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REVEIW ARTICLE

Probiotic-Derived Extracellular Vesicles as Immunomodulatory Agents in Autoimmune Disease Management

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ABSTRACT

Probiotics have been extensively studied for their ability to restore microbial balance, strengthen the intestinal barrier, and regulate immune responses in humans. Among their bioactive components, probiotic-derived extracellular vesicles (PEVs) have emerged as key mediators of host–microbe interactions. PEVs are lipid-bilayer-enclosed postbiotic secreted by both Gram-negative and Gram-positive probiotic species, carrying diverse molecular cargo including proteins, lipids, nucleic acids, and metabolites. These vesicles enable targeted communication between probiotics and host cells, allowing them to exert biological effects independent of the viability of the parent bacteria. However, the biological activity and therapeutic potential of PEVs are influenced by multiple factors, such as the bacterial species of origin, culture conditions, and the methods used for their isolation and purification. Growing evidence indicates that PEVs play pivotal roles in modulating inflammation, enhancing gut barrier integrity, regulating metabolic pathways, and shaping host immune responses. This review summarizes an overview of current knowledge regarding the characteristics, functional mechanisms, and biomedical applications of PEVs, with a particular emphasis on their emerging role in the management of autoimmune-mediated disorders.

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INTRODUCTION

Autoimmune diseases are a heterogeneous group of disorders in which the immune system mistakenly targets self-antigens and host tissues, resulting from loss of self-tolerance [1,2]. Conditions such as rheumatoid arthritis, type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, and various autoimmune disorders of the gut can cause significant health problems and place a heavy burden on healthcare systems [3]. Conventional treatments, including immunosuppressant (e.g., prednisone and cyclosporine) and biologic agents (e.g., infliximab, adalimumab), can effectively control unwanted immune responses. However, their clinical application is often constrained by adverse effects, including heightened susceptibility to infections, an increased risk of malignancy, metabolic disturbances, and organ-specific toxicities, particularly involving the liver and kidneys [4, 5].

Extensive research has demonstrated that the human microbiota and the immune system engage in highly intricate and dynamic interactions, with

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microbial communities exerting a significant influence on the development and progression of autoimmune diseases [6]. A key example involves disruptions to the gut microbiota, known as dysbiosis, alongside increases in intestinal permeability. These alterations permit microbial components or dietary antigens to translocate across the intestinal barrier, triggering aberrant immune responses and ultimately contributing to the breakdown of immune tolerance [7].

Probiotics have been widely explored for their ability to restore microbial balance, reinforce the intestinal barrier in humans and regulate immune responses, However, their clinical use faces several challenges, including differences between bacterial strains, limited survival rate through the gastrointestinal tract and safety concerns in immunocompromised individuals [8, 9]. To overcome these limitations, postbiotics which include non-viable microbial cells, their structural components, and secreted metabolites have emerged as a promising next-generation alternative for modulating gut and immunity [10]. Among these postbiotics entities, probiotic-derived extracellular vesicles (PEVs) have emerged as a promising therapeutic class. By encapsulation diverse bioactive molecules and modulating host cellular and immune pathways, PEVs offer a novel strategy for immune regulation [11]. This review examines the current evidence supporting the application of PEVs in the prevention and management of autoimmune diseases and discusses the key advancements, limitations, and translational challenges associated with their therapeutic development.

BIOGENESIS AND COMPOSITION OF PEVS

Generally, extracellular vesicles (EVs) are spherical, lipid bilayer–enclosed nanoparticles released by nearly all living cells, including both eukaryotic and prokaryotic organisms [12]. Historically regarded more as cellular waste products, EVs are now recognized as crucial mediators of intercellular communication, carrying diverse bioactive molecules that influence physiological and pathological processes [13]. EVs possess a selectively packaged molecular cargo from their parent cells, including proteins, lipids, and nucleic acids such as mRNA and microRNA, enclosed within a protective lipid bilayer rich in sphingolipids, phospholipids, and cholesterol [14, 15].Unlike mammalian EVs, which typically carry universal marker proteins such as tetraspanins (CD9, CD63, and CD81), probiotic-derived PEVs lack these markers, as tetraspanins are absent in bacterial systems [16, 17].

Table1. Summarizes the general differences between PEVs derived from gram negative and gram positive probiotics. PEVs from Gram-negative bacteria are typically referred to as outer membrane vesicles (OMVs) because they bud outward from the outer membrane. They possess a bilayer membrane containing components characteristic of the Gram-negative cell envelope, including lipopolysaccharides (LPS), outer membrane proteins (OMPs) such as OmpA, porins, phospholipids, and lipoproteins. Their luminal content is enriched with enzymes, nucleic acids (DNA/RNA), metabolic signaling molecules, antioxidants, and a diverse range of small regulatory RNAs [18]. In probiotic species such as Escherichia coli Nissle 1917 and Akkermansia muciniphila, OMVs have been shown to contain immunomodulatory factors that enhance epithelial barrier function and regulate host inflammatory signaling [19, 20]. Importantly, LPS associated with probiotic OMVs typically exhibits modified lipid A structures, producing lower endotoxic activity and enabling beneficial rather than inflammatory responses [21]. In contrast, Gram-positive bacteria, which lack an outer membrane but have a thick peptidoglycan layer, produce vesicles with different structural origins and compositions. PEVs from Gram-positive probiotics ,such as Lactobacillus, Bifidobacterium, and Bacillus species, are generated through cytoplasmic membrane protrusion across the peptidoglycan matrix [22, 23]. These vesicles are typically rich in lipoteichoic acid (LTA), membrane-associated proteins, surface adhesins, peptidoglycan fragments, and exopolysaccharides. They also enclose biologically active cargos including proteins, enzymes, metabolic intermediates, microRNAs-like molecules, and bacteriocins that contribute to probiotic antimicrobial and immunoregulatory effects [24]. Since Gram-positive vesicles lack LPS, they generally induce lower pro-inflammatory signaling and may be better suitable for therapeutic and nutraceutical applications where safety is critical [24,25].

Overall, while both Gram-negative and Gram-positive PEVs share core biochemical features, such as proteins, lipids, nucleic acids, and bioactive metabolites, their membrane composition,

Table 1. General comparison of PEVs Derived from gram-Negative and gram-positive postbiotics

Feature	Gram-Negative PEVs	Gram-Positive PEVs
Membrane origin	Mainly Budding from the outer membrane	Cytoplasmic membrane protrusion through thick peptidoglycan
Size range	20–400 nm	50–300 nm
Membrane components	Lipopolysaccharides (LPS), outer membrane proteins (OmpA, porins), phospholipids, lipoproteins	Lipoteichoic acid (LTA), surface adhesins, membrane proteins, peptidoglycan fragments, exopolysaccharides (EPS)
Cargo contents	DNA, RNA, small regulatory RNAs, enzymes, antioxidants, metabolic signaling molecules	Enzymes, metabolic intermediates, microRNA-like molecules, bacteriocins
Stability	Sensitive to environmental stress	More physically stable due to thick wall origin
Immunogenicity	Reduced endotoxin activity due to modified lipid A; moderate immune activation	Low inflammatory potential due to the absence of LPS
Example probiotic genus / strains	*Akkermansia muciniphila*, *Escherichia coli* Nissle 1917	*Lactobacillus*, *Bifidobacterium*, *Bacillus*
Biological functions	Enhancing epithelial barrier integrity, regulating inflammation, gut homeostasis, Obesity control Metabolic regulation, , microbiome communication	Antimicrobial activity, immune modulation, anti-inflammatory effects
Applications	Vaccine adjuvants, antimicrobial delivery, immunotherapy	Probiotic therapies, nutraceuticals

structural origin, and immunological properties differ substantially. These differences influence vesicle stability, cell targeting, and functional bioactivity, shaping their potential roles in modulating host metabolism, gut microbiota balance, and obesity associated inflammatory pathways [26-28].

ISOLATION AND CHARACTERIZATION OF PEV

Establishing universal and standardized methodologies for the isolation of PEVs is critical for overcoming the major technical and conceptual obstacles that currently hinder progress in PEVs research and development [29]. Several well-established isolation methods are currently used to purify PEVs, each method offers distinct advantages and limitations.

Differential ultracentrifugation (DUC), widely regarded as the gold standard, separates vesicles based on density and sedimentation rate through sequential high-speed centrifugation steps [30]. Size-exclusion chromatography (SEC) provides a gentle and highly reproducible approach for isolating PEVs especially in large scale preparations. By fractionating components according to hydrodynamic size, SEC efficiently isolates vesicles from soluble proteins, metabolites, culture media residues, and other low-molecular-weight contaminants. Importantly, because SEC does not involve harsh physical forces or chemical precipitation, it preserves vesicle morphology, membrane integrity, and biological activity. This makes SEC particularly suitable for functional assays and multi-omics characterization of probiotic-derived EVs [31].

Polymer-based precipitation techniques provide a rapid, simple, and scalable alternative for processing large fermentation volumes. These approaches aggregate vesicles by reducing their solubility, enabling collection via low-speed centrifugation. While advantageous for routine or high-throughput workflows, polymer precipitation may co-isolate protein aggregates, nucleic acids, and other impurities, compromising purity and downstream analyses. Consequently, additional cleanup steps—such as SEC or density-based separation, are often required to enhance vesicle quality [31, 32]. Ultrafiltration (UF) utilizing membrane filters with defined molecular-weight cutoffs (typically 100–300 KDa) represents another widely used and efficient concentration method for EVs from postbiotic samples. UF enables rapid volume reduction while avoiding high shear stress; however, it does not discriminate between vesicles and co-concentrated soluble

proteins or macromolecules. Therefore, UF is frequently combined with SEC, density gradient ultracentrifugation, or tangential-flow filtration to improve purity [33]. Immunoaffinity-based capture techniques employ antibodies or ligand-binding molecules to selectively isolate specific EV subpopulations based on surface markers. While offering high specificity and valuable mechanistic insight, this approach is limited by the lack of universally recognized biomarkers for probiotic-derived EVs and by relatively low recovery yields. As a result, immunocapture is better suited for analytical research rather than large-scale bioprocessing [31, 34]. Finally, microfluidic and lab-on-chip systems represent emerging technologies capable of isolating vesicles through physical characteristics such as electric charge, acoustic forces, or microchannel hydrodynamics. These platforms provide rapid processing, minimal sample handling, and compatibility with small sample volumes. However, their application to postbiotic matrices remains at an early stage, requiring further optimization to manage sample complexity and particle heterogeneity [35, 36].

Equally essential to the isolation of postbiotic PEVs is their comprehensive characterization, which confirms vesicle identity, purity, and functional potential particularly when transitioning toward large-scale production and industrial applications [13, 37]. Rigorous characterization is critical to ensure that the isolated particles are true vesicles rather than protein aggregates, cell debris, or other contaminants frequently found in bacterial culture supernatants [13, 25, 38].

Standardized analytical methods form the foundation of reliable PEVs assessment. Techniques such as nanoparticle tracking analysis to determine size distribution and concentration, transmission electron microscopy for visualization of vesicle morphology, proteomic and lipidomic profiling to define molecular composition, and functional bioassays to evaluate biological activity are indispensable for establishing batch-to-batch consistency and identifying critical quality attributes [39, 40]. As the field moves closer to clinical translation and industrial manufacturing, characterization frameworks aligned with emerging regulatory standards will be vital for ensuring the safety, reproducibility, and therapeutic efficacy of PEVs-based products [39, 41]. Typically, PEVs range from 20 to 300 nm in diameter, although their size varies according to bacterial species, environmental conditions, and vesiculation mechanisms [42,43]. Morphologically, PEVs appear spherical to cup-shaped under transmission electron microscopy, consistent with other biological vesicle types [25, 44, 45].

The molecular cargo of PEVs reflects their bacterial origin and is highly diverse. It typically includes cell-envelope proteins such as lipoproteins, porins, and adhesins; metabolic and stress-response enzymes; and chaperone proteins such as *GroEL* and *DnaK*. Immune-active components including lipoteichoic acid (LTA), peptidoglycan fragments, muramyl peptides, and various surface antigens enable activation of host immune pathways such as TLR2- and NOD-dependent signaling. Beyond proteins, PEVs contain a range of lipids and carbohydrates, including phospholipids, glycolipids, sphingolipids, and exopolysaccharides, which influence vesicle stability and interactions with host cells. They also encapsulate nucleic acids (DNA, mRNA, and small regulatory RNAs) as well as bioactive metabolites such as short-chain fatty acids, indoles, and vitamins [42,44,45]. This rich, multifaceted cargo endows PEVs with broad biological functions and therapeutic promise, including immunomodulatory and anti-inflammatory effects, strengthening of the intestinal barrier, antimicrobial activity, and metabolic regulation [9, 10].

STABILITY AND UPTAKE OF PEV

The stability of probiotic-derived PEVs is a critical factor influencing their biological functionality, storage feasibility and therapeutic potential. The lipid bilayer structure of PEVs provides intrinsic protection to encapsulated proteins, nucleic acids, and metabolites. This structure allows them to remain stable under a variety of environmental conditions, including fluctuations in pH, temperature, and enzymatic activity. However, vesicle stability may vary depending on the producing bacterial strain, membrane composition, and isolation method [46, 47].

PEVs exhibit considerable stability under gastrointestinal conditions, making them promising candidates for oral administration. However, extended exposure to strong acidity, mechanical shear stress, or repeated freeze–thaw cycles may cause them to aggregate, disrupt their membranes, or lose biological activity. To preserve their structural integrity during storage, methods such as lyophilization, cryopreservation with

cryoprotectants (e.g., trehalose or sucrose), and encapsulation in polymeric or lipid-based carriers are commonly used. Developing standardized stability testing frameworks is essential for defining optimal storage conditions, ensuring batch-to-batch consistency, and enabling clinical grade manufacturing and distribution [48, 49].

The cellular uptake of PEVs is a key mechanism underlying their biological activity and therapeutic potential. PEVs can interact with recipient cells through several pathways, including endocytosis (clathrin-dependent or caveolin-mediated), macropinocytosis, phagocytosis, and direct membrane fusion, depending on vesicle size, membrane composition, and surface ligand–receptor interactions. Surface molecules such as lipoteichoic acids, lipoproteins, and polysaccharides play essential roles in determining cellular targeting and internalization efficiency. After uptake, PEVs typically traffic through the endosomal pathway, where they can release their cargo including proteins, small RNAs, metabolites, and signaling molecules into the cytoplasm or other intracellular compartments, ultimately modulating gene expression, immune responses, metabolic pathways, and intercellular communication. Importantly, PEVs demonstrate efficient uptake in intestinal epithelial cells, immune cells, and adipocytes, supporting their relevance in both gut–immune and gut–metabolic interactions. Understanding the molecular determinants governing PEV uptake is crucial for optimizing targeted delivery strategies and enhancing their use in therapeutic and nutraceutical applications [50-53].

Variations in isolation procedures such as centrifugation conditions, filtration methods, chromatographic separation, and precipitation reagents can lead to inconsistencies in vesicle yield, purity, and biological function. Therefore, establishing harmonized and reproducible isolation protocols is crucial for ensuring cross-laboratory comparability, supporting quality-control standards, and enabling regulatory approval. Such standardization is essential for advancing PEVs research from fundamental studies toward preclinical evaluation and clinical translation, where scalable and GMP-compliant production processes are required [54-56].

MECHANISMS OF IMMUNOMODULATION BY PEVS

PEVs exert potent immunomodulatory effects through a range of interconnected mechanisms that collectively influence the entire immune system, including both innate and adaptive immune responses. These nanoscale vesicles contain a diverse repertoire of functional biomolecules that enable targeted interactions with host immune cells and signaling pathways [43].

PEVs regulate innate immunity primarily through the modulation of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I–like receptors (RLRs), C-type lectin receptors (CLRs), and DC-SIGN, located on various innate immune cells such as macrophages, dendritic cells, and epithelial cells .[57]Engagement of these receptors can attenuate pro-inflammatory signaling cascades, including NF-κB and MAPK pathways, resulting in decreased secretion of cytokines such as TNF-α, IL-6, and IL-1β. Concurrently, PEVs can promote anti-inflammatory cytokines—including IL-10 and TGF-β thereby fostering an immunoregulatory environment [58-60]. For example, EVs from Lactobacillus rhamnosus JB-1enriched in lipoteichoic acid have been shown to activate TLR2 signaling in dendritic cells, promoting IL-10 production and regulatory T-cell differentiation [61].

In adaptive immunity, PEVs influence T cell polarization and regulatory networks. Experimental studies demonstrate that pEV uptake by antigen presenting cells enhances the expansion and functional activity of regulatory T cells (Tregs), while suppressing differentiation of pro-inflammatory Th1 and Th17 subsets. This shift in T cell homeostasis contributes to the resolution of chronic inflammation and maintenance of immune equilibrium. Additionally, PEVs may modulate B cell antibody production and IgA responses in the gut, reinforcing immune surveillance [62].

Another critical mechanism involves the reinforcement of epithelial barrier function. PEVs have been shown to upregulate tight junction proteins (such as occludin and claudins), decrease intestinal permeability, and mitigate endotoxin translocation all of which reduce systemic inflammatory activation. PEVs mediated restoration of barrier integrity is particularly relevant in autoimmune disorders characterized by dysbiosis and compromised mucosal defenses [63]. Collectively, these mechanisms highlight the multifaceted roles of PEVs as modulators of immune homeostasis. Their capacity to orchestrate

coordinated responses across cellular, molecular, and barrier-level systems positions them as a promising therapeutic platform for autoimmune disease management [64].

PRECLINICAL AND CLINICAL EVIDENCE IN AUTOIMMUNE DISEASE MODELS

PEVs have been shown to interact with host immune cells and influence inflammatory pathways, indicating that they may serve as therapeutic postbiotics for immune-related conditions such as rheumatoid arthritis [65]. Several reviews and original research articles suggest that most existing evidence is derived from in vitro assays and preclinical animal models, particularly those focused on gastrointestinal inflammation. However, direct clinical evidence regarding isolated PEVs in human autoimmune diseases remains limited [11,29, 66]. Recent experimental investigations provide specific mechanistic insights in cellular and murine models. For example, studies have shown the inhibition of NF-κB signaling and the preservation of epithelial tight-junction proteins in colitis models. These findings serve as primary preclinical evidence supporting the anti-inflammatory properties of PEVs [67, 68]. Concurrently, reviews consistently highlight the lack of methodological consistency in PEVs research, including differences in isolation, characterization, dosing, and reporting. This inconsistency is a major barrier to comparing results across studies and advancing clinical use [29].

EVs originating from probiotic bacteria have demonstrated immunomodulatory and barrier-protective properties on intestinal epithelial cells in vitro. For instance, a study conducted by Kang et al. (2020) revealed that EVs from Lactobacillus kefirgranum PRCC-1301 significantly suppressed the expression of pro-inflammatory cytokines (IL-2, IL-8, TNF-α) in human intestinal epithelial Caco-2 cells subjected to DSS treatment. Furthermore, in the same investigation, PRCC-1301 EVs maintained epithelial barrier integrity; immunofluorescence analysis indicated a restoration of tight junction proteins ZO-1, claudin-1, and occludin in EV-treated Caco-2 cells in comparison to DSS-treated controls [67]. Additionally, EVs derived from Lactobacillus casei have been reported to influence immune-related signaling pathways in Caco-2 cells. Treatment with L. casei EVs at concentrations of 100 and 150 μg/mL led to a decrease in pro-inflammatory cytokines. Also, a reduction alongside an elevation in anti-inflammatory cytokines, such as IL-10 and IL-4, was observed when comparing the treated groups to untreated and bacteria-treated cohorts [69]. Furthermore, EVs derived from the probiotic strain Escherichia coli Nissle 1917 (EcN) demonstrated the capacity to mitigate inflammation-related responses in Caco-2 cells. In the context of IL-1β-induced inflammatory stimulation, EVs from EcN were found to downregulate the expression of the oligopeptide transporter PepT1 via the upregulation of miR-193a-3p, thereby suggesting a potential mechanism through which these EVs may diminish the uptake of pro-inflammatory peptides [70]. In summary, these in vitro results suggest that PEVs can directly influence epithelial cytokine secretion and preserve tight junction integrity, thereby providing essential mechanistic insights that warrant further preclinical and clinical exploration of pEV-based interventions in the context of autoimmune and inflammatory diseases.

In experimental models of gut-associated autoimmune and inflammatory diseases, PEVs have demonstrated significant protective effects. For example, EVs originating from Faecalibacterium prausnitzii (Fp-EVs) markedly reduced the severity of disease in a dextran sulfate sodium (DSS)-induced colitis model; treatment resulted in diminished weight loss, a lower disease activity index (DAI), reduced colon shortening, less histological damage, and decreased neutrophil infiltration, while also restoring epithelial barrier integrity, as evidenced by increased levels of ZO-1 and occludin, alongside an elevated proportion of Tregs in colon tissue. Furthermore, pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, IL-12a, IL-17a, IFN-γ, TNF-α, and GM-CSF, which were elevated due to DSS treatment, were subsequently downregulated; simultaneously, anti-inflammatory cytokines (IL-4, IL-10, TGF-β) exhibited upregulation. The same investigation indicated a suppression of pro-inflammatory signaling pathways, specifically the phosphorylation of NF-κB, JNK, p38 MAPK, alongside a concurrent modulation of oxidative stress related Nrf2/HO-1 pathways [71]. Pro-inflammatory cytokines, specifically IL-6, IL-1β, IL-2, and TNF-α, which were upregulated by dextran sulfate sodium (DSS), exhibited a significant reduction following treatment with Q7-EVs ($p<0.05$). In addition, 16S rRNA sequencing showed that Q7-EVs significantly altered gut

microbiota composition, helping to recover microbial diversity. This shift included a reduction in pro-inflammatory groups like Proteobacteria and an increase in anti-inflammatory microbes such as Bifidobacteria and Muribaculaceae [72]. These preclinical findings, derived from multiple independent studies, substantiate the assertion that PEVs can mitigate experimental colitis in murine models by attenuating inflammation, restoring epithelial barrier integrity, rebalancing immune responses, and modulating gut microbiota. This provides a compelling rationale for the continued exploration of PEVs in the context of autoimmune and inflammatory disease models.

In the preclinical literature, the evidence substantiating the therapeutic efficacy of probiotic-derived PEVs in models of systemic autoimmune diseases is notably limited and is primarily derived from a restricted number of studies. A salient example is the administration of EVs isolated from Propionibacterium freudenreichii MJ2 (PFEVs), which resulted in significant improvements in both clinical and histopathological outcomes within the collagen-induced arthritis (CIA) mouse model. The treated subjects demonstrated a reduction in clinical arthritis scores, diminished serum levels of collagen-specific IgG and IgG2a, decreased synovial inflammation and erosion of cartilage and bone, a lower count of TRAP-positive osteoclasts, and a notable shift towards an anti-inflammatory systemic cytokine profile [65,73]. Furthermore, additional preclinical investigations have established a correlation between the restoration of gut homeostasis through probiotic strains or their vesicles and a reduction in arthritis severity in murine models, thereby reinforcing the potential significance of the gut–joint axis in the effects mediated by PEVs [65, 74].

Emerging evidence indicates that bacterial EVs, including those derived from probiotics, may serve as a functional intermediary within the gut–joint axis in models of osteoarthritis. However, direct evidence specifically pertaining to probiotic-derived EVs in the context of osteoarthritis remains limited [75]. Currently, direct investigations into probiotic EVs in NOD mice or EAE models are notably few. Nevertheless, outer membrane vesicles from the probiotic Escherichia coli Nissle 1917 have been demonstrated to activate NOD1 signaling pathways in intestinal epithelial cells. This activation leads to NF-κB-dependent production of pro-inflammatory cytokines IL-6 and IL-8, enhancing epithelial barrier integrity and modulating innate immune responses without inducing excessive inflammation [76,77].

The implications of these NOD1-mediated responses are particularly significant, as dysregulation of the NOD1 signaling pathway is implicated in the pathogenesis of type 1 diabetes in NOD mice. This suggests that probiotic-derived EVs may theoretically contribute to the attenuation of autoimmune diabetes progression through mechanisms of gut-immune crosstalk [76,78].

In a similar vein, while isolated PEVs have yet to undergo direct testing in EAE, probiotic bacterial strains, whose immunomodulatory properties are increasingly ascribed to their secreted EVs, consistently demonstrate a reduction in clinical scores, a limitation of CNS inflammatory infiltration, a decrease in demyelination, and an expansion of Tregs, alongside an elevation of IL-10 and TGF-β in EAE models. These mechanisms are congruent with the established bioactivity of PEVs [79, 80].

Conversely, the predominant focus of the existing in vivo studies on PEVs remains on gastrointestinal inflammation, specifically in models of DSS- or TNBS-induced colitis, rather than on established systemic autoimmune models such as EAE, NOD mice, or lupus-prone strains. Several recent reviews have explicitly underscored this deficiency of data in the context of systemic autoimmunity as a significant translational gap [29, 65].

Nonetheless, the findings from mechanistic studies offer a compelling biological foundation for the prospective systemic efficacy of PEVs. Specifically, certain PEVs have been shown to inhibit pivotal inflammatory signaling pathways, including NF-κB, MAPK, and JAK-STAT. Additionally, they facilitate M2 macrophage polarization, promote the differentiation of Tregsand enhance the production of anti-inflammatory cytokines following systemic or ex vivo administration, mechanisms that are closely correlated with the beneficial effects observed in the collagen-induced arthritis (CIA) model [65,73].

In summary, while PEVs have exhibited significant therapeutic efficacy in at least one well-characterized experimental model of arthritis, and there is indirect evidence suggesting a broader gut-immune communication pertinent to the pathophysiology of NOD and EAE, there remains a notable absence of robust direct evidence in

these traditional systemic autoimmune in vivo models. This highlights the urgent necessity for further targeted preclinical investigations prior to the confident advancement of PEVs as immunomodulatory postbiotics for the treatment of human systemic autoimmune disorders.

PEVs have demonstrated promising results across various preclinical milieus; however, their application in human autoimmune disorders remains nascent and inadequately explored. While PEVs present attractive characteristics as non-cellular immunomodulators characterized by low immunogenicity and high stability, there is an absence of documented or registered clinical trials that evaluate the efficacy of purified PEVs specifically for autoimmune conditions, a concern frequently highlighted in recent expert reviews concerning methodology and translational challenges [29, 65]. Moreover, the existing preclinical literature on PEVs is characterized by significant methodological heterogeneity, which includes variations in techniques for EVs extraction and purification, inconsistent dosage protocols, and diverse delivery methods. These factors hinder the comparability of findings across different studies and impede the development of standardized therapeutic protocols. This issue of inconsistency has emerged as a persistent theme in comprehensive evaluations of PEV research [65,75, 81].

Consequently, to advance the transition to clinical applications, essential priorities for forthcoming studies encompass: (i) the establishment of stringent protocols for the manufacturing and profiling of EVs to guarantee reproducibility; (ii) the execution of comprehensive biodistribution and pharmacokinetic evaluations to ascertain the efficacy of PEVs in targeting tissues beyond the gastrointestinal tract; (iii) the conduct of meticulous assessments regarding immunotoxicity and long-term safety profiles; and (iv) conducting rigorous in vivo experiments within validated models of systemic autoimmunity. These steps are considered essential foundational prerequisites prior to the establishment of PEVs as dependable therapeutic interventions for autoimmune disorders in human subjects [29,65, 82].

In conclusion, while PEVs consistently exhibit significant immunomodulatory effects in preclinical models of intestinal inflammation and, to a lesser degree, in experimental arthritis, the evidence in traditional systemic autoimmune models (such as EAE, NOD mice, and lupus) is either limited or indirect [83]. The existing data offer a substantial mechanistic basis; however, they do not sufficiently demonstrate disease-modifying efficacy in organ-specific autoimmunity beyond the gastrointestinal system, compounded by enduring methodological heterogeneity and the total lack of registered clinical trials [29, 65]. Nonetheless, swift progress in EVs engineering, standardized production methodologies, and an enhanced understanding of systemic bioavailability present a viable avenue for clinical translation [83]. As articulated in a recent thorough review, "Strategies manipulating gut microbiota, such as fecal microbiota transplantation (FMT), antibiotic therapies, and dietary interventions, could optimize immunotherapy response and prognosis." a principle that directly pertains to probiotic-derived EVs as novel postbiotic therapeutics for autoimmune disorders [84]. Methodologically rigorous, MISEV-compliant preclinical investigations in appropriate systemic models, succeeded by early-phase clinical trials, will ultimately ascertain the potential of PEVs to realize their promise as a new category of safe, targeted, and microbiota-inspired immunomodulators.

SAFETY, REGULATORY, AND TRANSLATIONAL CONSIDERATIONS

Although PEVs reduce the toxicity of probiotics and their moderate consumption can beneficially modulate immune responses, their use still demands rigorous safety evaluation because they retain intrinsic immunogenicity and may pose risks to vulnerable populations such as infants, severely immunocompromised individuals, and patients with autoimmune disorders [92]. Individuals with autoimmune diseases warrant particular caution, as they have impaired immune tolerance and heightened sensitivity to microbial factors [93]. While PEVs frequently promote regulatory immune pathways, growing evidence indicates that they may also provoke adverse effects if they elicit excessive inflammatory responses. In these populations, even small amounts of PEVs could overstimulate pattern-recognition receptors or cytokine networks already prone to dysregulation, potentially aggravating underlying disease activity [11].

EVs can carry immunogenic cargo, including proteins, lipids, nucleic acids, and DAMPs capable of activating immune responses. In autoimmune

Table 2. Summary of Some Key Preclinical Studies on PEVs in Autoimmune Disease Models

Autoimmune Disease Type	Probiotic Source	Study Type	Key Observation	References
Gut epithelial inflammation (IBD/autoimmunity)	*Escherichia coli* Nissle 1917	In Vitro (Caco-2 cells)	NOD1 signaling ↑; IL-6/IL-8 ↑; strengthened gut barrier	(76)
Neuroinflammation (MS/autoimmunity)	Lactobacillus-derived (unspecified)	In Vitro (microglia)	LPS-induced inflammation ↓; activation of inflammatory proteins ↓	(68)
Inflammatory Bowel Disease (IBD)	Lactobacillus plantarum	In Vivo (DSS-colitis model)	DAI ↓; alleviated colitis; modulated microbiota	(81)
Rheumatoid Arthritis (RA)	*Propionibacterium freudenreichii* MJ2	In Vivo (CIA arthritis model)	arthritis scores ↓; IgG ↓; anti-inflammatory cytokines ↑	(65)
Food allergy (autoimmune-like)	*Bifidobacterium longum*	In Vivo (food allergy model)	Alleviated symptoms; induced mast cell apoptosis	(85)
Multiple Sclerosis (MS)	Lactobacillus mixture	In Vivo (EAE model)	IL-10-producing Tregs ↑; EAE progression ↓	(79)
Metabolic inflammation / T2D-related autoimmunity-like gut barrier dysfunction	*Akkermansia muciniphila*	In Vitro (Caco-2) & In Vivo (HFD mice)	tight junction proteins ↑; gut permeability ↓; improved glucose tolerance	(86)
Intestinal inflammation (IBD/autoimmunity)	*Akkermansia muciniphila*	In Vitro (Caco-2)	ZO-1, occludin, claudin-4 ↑; modulated TLR2/TLR4	(87)
Immune modulation in TME (autoimmunity-like)	*Akkermansia muciniphila*	In Vitro & In Vivo (RM-1 PCa mice)	M2→M1 polarization; $CD8^+$ $GZMB^+$ T cells; ~60% ↑ tumor growth inhibition	(84, 88)
Systemic inflammation (autoimmunity-like)	*Lactobacillus rhamnosus GG*	In Vitro & In Vivo (RAW264.7 & LPS mice)	NF-κB, TNF-α, IL-6 ↓; IL-10 ↑	(89)
Inflammatory Bowel Disease (IBD)	*Lactobacillus plantarum*	In Vitro & In Vivo (HT-29 & DSS mice)	ZO-1 ↑, occludin; IL-6 ↓, TNF-α; DAI ↓	(90)
Inflammatory Bowel Disease (IBD)	*Akkermansia muciniphila*	In Vivo (DSS-colitis mice)	IgA, IL-10 ↑; restored microbiota; IFN-γ ↓	(91)

patients, such stimulation can lead to pro-inflammatory cytokine release, activation of APCs, and expansion of effector T cells instead of Tregs [94]. Moreover, EVs may present self-antigens to the immune system, potentially inducing autoantibody production; formation of EV–autoantibody immune complexes can result in their deposition in tissues such as kidneys or vascular walls, contributing to immune-complex-mediated pathology as observed in SLE-associated nephritis [95,96]. Microbiota-derived EVs can also influence intestinal barrier integrity and gut immune signaling. Under conditions of dysbiosis, bacterial EVs may carry pro-inflammatory molecules such as LPS, exacerbating gut inflammation and worsening diseases such as inflammatory bowel disease (IBD) in susceptible patients [97].

The safety profile of EVs is strongly influenced by their source, molecular cargo, and preparation procedures, including isolation, purification, and storage. In the absence of standardized protocols, an EV formulation that appears safe in healthy individuals may trigger adverse or exaggerated immune responses in autoimmune patients [43]. Limited standardization and reproducibility mean that repeated EV administration could result in cumulative immunogenicity, autoantibody formation, or unpredictable immune alterations. Therefore, preclinical studies in autoimmune models and careful design of early clinical trials are essential for this population. These observations emphasize the importance of establishing well-defined therapeutic windows through careful dose–response studies in relevant in vitro and in vivo models.

To comprehensively assess the safety of PEVs in host cells, it is necessary to investigate whether the dose administered produces the necessary intervention effect or whether excessive consumption may cause adverse effects or dependence. Beyond understanding the distribution and metabolic duration of PEVs in the body, animal studies, particularly those involving oral administration, should also establish the optimal dosage, frequency, and treatment period, as well as assess whether long-term use could lead to potential adverse effects or dependence [43, 92]. Chen et al. have pointed out the limitations of the

dosing regimen of *Lacticaseibacillus rhamnosus* CMVs in the treatment of osteoporosis in mice [98]. The survival and clearance of PEVs in the gastrointestinal tract is also an important issue. Studies have shown that PEVs are able to resist digestive juices after oral administration [99], but the effects of dilution of digestive juices, intestinal absorption rate, and clearance of PEVs by other host microorganisms must also be considered. In this regard, Kuhn et al. have improved the survival of PEVs in the gastrointestinal tract by designing a particle molecule that binds to PEVs membrane proteins [100].

Additionally, in vitro safety assays such as cytotoxicity assays, epithelial barrier integrity testing, and immune cell activation panels should be performed before in vivo studies to establish initial safety. Strain-specific variations further complicate safety assessment, and environmental factors, including growth stage, culture medium composition, and stress conditions, can alter PEVs cargo, requiring multi-omics profiling for each batch to minimize the risk of presenting harmful or overly immunogenic vesicles [101].

MANUFACTURING AND STANDARDIZATION

As with all other bacterial EVs (BEVs), EVs derived from probiotics are most commonly isolated from cell-free culture supernatants using sequential centrifugation and filtration steps. Therefore, large-scale manufacturing of PEVs depends on robust and standardized isolation workflows, which often integrate ultracentrifugation, size-exclusion chromatography (SEC), tangential flow filtration (TFF), or ultrafiltration to achieve acceptable balances between yield, purity, and scalability. Despite these advances, contamination with soluble proteins, metabolites, and cellular debris remains a major challenge [43]. Ensuring reproducibility is equally demanding because PEVs composition is highly sensitive to culture and production conditions, including medium formulation, growth phase, oxygen availability, and environmental stress. Even minor deviations in these factors can alter EV cargo profiles and thus change their biological activity, resulting in significant batch-to-batch variability. This highlights the need for tightly controlled production conditions and harmonized harvesting protocols [102, 103].

Effective quality control (QC) requires integration of biophysical, biochemical, and multi-omics assays. Particle size and concentration are typically assessed using nanoparticle tracking analysis (NTA) or dynamic light scattering (DLS), while morphological validation is obtained through electron microscopy techniques such as TEM, SEM, or cryo-EM. Surface markers, including LTA in Gram-positive species and LPS in Gram-negative bacteria, are commonly evaluated using western blotting. In addition, proteomic, lipidomic, and metabolomic profiling are increasingly used to confirm cargo composition and detect batch deviations [43,104]. However, the absence of universally accepted QC standards limits cross-study comparability and presents a major bottleneck for regulatory approval. Overall, improving manufacturing pipelines through harmonized isolation procedures, controlled production conditions, and validated QC metrics will be essential for producing scalable, reproducible, and clinically reliable PEV preparations.

To enable clinical application, PEVs production must comply with Good Manufacturing Practice (GMP) standards. Key considerations include:

- **Culture conditions:** Transitioning from research-grade media to xeno-free or chemically defined formulations is required to minimize contaminants and reduce immunogenic risk.
- **Closed and sterile bioprocessing:** Open laboratory procedures are inadequate for clinical production; instead, aseptic workflows and controlled bioreactor systems must be used to prevent microbial contamination [105,106].
- **Batch-to-batch consistency and traceability:** Each batch must undergo full documentation, standardized characterization, and predefined quality-control testing to ensure reproducibility and consistent biological activity [107].
- **Sterility and pyrogenicity testing:** Due to the bacterial origin of PEVs, especially those from Gram-negative species containing LPS or other PAMPs, stringent sterility and endotoxin testing is mandatory [108, 109]. Although GMP-compliant workflows are well established for mammalian cell-derived EVs, the process for bacterial EVs requires additional validation because of their distinct composition and potential immunogenicity.

FUTURE PERSPECTIVES AND RESEARCH DIRECTIONS

Advances in bioengineering now enable the design of PEVs with enhanced disease-specific targeting capacities. This includes

surface modification with ligands, antibodies, or peptide motifs that selectively bind to immune cells, inflamed tissues, or receptors implicated in specific autoimmune disorders. Engineering the vesicular cargo such as loading regulatory microRNAs, immunomodulatory proteins, or anti-inflammatory metabolites allows PEVs to modulate pathological immune pathways more precisely while minimizing systemic effects. Such refined targeting strategies can substantially improve therapeutic potency and reduce off-target immune activation, positioning engineered PEVs as a next-generation immunomodulatory platform for autoimmune disease management [43,110-112]. More over Integrating PEVs-based therapies with dietary or pharmacological interventions represents a promising multidimensional approach [110]. Dietary components such as prebiotics, polyphenols, and microbiota-modulating nutrients can influence endogenous PEVs production and enhance gut microbial balance, thereby synergizing with administered PEVs [113]. Moreover, co-administration of PEVs with established pharmacological agents including anti-inflammatory drugs, biologics, or metabolic regulators may amplify therapeutic responses by acting on parallel immune pathways. This combined strategy leverages both host-directed and microbiome-mediated mechanisms, offering a more comprehensive method for controlling autoimmune inflammation and improving patient outcomes [114,115].

Despite strong preclinical evidence supporting the immunomodulatory capacity of PEVs, translation to clinical practice requires rigorously designed human studies. Standardization of PEVs isolation, purification, storage stability, and dose quantification is essential to ensure reproducibility and safety [25,103,116]. Early-phase clinical trials should assess pharmacokinetics, biodistribution, immunogenicity, and short-term safety in autoimmune populations, followed by larger trials evaluating clinical efficacy and long-term outcomes [25]. Establishing validated manufacturing protocols and regulatory pathways will be critical for positioning PEVs-based therapeutics within the clinical landscape and enabling broader therapeutic deployment [25,116].

As autoimmune diseases are heterogeneous in origin and presentation, incorporating PEVs therapies into personalized medicine frameworks may significantly enhance therapeutic success [43,112]. Detailed gut microbiome profiling, immune signature analysis, and patient-specific biomarker evaluation can guide the selection or engineering of PEVs tailored to individual inflammatory profiles [43]. Personalized PEVs regimens may also reduce adverse effects by aligning therapeutic activity with each patient's microbial composition and immune characteristics. Leveraging precision-medicine methodologies in combination with PEVs can therefore optimize treatment responses, increase therapy specificity, and contribute to long-term disease management strategies [117,118].

CONCLUSION

PEVs are emerging as potent postbiotics capable of modulating host immune responses. By transporting bioactive molecules, they facilitate targeted communication between probiotics and host cells, influencing inflammation, strengthening the intestinal barrier, and promoting immune balance. Growing evidence from in vitro, in vivo, and early translational studies indicates that PEVs can reduce excessive immune activation and support immune tolerance in autoimmune disorders. Despite their promise, challenges such as variability in PEVs composition, lack of standardized isolation methods, and limited understanding of their bio distribution and mechanisms still hinder clinical translation. Continued progress in vesicle engineering, purification technologies, and mechanistic research will be essential to fully realize their therapeutic potential. Future studies should prioritize methodological standardization, deeper mechanistic insight, and rigorous assessment of safety and bio distribution, alongside disease-specific investigations and early clinical trials to clarify their viability as next-generation immunomodulatory agents.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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