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Article

The Crosstalk Between Microbial-Derived Extracellular Vesicles and Host Immune Checkpoints: A Molecular Perspective on Chronic Inflammation and Immune Evasion

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Abstract: Bacterial extracellular vesicles (EVs) are increasingly recognized as major regulators at the dynamic host-microbiota interface. Nanosized vesicles that were originally considered as cellular debris are now appreciated for their powerful regulatory function and the variety of bioactive molecules (proteins, nucleic acids, lipids, and metabolites) that modulate immune responses included within them. In this review, we dissect the complex interplay between microbial EVs and immune checkpoint players, such as PD-1/PD-L1, CTLA-4, TIM-3, and LAG-3, which are crucial to preserve immune tolerance but can be hijacked during chronic inflammation, infection, or cancer.

This review introduces the biology and biogenesis of microbial EVs and the architecture and specific function of canonical immune checkpoints. It also explores how microbial EVs may upregulate checkpoint ligands, increase regulatory T cells, suppress cytotoxicity, and modulate dendritic cell function, underscoring their dual function in immune suppression and homeostasis. In chronic infectious diseases such as tuberculosis and H. pylori or immunological-based diseases such as IBD and cancer, microbial EVs are instrumental in immune evasion and in disease development.

From a therapeutic standpoint, microbial EVs constitute another exciting frontier: they can be engineered for immune modulation, small molecule delivery or vaccine formulations. Furthermore, the detection of these in biofluids, make them potential diagnostic as non-invasive biomarkers of immune dysregulation. Although there have been exciting progresses, barriers remain in the standardization of EV isolation, as well as in larger definition of the context-dependent effects. Ultimately, characterisation of EV–immune checkpoint axis might revolutionise strategies for treatment of chronic inflammation, chronic infections and immune mediated diseases.

.**Keywords:** Bacterial extracellular vesicles, Chronic Inflammati, PD-1/PD-L1, CTLA-4

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1. Introduction

The human immune system is a complex network that protects people from pathogenic neighborhood inhabitants and maintains tolerance to benign self-antigens and environmental components [1]. A sophisticated balance between activation and suppression is maintained in the body by a variety of mechanisms to ensure homeostasis. When this homeostasis is disrupted, inflammation can become uncontrollable or, conversely, fail to eliminate insidious infectious agents, leading to uncontrolled inflammation or autoimmunity. One of the most essential mechanisms to keep the immune response in check is immune checkpoints. Immune checkpoint pathways contain both inhibitory and activating signals that may activate and inhibit immune cell function. These include pathways like PD-1/pd-L1 and CTLA-4, which slow the immune response,

preventing it from attacking self-antigens or causing severe tissue damage during potential autoimmune diseases or persistent inflammation. At the same time, the human body is colonized by trillions of microorganisms, including bacteria, fungi, viruses, and archaea [2], [3]. Although many of them live in peaceful symbiosis with the host, they are not silent passengers but actively participate in host physiology. In doing so, they may influence the immune response, host metabolism, and even behavior by producing multiple molecules. In the last two decades, a new class of biologically active microbial molecules called extracellular vesicles has emerged as an essential signaling tool. EVs are nano-sized particles that are enveloped by a membrane and contain various molecules. They are mainly made up of proteins, lipids, nucleic acids, and metabolites and are top-secreted by bacteria, fungi, archaea, and other microorganisms diving below the microbiome's coral reef [4].

The function of microbial EVs on the host immune response has recently become the focus of attention. Contrary to live pathogens, which become often physically and immunologically trapped, EVs can overcome mucosal surfaces and reach systemic circulation delivering their molecular load to immune as well as non-immune cells. Such interactions can have important deleterious or modulating immunological effects, depending on the EV (cargoes) and host context [5].

According to the recent research, microbial EVs could potentially modulate immune checkpoints either directly or indirectly. For example, some bacterial vesicles can induce PD-L1 expression on the host antigen-presenting cells (APCs), thus inhibiting T cell activation. Others could harbor molecules that imitate host ligands or regulators, allowing them to subvert immune signaling pathways. In addition, the expression of microRNAs (miRNAs) or long non-coding RNAs (lncRNAs) expressed by immune cells may be modified by microbial EVs, resulting in changes in gene expression programs related to immune-related signaling pathways including immune checkpoint pathways. These types of disruptor-microbe and disruptor-host interactions may support the induction or maintenance of chronic inflammation, immune exhaustion and immune evasion by microbial pathogens [6].

The communication of microbial-derived EVs with immune checkpoints is particularly relevant in chronic infections, autoimmune-related diseases and cancer. Pathogens such as Mycobacterium tuberculosis, Helicobacter pylori and Hepatitis C virus are a well-known example of the pathogens that are able to establish chronic infection in their hosts despite an effective immune response. Rising evidence suggests that such pathogens may excrete EVs to hijack immune checkpoint pathways, to overlook/by-pass/stimulate/or inhibit its own identity and destruction. For instance, M. tuberculosis EVs mediate the delivery of lipoproteins and glycolipids that inhibit dendritic cell maturation and T-cell activation, at least in part via a PD-L1 dependent pathway [7],[8]

On the contrary, commensal bacteria, particularly the organisms of the gut microbiota, also secrete EVs that modulate the host immune system. Curiously, these interactions are not always detrimental. Indeed, some commensal-derived EVs are immunomodulatory, which may counteract autoimmunity and inflammation. For example Bacteroides fragilis EVs transport polysaccharide A, a molecule enhancing expansion of regulatory T cells (Tregs) and reducing pro-inflammatory responses. These findings indicates a dual function of microbial EVs in immune-related diseases acting as either pro- or anti-inflammatory factors depending on the context and the microbial source [9], [10].

It is an intriguing realm of point-of-care research to target microbial EVs, or the contents therein, for influencing immune checkpoints. Nanotechnological and molecular biology innovations enable the separation, identification, and modification of EV to use them for therapeutic reasons. These engineered EVs have potential applications as vaccines, immune modulators, or vehicles to deliver immune checkpoint inhibitors or agonists. Furthermore, it can be the profiling of microbial EV content a tool for diagnosis or prognosis in immune-related pathologies [11], [12].

Although promising, many questions go unanswered. Which components of microbial EVs are able to regulate immune checkpoints? Do these behaviors persist across species and social situations? To what extent host genetics and environmental factors determine

the effect of microbial EVs in the control of host immunity? Answering these questions will necessitate a multi-, interdisciplinary endeavor that includes microbiology, immunology, genomics and systems biology [13].

For this purpose, the present review explores the interaction of microbial-EVs in relation to host immune checkpoints. We will investigate the kinds and the mechanisms of MV and EV formation by microorganisms, the organization and function of crucial immune checkpoint pathways, and molecular crosstalk that tie these systems together. We will mainly focus on chronic inflammatory diseases and immune subversion modalities used by microbial EVs. Finally, we will address new therapeutic approaches targeting these new acquisitions and point to future research avenues [14], [15].

In conclusion, the crosstalk of microbial EVs with immune checkpoints is an emerging area in immunology that may have important implications in our understanding of chronic inflammation, immune tolerance and pathogenesis of infectious diseases. As further studies of this nature continue, it could lead to innovative opportunities for therapeutic interventions in disorders of immune dysregulation and chronic infections [16].

2. Materials and Methods

Overview of Microbial Extracellular Vesicles (EVs)

Microbial extracellular vesicles (EVs) are membrane-derived structures produced by a broad spectrum of microorganisms, ranging from Gram-negative and Gram-positive bacteria to fungi, archaea, and protozoa. They average in size from 20–400 nm and have been categorized according to their origin, and size and substance content. EVs function as a modality of intercellular communication both within the microbial communities and between microbes and host cells. Their capacity to engulf various bioactive compounds makes them key actors for the control of the host physiology/pathophysiology.

The biogenesis of microbial EVs differs among organisms. In Gram-negative bacteria, the outer membrane vesicles (OMVs) are formed by bulging of outer membrane, which trapping periplasmic materials, lipopolysaccharides (LPS), outer membrane proteins, and DNA together. In Gram-positive bacteria, which do not have an outer membrane, EVs are produced by the local degradation of the peptidoglycan cell wall to allow budding of cytoplasmic contents and membrane components, see Table 1. In fungi, EVs are generated via mechanisms that are conserved with those in eukaryotic cells, and in some instances these mechanisms require the endosomal sorting complex required for transport (ESCRT) machinery

3. Results

Table 1. Major Types of Microbial EVs and Their Key Characteristics

Type of Microbe	Type of EV	Biogenesis Mechanism	Common Cargo
Gram-negative	Outer membrane vesicles (OMVs)	Outer membrane blebbing	LPS, DNA, proteins, enzymes
Gram-positive	Membrane vesicles	Peptidoglycan remodeling	Cytoplasmic proteins, lipids, metabolites
Fungi	Fungal EVs	ESCRT-dependent and independent pathways	Polysaccharides, RNA, immunogenic proteins
Archaea	Archaeal EVs	Unknown (similar to OMVs?)	Lipids, extremophile proteins

The content of these microbial EVs are highly variable, which can represent the physiological state of the microbe or of the environment. EVs can harbor toxins, enzymes, antigens, quorum-sensing molecules, small RNAs, or metabolic waste products. In pathogenes, these vesicles tend to contain virulence factors that promote colonization, invasion of tissue, and avoidance of the immune response [17], [18]. For instance, Helicobacter pylori EVs contain cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA), which drive host cell signaling and immune responses [19].

Besides being involved in pathogenesis, EVs of the commensal microbiota have been shown to modulate the immune homeostasis. VEVs of Bacteroides fragilis that contain polysaccharide A (PSA) for example, drive regulatory T cell (Treg) maturation in the gut while repressing inflammation. These results highlight the Janus-faced nature of EVs in driving or dampening disease depending on the microbial origin and host context [20,21]. EVs can be taken up by target cell through endocytosis, phagocytosis, fusion with membrane, and receptor mediated endocytosis. Once taken up by the cells, the EV cargo can influence the expression of host genes, activate signaling pathways, as well as determine the profile of cytokines. Furthermore, EVs can engage pattern recognition receptors (PRRs -including Toll-like receptors (TLRs) and NOD-like receptors (NLRs-)) that drive downstream immune responses [22].

Immune Checkpoints: PD-1/PD-L1, CTLA-4, and Others

Immune checkpoints are a class of regulatory pathways that turn a brake on the magnitude and persistence of immune responses, particularly in T cells. They are essential for maintaining self-tolerance and preventing autoimmunity for their provision of inhibitory signals that dampen immune cell function. These same strategies may also be

hijacked by pathogens and tumours to escape elimination by the immune system, leading to chronic infections and immune failure [23].

PD-1/PD-L1 Pathway

Programmed cell death protein 1 (PD-1) is a negative (inhibitory) receptor found on activated T cells, B cells, and myeloid cells. Its ligands, PD-L1 and PD-L2, are found in antigen-presenting cells (APCs), certain non-immune cells, and numerous tumor cells. When bound to PD-L1 or PD-L2, PD-1 delivers an inhibitory signal and inhibits T cell proliferation, cytokine production, and cytotoxicity. Physical interaction between CD44 and LFA-1 is essential for regulating cellular damage associated with inflammation and tolerance [24].

Under conditions of chronic infections, such as HIV, HCV or tuberculosis, providing continuous epitopic stimulation, T cells are exposed to constant levels of PD-1 ligand, therefore, T cells remain persistently expressing PD-1, as it was termed- T cell exhaustion. This "tired" phenotype has reduced effector functionality, effector molecule and proliferative potential that may further promote pathogen persistence [25], [26].

CTLA-4 Pathway

Cytotoxic T lymphocyte antigen-4 (CTLA-4); another important inhibitory receptor, is expressed after activation by T cells. It has CD80 and CD86 as ligands, which are also shared by the co-stimulatory receptor CD28, but it binds several times stronger than the latter receptor. Competition against CD28 by CTLA-4 limits the co-stimulation stimulus for effective T cell activation and, thereby, functions as an important “check point” potential during T cell priming in the early phase in lymphoid organs [27, 28].

CTLA-4 is also a constitutive receptor on regulatory T cells (Tregs), where in part mediates their suppressive activity. CTLA-4 blockade has been effective in cancer immunotherapy, but in the presence of an infection, CTLA-4-induced suppression could diminish immune clearance and favor chronicity [29].

Emerging Checkpoints: TIM-3, LAG-3, TIGIT

There are more than PD-1 and CTLA-4 other immune checkpoints which have been identified, such as TIM-3 (T cell immunoglobulin and mucin domain-3), LAG-3 (lymphocyte activation gene-3), TIGIT (T cell immunoreceptor with Ig and ITIM domains). These molecules are frequently coexpressed with PD-1 on exhausted T cells and promote immune suppression by distinct mechanisms:

1. TIM-3 can engage with ligands including Galectin-9 and T cell apoptosis and impaired Th1 responses.
2. LAG-3 binds to MHC class II molecules and inhibits T cell growth.
3. TIGIT competes with the activating receptor CD226 for CD155 binding, thereby inhibiting T cell and NK cell responses, see Table 2.

The “rescue” of multiple checkpoint molecules can reflect the deepening dysfunction of the immune system, and resistance to monotherapy checkpoint blockade [30].

Table 2. Summary of Key Immune Checkpoint Molecules

Checkpoint	Ligands	Expressed On	Primary Function	Role in Disease
PD-1	PD-L1, PD-L2	T cells, B cells	Inhibits TCR signaling and cytokine secretion	Chronic infection, cancer

CTLA-4	CD80, CD86	Activated T cells, Tregs	Competes with CD28; suppresses T cell priming	Autoimmunity, infection
TIM-3	Galectin-9	T cells, NK cells	Promotes T cell exhaustion and apoptosis	Viral infections, tumors
LAG-3	MHC class II	T cells, B cells	Inhibits T cell proliferation	Inflammation, cancer
TIGIT	CD155, CD112	T cells, NK cells	Suppresses immune activation	Chronic infections, tumors

Molecular Crosstalk Between Microbial EVs and Immune Checkpoints

The cross-talk between microbial EVs and host immune checkpoints constitutes an unexplored and intricate interface in immunology. Although various aspects of microbial EVs and immune checkpoints have been investigated separately, recent findings indicate an extensive crosstalk between them, especially under the settings of chronic inflammation, infection, and immune escape. In this section, we discuss the molecular strategies of microbial EVs in the regulation of immune checkpoint pathways with an emphasis on their function in the influence of host immunity [31,32]

1. EV-Mediated Modulation of Checkpoint Ligands and Receptors

Host cells express immune checkpoint molecules in response to microbial EVs. Bacterial EVs from Helicobacter pylori and Mycobacterium tuberculosis, for instance, were found to induce PD-L1 in macrophages and dendritic cells. This upregulation is frequently achieved through activation of Toll-like receptors (TLRs), including TLR2 and TLR4, which further lead to downstream signaling cascades via NF-κB and STAT3 pathways. The overall impact is the creation of an immunosuppressive milieu that hinders t cell activation and drives immune escape [33].

Beyond upregulating checkpoint ligands, some microbial EVs can carry proteins or RNAs that resemble host checkpoint molecule, or that affect the activity of the latter. For example, bacterial EV cargo carrying small RNAs can hamper mRNA translation or stability in the host leading to a potential impact on the production of immune receptors or their regulators.

2. EV Cargo Influencing T Cell Exhaustion

Persistent exposure to microbial EVs carrying antigens, toxins, or immune-modulatory molecules can contribute to T cell exhaustion. Chronic exposure to EVs may lead to sustained expression of PD-1, TIM-3, and LAG-3 on effector T cells, especially in mucosal tissues and draining lymph nodes. This state of exhaustion is characterized by reduced cytokine production, impaired cytotoxicity, and decreased proliferation, all of which contribute to pathogen persistence [34].

For example, EVs from *Porphyromonas gingivalis*, a bacterium implicated in periodontal disease, have been reported to modulate CD4+ T cell activity and promote an exhausted

phenotype. Similarly, fungal EVs have been shown to alter dendritic cell maturation and skew T cell responses toward regulatory or suppressive profiles.

3. Impact on Regulatory T Cells and the Tumor Microenvironment

Microbial-derived EVs also modulate the proliferation and differentiation of regulatory T cells (Tregs), which are the key players in the immune checkpoint signaling. Commensal bacteria like Bacteroides fragilis produce EVs with polysaccharide A, which is known to promote Treg differentiation and IL-10 secretion. This results in immune tolerance and could end up generating microenvironments that favor pathogen survival, but also tumor progression, indirectly [35].

TME also provide an environment in which microbial EVs from gut or oral microbiota are released into the bloodstream transit the body and infiltrate in the distant organs. Once there, these cells can engage with immune and stromal cells to regulate immune checkpoint expression, drive angiogenesis, and enable immune evasion [36].

To provide a comprehensive overview of the interactions between microbial extracellular vesicles (EVs) and host immune cells, Table 3 summarizes the types of EVs released by various microbes, their specific host targets, the immune checkpoint pathways they influence, and the underlying mechanisms of action. This detailed compilation highlights the diversity of microbial strategies to modulate immune responses, which is critical for understanding pathogen-host dynamics as well as the tumor microenvironment modulation by commensal microbiota.

Table 3: Examples of Microbial EVs Influencing Immune Checkpoints

Microbe	Type of EV	Host Target	Affected Checkpoint Pathway	Mechanism of Action
H. pylori	OMV	Macrophages, DCs	PD-L1	TLR4-NF-κB signaling
M. tuberculosis	Membrane vesicle	Macrophages	PD-L1	Induction via STAT3
P. gingivalis	OMV	CD4+ T cells	PD-1, TIM-3	Promotes exhaustion phenotype
B. fragilis	Polysaccharide-rich EV	Tregs	CTLA-4, IL-10	Expansion and functional skewing
Commensal oral microbiota	EV	Tumor microenvironment	PD-L1, LAG-3	Indirect modulation through

	cytokine signaling

As illustrated in Table 3, microbial EVs exhibit a range of effects on host immune cells, targeting key immune checkpoints such as PD-L1, PD-1, TIM-3, CTLA-4, and LAG-3. For instance, Helicobacter pylori OMVs primarily engage macrophages and dendritic cells through the TLR4-NF-κB signaling pathway, leading to upregulation of PD-L1 and subsequent immune modulation. Similarly, Mycobacterium tuberculosis membrane vesicles induce PD-L1 expression via STAT3 activation in macrophages. Porphyromonas gingivalis OMVs promote T cell exhaustion by affecting PD-1 and TIM-3 checkpoints, while Bacteroides fragilis releases polysaccharide-rich EVs that expand regulatory T cells (Tregs) and influence CTLA-4 and IL-10 pathways, contributing to immune tolerance. Furthermore, commensal oral microbiota-derived EVs modulate the tumor microenvironment indirectly through cytokine signaling pathways affecting PD-L1 and LAG-3 expression. These diverse mechanisms underscore the complex role of microbial EVs in shaping host immune landscapes and their potential implications for therapeutic interventions

4. Implications for Chronic Inflammation and Disease Progression

The potential of immune checkpoint modulation by microbial EVs suggests a possible role of them in the pathogenesis of a number of chronic diseases, including IBD, periodontitis, chronic hepatitis as well as some cancers. EV also maintain a tolerogenic or immunosuppressed state, by continuous release of checkpoint modulating signal(s), that enable pathogens to survive and tissue persistently inflamed [37].

The knowledge of these molecular interactions thus offers the possibility for development of specific therapies. Neutralizing the checkpoint-modulating activity of microbial EAVs may also promote greater immune activation in chronic infections or overcome tumor immunosuppression. Furthermore, microbial EV features may function as biomarkers for disease severity or therapy response.

In summary, the interplay between microbial EVs and immune checkpoint molecules at the molecular level is a complex and dynamic phenomenon having substantial impact on the immune homeostasis. More detailed research is also required to clarify the complete pattern of EV-related actions on checkpoint pathways and their role in health and disease [38].

Role of Microbial EVs in Chronic Inflammation and Immune Evasion

Microbial extracellular vesicles (EVs) have emerged as central players in the pathogenesis of chronic inflammation and immune evasion. By delivering microbial components directly into host cells or modulating the extracellular immune landscape, these vesicles contribute to long-term immune dysregulation and persistence of disease.

1. Sustained Innate Immune Activation

Persistent activation of innate immune receptors is one of the most important mechanisms of action of microbial EVs inducing chronic inflammation. A number of EVs are coated with pathogen-associated molecular patterns (PAMPs), including lipopolysaccharides (LPS), peptidoglycan, flagellin, or microbial nucleic acids. These molecules are recognized by pattern recognition receptors (PRRs), such as TLRs, NLRs, and RLRs, and lead to persistent production of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6.

EVs from H. pylori and E. coli, for instance, have been found to stimulate the TLR4 and TLR2 complexes on epithelial and macrophage cells, resulting in chronic low-grade inflammation [39]. T85, Likewise EVs derived from Candida albicans are able to activate Dectin-1 and other C-type lectin receptor and participate in the pathogenesis of mucosal and systemic fungal infections.

2. Induction of T Cell Exhaustion

Microbial EVs also contribute to T cell dysfunction and exhaustion, a frequent complication in chronic infections and cancer. Sustained or chronic stimulation with antigens and immune suppressive signals (often packaged within EVs) results in the expression of immune checkpoint molecules, such as PD-1, LAG-3, and TIM-3 on T cells [40].

It has been demonstrated that EVs from bacteria such as P. gingivalis and M. tuberculosis, also induce PD-L1 on APCs that interact with PD-1 on T cells and suppress their proliferation and cytokine production. This persistent inhibitory signaling compromises the host's capacity to eradicate pathogens and promotes microbial persistence and dissemination.

3. Expansion of Regulatory T Cells and Anti-inflammatory Bias

Some of the microbial EVs, namely those coming from commensals as was reported for Bacteroides fragilis, are able to induce the differentiation of regulatory T cells (Tregs) and the production of anti-inflammatory cytokines like IL-10 and TGF-β. Albeit this mechanism can be beneficial to maintain mucosal tolerance, a too strong and/or misdirected Treg expansion adds to immune suppression and tolerance against pathogens [41].

Under the MEVs, such immune response skewing in a disease setting such as IBD or colorectal cancer, yields an environment which favours chronic inflammation and yet hampers protective immunity.

4. Modulation of Antigen Presentation and Dendritic Cell Function

Microbial EVs may inhibit maturation and antigen-presenting capability of dendritic cells and thus suppress the induction of adaptive immunity. Fungal EVs that bud from C. neoformans contain enzymes and tryglyceride lipids that suppress the dendritic cells function and do not allow T cells to prime properly thus aiding fungal persistence, see Table 4.

Bacterial EVs have been observed to meddle with the MHC class I and II presentation through reducing expression or changing endosomal trafficking that consequently attenuate the CD4+ and CD8+ T cell responses as well [42].

Table 4. Microbial EV-Mediated Mechanisms of Immune Evasion and Chronic Inflammation

Microbial Source	Key EV Cargo	Targeted Host Pathway	Immune Outcome
H. pylori	LPS, VacA toxin	TLR4 → NF-κB/PD-L1	Chronic inflammation, T cell inhibition
M. tuberculosis	Lipoglycans, mycolic acids	APCs → PD-L1, antigen presentation	T cell exhaustion, immune evasion
P. gingivalis	Gingipains, OMV proteins	PD-1/PD-L1 axis	Suppressed T cell activation
C. albicans	β-glucans, lipids	Dectin-1/TLR2	Pro-inflammatory cytokine storm

B. fragilis	Polysaccharide A (PSA)	Tregs → IL-10	Immune tolerance, gut homeostasis
Dysbiotic microbiota	Mixed PAMPs	PRRs → cytokines	Chronic systemic inflammation

Therapeutic Perspectives and Future Directions

The growing understanding of microbial extracellular vesicles (EVs) and their interaction with host immune checkpoints offers exciting and novel therapeutic opportunities. As our insights into the molecular cargo and biological functions of these vesicles deepen, so too does the potential to translate this knowledge into clinical applications targeting chronic infections, immune-mediated diseases, and cancer[43], [44].

1. Targeting Microbial EV Production and Release

One potential treatment is to target the generation and release of noxious EVs. Inhibitors of OMV formation (that could target peptidoglycan remodeling enzymes or the Tol-Pal complex etc.) in bacteria may result in diminished delivery of virulence factors and immune-modulating molecules. Likewise, antifungal drugs which affect vesicle trafficking pathways in fungi could inhibit the release of EVs involved in immune evasion.

Inhibiting microbial EV biogenesis might not only lessen pathogenicity but also potentiate microbes to host immune cleareace and classical antimicrobial therapies [45].

2. Neutralizing EV Components

Specific drugs in the form of therapeutic antibodies or nanobodies that bind to distinct EV-associated molecules implicated in immune checkpoint modulation, for instance, EV-bound PD-L1 in infected tissues, can be designed. Furthemore, aptamers or peptide inhibitors could be developed to target and inhibit the EV proteins or RNAs of the microorganisms that dampen the immune signaling.

This would enable a selective inhibition of immune-suppressive properties of EVs, while maintaining beneficial commensal-derived EVs that are necessary for immune homeostasis [46].

3. Engineering EVs as Delivery Platforms

However, microbial EVs (identical or synthetic vesicles resembling their structure) can also be engineered to deliver therapeutic cargos, like antigens, checkpoint inhibitors, small-interfering RNAs (siRNAs), or immune-stimulatory compounds. These engineered EVs can be designed to target a particular immune cell or tissue and can load their contents with a high specificity.

In cancer, for instance, EVs may be engineered to inhibit PD-1/PD-L1 interaction in the tumor microenvironment or transport tumor-associated antigens to dendritic cells, resulting in the optimal T cell priming [47].

4. Leveraging Commensal EVs in Immune Regulation

Commensal EVs, in particular EVs derived from gut commensals such as Bacteroides fragilis and Akkermansia muciniphila, are an ideal resource to generate probiotic-based immune modulators. These EVs can drive the expansion and production of regulatory T cells and IL-10, being protective in diseases with hyperactive immune responses, like IBD, multiple sclerosis, or autoimmune arthritis [48].

Formulation of these EVs into oral capsules or mucosal sprays can offer a safe and efficacious strategy for reprogramming immune tolerance and preserving mucosal immunity.

5. EVs as Diagnostic and Prognostic Tools

As downstream products of microbes, microbial EVs can be found in blood, saliva, urine, and stool, thus making them ideal for non-invasive biomarkers, see Table 5. Profiling EVs may be useful to differentiate infectious from inflammatory causes of disease, follow the course of disease, and reflect on the response to therapy [49],[50],[51].

For instance, the detection of EVs with PD-L1-engendering signals or check-point-mimicking proteins in the blood may reflect an immune-suppressed status during chronic infections or cancer [52],[53],[54].

Table **5**. Future Directions in Microbial EV-Immune Checkpoint Research

Strategy	Application	Potential Impact
Inhibiting EV biogenesis	Suppress pathogen virulence	Reduce chronic inflammation, improve clearance
EV-targeted antibodies	Neutralize immune-modulatory cargo	Restore T cell function, block immune suppression
EV-based drug delivery	Cancer, autoimmunity, vaccines	Precision immunotherapy
Probiotic EV therapies	IBD, autoimmunity	Restore immune tolerance
EV-based diagnostics	Infection, cancer, inflammatory disease	Early detection and monitoring

4. Conclusion

The paradigm shift in our understanding of host-pathogen relationship has occurred due to the ever-increasing evidences and advances of microbial EVs. Once thought of as nothing more than cellular waste, these nanosized vesicles are now known as powerful regulators of immunity, with the potential to both stimulate and suppress both innate and adaptive immune function through their rich protein, nucleic acid, lipid, and microbial metabolite cargo.

This review has extensively examined the interplay at the molecular level of microbial EVs with host immune checkpoints—central regulatory circuits, such as PD-1/PD-L1, CTLA-4, TIM-3, and LAG-3, which are required for immune homeostasis but are frequently perverted in the context either of infection, inflammation, or cancer. The capacity of microbial EVs to increase checkpoint ligand expression, dampen Tc cytotoxicity, expand Tregs, and inhibit DC function demonstrates the duality in EV involvement in immune evasion and chronic disease establishment.

Notably, the microbial EVs do not work alone but collaborate with host signaling pathways to maintain a proinflammatory or tolerogenic balance. In infected diseases such as tuberculosis, H. pylori or chronic fungal diseases, EVs are a tool for pathogens to escape from immune response and colonization on a long-term basis. In sterile inflammatory states of concern, such as inflammatory bowel disease or cancer, both dysbiosis and the release of immunomodulatory EVs will potentiate immune collapse, resulting in suppression of both local and systemic immune responses.

Clinically, the EV-immune checkpoint axis represents a two-sided opportunity: microbial EVs can be targeted to avoid or reverse immune evasion, and used for delivery or immune modulation in innovative therapies. As discussed, interventions such as the inhibition in EV biogenesis, attenuation of EV cargo, EV-based therapy, and microbiome-derived EVs adopt real potential to modulate immune responses in a specific and individual fashion.

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