combined with metabolic reprogramming of iron and lipid metabolism, forming a unique regulatory network. Their regulatory patterns exhibit significant heterogeneity-some rely on molecules like LPS, while others adopt unique approaches such as efferocytosis inhibition-profoundly influencing the infection process and immune outcomes. These findings clarify the core role of EVs in bacteria-host interactions and provide multiple targeted strategies for infection intervention, including "receptor-pathway-phenotype," "metabolic pathway," and "specific molecular target," laying the foundation for anti-inflammatory therapies, vaccine development, and engineered EV applications. EVs hold enormous potential for clinical translation: they can serve as a source of early diagnostic biomarkers and engineered drug delivery carriers, demonstrating application value in early infection detection, vaccine development, and targeted drug administration. Currently, EV research still faces challenges such as heterogeneity and the lack of standardized isolation methods, and the exact metabolic mechanisms by which EVs regulate macrophage polarization require further exploration. Future studies should focus on regulatory heterogeneity at the single-cell level, the systemic effects of microbiome-derived EVs, and the optimization of targeted delivery systems to promote the translation of basic research into clinical practice, providing new strategies for the prevention and treatment of infectious diseases.

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