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REVIEW

Extracellular vesicles regulate immune responses and cellular function in intestinal inflammation and repair

Triet M. Bui[†], Lorraine A. Mascarenhas[†], and Ronen Sumagin

Northwestern University, Feinberg School of Medicine, Department of Pathology, Chicago, IL, USA

ABSTRACT

Tightly controlled communication among the various resident and recruited cells in the intestinal tissue is critical for maintaining tissue homeostasis, re-establishment of the barrier function and healing responses following injury. Emerging evidence convincingly implicates extracellular vesicles (EVs) in facilitating this important cell-to-cell crosstalk by transporting bioactive effectors and genetic information in healthy tissue and disease. While many aspects of EV biology, including release mechanisms, cargo packaging, and uptake by target cells are still not completely understood, EVs contribution to cellular signaling and function is apparent. Moreover, EV research has already sparked a clinical interest, as a potential diagnostic, prognostic and therapeutic tool. The current review will discuss the function of EVs originating from innate immune cells, namely, neutrophils, monocytes and macrophages, as well as intestinal epithelial cells in healthy tissue and inflammatory disorders of the intestinal tract. Our discussion will specifically emphasize the contribution of EVs to the regulation of vascular and epithelial barrier function in inflamed intestines, wound healing, as well as trafficking and activity of resident and recruited immune cells.

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Mucosal integrity

Maintenance of the intestinal barrier integrity and rapid resealing of mucosal wounds is critical for proper function of the gastrointestinal (GI) tract. Intestinal epithelial cells (IECs) lining the intestinal lumen form a selectively permeable barrier to separate luminal contents from the underlying tissues. Through complex communication with the microbiome and the immune system, IECs maintain gut homeostasis. Dysregulation of the immune cell composition during gut injury results in impairment of the intestinal barrier and underlies a wide spectrum of inflammatory disorders of the GI tract, including Inflammatory Bowel Disease (IBD).[1,2]

Basis of epithelial healing

IECs are constantly exposed to a repertoire of dietary substances, foreign antigens, commensal and pathogenic bacteria, and thus are susceptible to injury.[3] Rapid resealing and repair of mucosal wounds is essential for reestablishing the intestinal barrier and limiting antigen leakage into underlying tissues. If not efficiently repaired, bacterial translocation and antigenic exposure associated with a breached barrier inevitably results in aberrant immune response and augmentation of epithelial injury.[3] Wound healing requires efficient tissue remodeling, where IECs proliferate and migrate into the wound bed to cover denuded surfaces.[4] Epithelial cells migrate as cohesive sheets and require actin cytoskeleton-driven depolarization and dynamic turnover of focal cell-matrix associations.[4] To reestablish barrier function and tissue homeostasis, wound healing is terminated by resolution of inflammation and removal of damaged cells, a process in which resident and recruited immune cells are key players (summarized in).[5]

Innate immune cells are key contributors to the healing process

It is well-established that an innate immunity is a critical component of wound healing and gut homeostasis. Coordinated recruitment of leukocytes in response to chemotactic gradient generated at injury site is critical for host defense, resolution of inflammation, and tissue

CONTACT Ronen Sumagin, PhD. ronen.sumagin@northwestern.edu Northwestern University, Tarry Research Building, Room: 3–707, 303 E. Chicago Avenue, Chicago, IL 60611, USA.

[†]Equal contribution.

regeneration.[6] Among the immune cells, neutrophils (polymorphonuclear, PMNs) are the first to respond to insult and the ensuing chemotactic cues.[7,8] As the predominant players during the onset of injury-induced inflammation, tissue-infiltrating PMNs elicit "in-danger" cues that amplify and sustain inflammation by promoting recruitment of other inflammatory effector cells, including monocytes/macrophages and T helper 17 (Th17) cells.[9] At the site of injury, PMNs produce reactive oxygen species (ROS) and lytic enzymes critical for host defense,[10] but harmful to surrounding tissues.[11] As a consequence, the presence of PMNs in tissues is often viewed as detrimental and regarded as the hallmark of many inflammatory diseases, including IBD. However, emerging evidence demonstrates increased PMN plasticity, life-span, and phenotypic heterogeneity in inflamed tissues.[12] As such, in addition to their phagocytic activity that protects against pathogens and removes apoptotic/necrotic cells and cellular debris, PMNs are capable of producing a milieu of pro-resolving mediators, including antibacterial peptides,[13] resolvins,[14] defensins,[15] and cationic peptides such as LL-37,[16] nitric oxide,[17] and transforming growth factor *beta* (TGFβ)[18] in order to promote epithelial repair.[19] PMNs can further physically interact with epithelial receptors such as intercellular adhesion molecule-1 (ICAM-1) to direct IEC proliferation,[20] repair of blood vessels,[21] and sequential recruitment of pro-resolving macrophages to assist wound closure.[22] Macrophages replace PMNs at the late phase of the healing process and provide additional protection against pathogens, contributing to wound debridement, clearance of dead and inflammatory cells, and resolution of inflammation.[23] In this regard, PMNs and macrophages play a context-dependent role in wound repair and tissue regeneration. It is important to note that as with the innate immune cells, adaptive immunity counterparts also contribute significantly to intestinal homeostasis, where regulatory T-cells, for example, play key roles in resolution of inflammation and wound repair as seen in ulcerative colitis and Crohn's disease.[24]

The biogenesis and composition of extracellular vesicles (EVs)

To maintain tissue homeostasis, immune cells communicate with IECs via physical interaction or in a paracrine fashion by exchanging soluble effectors such as, cytokines, chemokines, small peptides, and lipid mediators.[2,22] Intriguingly, substantial evidence supports an emerging way of cell-to-cell crosstalk in the form of extracellular vesicles (EVs).[25,26] EVs are secreted by almost every cell type, and serve to shuttle, and protect bioactive effectors as well as transport genetic information between cells, in healthy tissue and disease.[25,26,27] As such, EVs emerged as important contributors to the coordinated signaling events and communication between the microbiota,[28] IECs,[29] endothelial cells,[30] and immune cells[31] during homeostasis, immune activation, and inflammation.

EVs are lipid vesicles with sizes ranging from 50 to 1000 nm in diameter.[32,33] Based on size and biogenesis, EVs can be further subdivided into *exosomes* and *microvesicles*, or in the case of immune cells often referred to as *ectosomes or microparticles (MPs)*.[33] Exosomes are vesicles with diameter of 40–150 nm and are derived during the inward budding of early endosomes to form multivesicular bodies (MVBs) and later released when these compartments fuse with the plasma membrane (Fig. 1A).[33,34] Recent *proteomic* analyses of exosomes suggested enrichment of tetraspanin proteins, as well as different classes of lipids, including cholesterol, sphingomyelin, ceramide, and phosphatidylserine.[34,35] Similarly, endosomal proteins (ESCRT, ALIX), tetraspanins (CD9, CD63, and CD81), and heat-shock proteins (HSP-70, HSP-90) were shown to be highly concentrated in exosomes of various cell types, and are currently used as universal markers for these vesicles.[35,36,37] Possibly, one of the key functions of exosomes is to transport regulatory microRNAs (miRNAs),[38,39,40] which are otherwise extremely unstable and are rapidly degraded in the tissues.[41]

Ectosomes or MPs, on the other hand, are larger particles with diameters ranging from 200 to 1000 nm that are generated by the outward budding of the plasma membrane[33,34,37] (Fig. 1B). Since ectosomes are primarily membrane-derived, they contain lipids and many of the surface molecules characteristic of parental cells they were originated from.[34] Electron microscopy and proteomic analyses confirmed size and composition heterogeneity of ectosomes[42,43,44] and established phosphatidylserine as a reliable ectosome marker.[45,46] Importantly, while the heterogeneous content of EVs reflects the parental cell phenotype, the composition, including, levels and the bioactivity of specific mediators is stimulus-dependent and is dramatically altered as a result of stimulatory conditions and the environmental milieu.[34,40,42] Thus

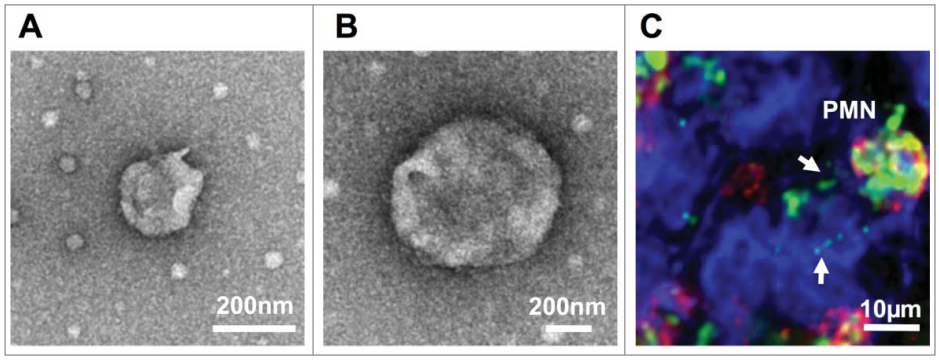

Figure 1. Characterization of PMN-derived EVs. (A-B) PMNs were stimulated with fMLF (1μM) to produce EVs. EVs were isolated by serial centrifugation and analyzed by transmission electron microscopy. (A) A representative EV with the size of exosomes (< 100 nm). (B) A representative microparticle/ectosome with the size of ~600 nm. (C) PMNs (immunolabeled for CD11b, *red* and myeloperoxidase, *green*) release myeloperoxidase-containing EVs (shown by arrows) following adhesion to and migration across IECs (surface stain, blue).

not surprisingly, EV contribution to cell function is context-dependent, and has been assigned both pro- and anti-inflammatory effects.[47,48] Finally, EVs by yet unknown mechanism can bind target cells to modulate the expression and localization of surface proteins by way of MMPs[49] and/or be internalized by target cells, resulting in the release of their content. The processes of EVs uptake is dependent on the recipient cell type, and involves clathrin-, cholesterol- and lipid raft-dependent endocytosis by immune cells.[34,37]

The biological activity of EVs in healthy and inflamed intestine

Proteins, lipids, mRNAs and miRNAs that are shuttled by EVs among neighboring cells serve as secondary messengers to temporally and spatially modulate and coordinate cellular responses.[33,34] As such, EV-associated matrix metalloproteinases (MMPs), growth factors, chemokines and miRNA can rapidly and in a localized fashion help reorganize the extracellular matrix and junctional complexes, promote cell growth and migration, as well as facilitate recruitment of immune cells. The specific contribution of EV-associated miRNAs to the regulation of these key processes mediating tissue injury and repair[47] are summarized in Table 1. EVs can be readily isolated from bodily fluids, such as serum,[50] saliva,[51] urine,[52] and in the intestine from luminal aspirates.[53] The number of EVs and their composition can reflect both healthy and pathological states.[54] Specifically, in IBD an increased number of EVs in the serum and the intestinal lumen[53] was correlated with disease severity and were shown to contain immune cell- and IEC-specific markers as well as many inflammatory markers associated with the diseases.[55] PMN-derived MPs, in particular, have been shown to be highly enriched at sites of inflammation.[56,57,58] Since EVs can be released by immune cells, endothelial, and epithelial cells, understanding the biogenesis and function of EVs in inflamed tissue will help decipher mechanisms governing the complex interplay of these cells in maintaining barrier integrity and facilitating tissue repair. In the following sections, we will discuss the contribution of EVs to intestinal homeostasis and immune cell function, specifically focusing on epithelial barrier, wound healing, and leukocyte recruitment to sites of inflammation.

EVs regulate epithelial barrier integrity

IECs via apical junctional complexes (AJCs) form a barrier to separate luminal content from the underlying tissue. Disruption of IEC junctions leads to loss of barrier integrity, a feature that underlies intestinal injury and IBD. PMN migration across IECs is a hallmark of intestinal inflammation, and is often associated with the loss barrier function.[59,60]As such, mislocalization/loss of several key components of the AJCs including, E-cadherin, Occludin, Claudin-1, Zonula Occluden-1 (ZO-1), and Junctional Adhesion Molecule-A (JAM-A) adjacent to clusters of transmigrating neutrophils was reported in clinical samples obtained from patients with IBD and cultured IECs.[59,61,62] Importantly, while most of the pathological effects of PMNs were attributed to PMN-derived soluble mediators, recently PMN-derived EVs have been implicated in contributing to these processes.[56,57] We recently reported abundant association of MMP-9 with PMN-derived MPs, released during

Table 1. A summary of miRNAs that have been shown to be transported by immune, epithelial, and endothelial cell-derived EVs and their contribution to cellular signaling and intestinal homeostasis.

	Target genes	Effects	Reference
miR-146	TLR4, TRAF6, IκBα	– Suppressed NFκB signaling – Increased cell survival. – Increased monocytic IL-10 production – Decreased IL-8 and CCL5 production	*87, 88, 89, 125, 14 2*
miR-21	RhoB, Cdc42 PTEN, PDCD4	– Increased colonic epithelial permeability. – Modulate PTEN/ PI3K/ Akt axis. – Decreased tight junction proteins, i.e. occluding and E-cadherin – Increased IL-6 and IL-8 production	*82, 83* *80*
miR-29a	Glutamine synthetase, integrin-β1, claudin-1 LRP6, HuR Mcl-1	– Impaired intestinal barrier functions. – G1-phase arrest and impaired proliferation – -IEC apoptosis	*77, 78*
miR-16	Cingulin, claudin-2, occludin	– Impaired tight junction integrity – Altered cytokine secretion by degradation of TNFα, IL-8, and IL-6 mRNAs.	*90, 91*
miR-223	IKKα STAT3 NLRP3 ARNT	– Suppressed NFκB signaling – Decreased IL-6 and IL-1β production – Decreased NLRP3 inflammasome activity – - Suppressed AHR-mediated Notch signaling	*110, 111*
miR-155	SOCS1	– Increased cytokine production of IL-6/ IL-8 – Upregulation of VCAM1 and ICAM1, followed by Increased adhesion of monocytes/T-cells to endothelial cells	*124, 128*
miR-206	A3AR	– Increased NFκB/p65 signaling – Increased IL-8/ IL-1β secretion – Increased DSS-induced colitis severity	*138, 139*
miR-141	CXCL12β	– Reduced leukocyte trafficking – Alleviated experimental colitis	*141*
miR-221	p27 DDIT4 TIMP2	– Increased cell growth – Dysregulation of mTOR signaling – Increased MMP-2/MMP-9 expression followed by remodeling of junctions and ECM	*143*
miR-320	NOD2	– Suppressed NFκB/p65 signaling – Decreased cytokine production	*143*

transepithelial migration (TEM).[49] PMN-MPs were found to bind IECs and potently cleave desmoglein-2 (Dsg-2), a key desmosomal cadherin, and destabilize epithelial cell-to cell adhesions.[49] Loss of Dsg-2 has been previously correlated with perturbed epithelial permeability[63] and mislocalization of other IEC junctional components associated with known function in regulating epithelial permeability,[64] including ZO-1 and coxsackie and adenovirus receptor (CAR).[65] Thus, tissue-infiltrating PMNs via the release of EVs can exacerbate barrier dysfunction and drive acute inflammatory responses and tissue injury in inflamed intestines. Similarly, MMP-9 was shown to degrade/cleave key adherens junction protein, E-cadherin.[66,67] Since assembly of adherens junctions is required for proper organization of barrier regulating tight junctional proteins,[68] PMNs via the release of EVs can affect the assembly of IEC junctional complexes and IEC permeability. In contrast, tissue macrophages that contribute to tissue homeostasis release EVs during differentiation that contain high levels of galectin-3.[69] Galectin-3 functions to stabilize Dsg-2 at cell junctions, enhancing the integrity of the IEC monolayer.[70] Intriguingly, PMN-derived ectosomes were found to be taken up by macrophages, inducing NFκB inhibition, and polarizing them to pro-resolving phenotype.[71,72] Thus, during injury, tissue-infiltrating PMNs may contribute to the reestablishment of epithelial barrier through reprograming of macrophages.

As with MMPs, inflammatory cytokines can promote epithelial damage and barrier dysfunction. Granulocyte-derived MPs that were isolated from intestinal luminal aspirates of IBD patients were found to contain inflammatory cytokines, including IL-6, IL-8 and TNFα.[53] IL-6 and TNFα are known to increase epithelial permeability via downregulation or mislocalization of tight junction proteins, including ZO-1, Claudins, Occludins and JAM-A.[73,74,75] As we have discussed above, EVs serve to transport regulatory miRNAs,[38,39,40] which can post-transcriptionally alter protein expression in target cells. As such, EV-associated miRNAs have been implicated in targeting IEC junctional components and modulating barrier function

during intestinal inflammation.[76] For example, increased intestinal permeability in a subset of patients with Irritable Bowel Syndrome (IBS) has been correlated with an increased number of miR-29a-rich EVs in blood and the intestinal tissue.[77] Increased intestinal permeability in these patients was suggested to be due to miR-29a-mediated downregulation of glutamine synthetase.[77] miR-29a was further shown to downregulate Claudin-1, causing increases in epithelial permeability.[78] miR-29a was also found in EVs released by dendritic cells (DCs) into the extracellular environment during cognate T-cell-DC interactions.[79] Since DCs act as sentinels in the intestinal mucosa to prime T cells activation in the case of injury or bacterial infection, EVs and miR-29a can contribute to intestinal function and barrier integrity.

miR-21 is another miRNA that is released within EVs by DCs, macrophages, and PMNs (unpublished observations) during intestinal inflammation, and can have profound effects on IEC permeability. Increases in miR21 were reported in mucosa and serum of IBD patients.[80,81] In cultured Caco-2 IECs, miR-21 impaired intestinal permeability by targeting Ras-related small GTP-binding protein B (RhoB) and cell division control protein 42 (CDC42).[80] miR-21 has also been suggested to increase intestinal epithelial tight junction permeability through activation of PTEN/PI3K/Akt signaling pathway, and knockout of miR-21 in mice led to increased intestinal permeability and apoptosis of epithelial cells.[82,83] Furthermore, miR-21 overexpression significantly downregulated Occludin and E-Cadherin, while increased IL-6 and IL-8 production,[83] confirming an important contribution of miR-21 to barrier integrity and immune cell recruitment.

Immune cells and IEC-derived miRNAs transported by EVs can further alter IEC function and intestinal barrier by modulating the activity of inflammatory transcription genes, such as NFκB and cytokine production. For example, miR-146a released in EVs by monocytes and macrophages can target interleukin-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6) to suppress NFκB signaling.[84,85,86] If taken up by either gut immune cells or IECs, miR-146a can potently suppress the release of barrier-altering cytokines, including TNFα and IL-6, reducing the inflammatory response and improving intestinal barrier.[86,87] Indeed, in IECs, miR-146a protects small intestine against ischemia/reperfusion injury by downregulating Toll-like Receptor 4 (TLR4)/TRAF6/NF-κB pathway.[88] Epithelial cell-derived miR-146a was further found to promote IL-10 released by monocytes and limit nasal inflammation.[89] Similarly, miR-16 expressed by epithelial cells[91] and released in EVs can facilitate rapid degradation of RNAs containing AU-rich elements within their 3′UTRs, causing downregulation of inflammatory cytokine, such as TNFα, IL-8 and IL-6.[90,91]

IEC-derived EVs help protect against pathogenic infections

Enteropathogenic infections and the resulting inflammation can present itself with symptoms similar to IBD, causing epithelial injury and barrier disruption.[92,93] Rapid activation of the immune system is required for pathogen clearance and reestablishment of the barrier integrity.[3] Interestingly, emerging evidence suggest that EVs may confer the ability of static epithelial cells to act at a distance to both limit bacterial spreading and inform local innate and adaptive immune responses to luminal pathogens. Indeed, the release of exosomes from the epithelium into the intestinal lumen was increased following infection by the protozoan parasite Cryptosporidium parvum.[94] IEC-derived exosomes carried antimicrobial peptides, including cathelicidin-37 and beta-defensin 2, and were found to bind and help eliminate invading pathogens.[94] Thus, IEC-derived EVs can help protect against pathogenic infections. Intriguingly, electron microscopy examination of the luminal IEC surface revealed a layer of EVs up to 50 nm in diameter between the microvilli and mucous gel, suggesting that EV layer can act as an additional barrier to limit adherence by both commensal and pathogenic bacteria.[95]

EVs released apically or basolaterally by IECs were also found to contain MHC class II and other accessory molecules involved in antigen presentation, suggesting that they can act as antigen-presenting vesicles to prime adaptive immune cells for immunogenic responses in the mucosa.[29,96] Similarly, epithelial cell-derived exosomes entrapped and transported $\alpha v\beta 6$ integrin and food antigens to DCs, resulting in production of active TGFβ by DCs and generation of antigen-specific regulatory T cells.[97] In contrast, EVs released by an enteric pathogen, Giardia intestinalis,

can potentially promote inflammation and IEC injury by facilitating attachment of Giardia to IECs.[98]

EVs contribution to intestinal injury and repair

Emerging evidence suggests that following injury and the ensuing inflammation, immune cell, stroma cell, and IEC-derived EVs can locally alter the production of cytokines, growth and transcription factors in wounded mucosa to regulate cell migration, proliferation, and differentiation. As such, EVs have been suggested to both impede and promote resolution of inflammation and tissue repair.

PMNs are the first immune cells to infiltrate the intestinal mucosa following injury. During TEM, PMNs were found to release microparticles (MPs) that contain abundant levels of myeloperoxidase (MPO).[99] The binding of PMN derived, and MPO containing EVs to IEC is shown in (Fig. 1C). While MPO primarily functions in bacterial killing, during PMN activation, it is mobilized to the cell surface and is released in association with MPs. MP-associated MPO is enzymatically active, and when delivered to IECs, can impair actin dynamics, migration, and proliferation, significantly impeding IEC wound closure.[99] PMN-derived EVs were further suggested to potentiate endothelial cell injury via deposition of MPO-rich EVs,[100] increased production of reactive oxygen species,[101] and increased pro-inflammatory activity of metalloproteinase domain containing proteins 10 and 17 (ADAM10 and ADAM17).[102] PMN-derived EVs binding to endothelial cells facilitated leukocyte recruitment by inducing IL-6, IL-8 and MCP-1 release and upregulating adhesion molecules by inflamed endothelial cells, thus aggravating tissue injury.[103,104]

In contrast, granulocytic myeloid-derived suppressor cells (MDSC)-derived exosomes were found to attenuate dextran sulfate sodium (DSS)-induced epithelial injury by reducing the number of Th1 cells and increasing T regulatory cells in a TGFβ-dependent manner.[105] DC-derived EVs may similarly act to suppress inflammation and tissue damage, as they contain abundant amounts of milk fat globule EGF/factor VIII (MFG-E8),[106] which has been shown to promote dead cell clearance and inhibition of NFκB-dependent release of pro-inflammatory cytokines in experimental colitis.[107] Intriguingly, given the well-established cross-communication between various immune cells in inflammation,[108] PMN-derived EVs were suggested to suppress inflammatory responses and promote pro-repair function of macrophages in injured tissue. PMN-derived EVs were shown to inhibit NFκB signaling and increase the release of TGFβ[71] and pro-resolving lipid mediators, such as Resolvin D1 and Resolvin E2.[109] Moreover, in addition to serving as an important source of pro-resolving mediators and their role in wound debridement, macrophages via the release of miR-223-containing EVs can potentially promote wound healing by enhancing epithelial cell migratory behavior, as has been shown for breast cancer cells.[110] Indeed, *miR-223*$^{-/y}$ mice presented exacerbated, myeloid cell-driven experimental colitis with heightened clinical, histopathological, and inflammatory cytokine readouts.[111] Injured IECs were also shown to release EVs, which served to promote resolution of inflammation and healing. A recent work elegantly demonstrated that IEC-derived exosomes containing Annexin A1 (ANXA1), promoted wound closure by binding to formyl peptide receptors (FPRs) and FPR-dependent generation ROS.[19] Physiological relevance of these findings was confirmed by the observation of elevated ANXA1-containing EVs in patients with active IBD.[19] Interestingly, in the circulation, PMNs were similarly shown to release MPs containing ANXA1, where they served to limit PMN adhesion to the endothelial cells and inhibit inflammatory recruitment of PMNs.[112] Because the release of MPs by PMNs is stimulus-dependent, whether this is also true in the setting of intestinal injury, and whether by function of ANXA1 PMN-MPs could promote IEC repair remains to be determined. Similarly, TGFβ-containing exosomes from injured epithelial cells were found to activate fibroblasts and promote tissue repair by increased matrix deposition and fibrosis.[113] TGFβ mRNA transported by exosomes induced proliferation, α-smooth muscle actin expression, and F-actin expression in fibroblasts. It is reasonable to speculate that if taken up by neighboring IECs, these exosomes could similarly promote IEC migration and proliferation; however, this would need to be experimentally confirmed. Furthermore, fibroblasts in the wound bed can promote epithelial cell motility, which is an essential component of wound healing,[114] via the release of CD81-containing EVs, as has been shown for breast cancer cells.[115] Epithelial exosomes also contain the A33 antigen, which is a transmembrane protein expressed predominantly in intestinal epithelium and is associated with the regulation of IEC migration and

proliferation.[116] Mice lacking A33 antigen expression were compromised in their ability to resolve hapten-induced mucosal damage, exhibiting impaired IEC proliferation.[116] Thus, through the activity of A33, IEC-derived EVs may promote intestinal wound repair. Finally, Wnt5a, which is significantly increased at the wound bed and contributes to epithelial healing,[117,118] has been detected in the exosomal fraction of Caco-2 IECs.[119]

EVs contribution to leukocyte trafficking in inflamed tissue

Immune cells in the intestinal mucosa and other tissues play critical roles in host defense, and as discussed above contribute significantly to tissue injury and resolution of inflammation. Recruitment of immune cells is dependent upon the generation of local gradients of chemokines, growth factors, and cytokines produced by resident and recruited cells. EVs by way of miRNAs can regulate the expression of these chemotactic factors, or directly shuttle them to the surrounding tissue to promote/limit leukocyte recruitment. Leukocytes recruited to sites of inflammation must first cross the endothelial barrier, a process that is mediated by several classes of adhesion molecules and chemotactic cues.[120] In the circulation, EVs released by activated monocytes were found to induce expression of ICAM-1 (a key leukocyte adhesion molecule)[121] and the release of CCL2 (a potent monocyte chemoattractant)[122] to promote leukocyte recruitment.[123] These effects were attributed to the presence of pro-inflammatory miR-155 in these EVs and its delivery to endothelial cells. Indeed, endothelial miR-155 has been confirmed to positively regulate expression of ICAM-1 and VCAM-1.[124] miR-155 is one of several miRNAs, that were found to be highly enriched in IBD and was further suggested to regulate cytokine production.[125,126] As such, miR-155 was found to inhibit suppressor of cytokine signaling 1 (SOCS1), an important anti-inflammatory gene,[127] resulting in elevated production of leukocyte agonists and chemoattractants, including IL-6 and IL-8, by intestinal myofibroblasts.[128] Similarly, serum EVs, presumably produced by the immune and endothelial cells in a mouse model of colitis were found to promote inflammatory activation of gut macrophages and increased production of TNFα.[129] TNFα can impair both endothelial[130] and epithelial barriers[131,132] and enhance leukocyte recruitment.[133,134] Macrophage activation by EVs, leading to pro-inflammatory cytokine release and increased recruitment of inflammatory cells, was also substantiated in lung injury.[135] EV numbers were found to be increased in Crohn's patients, with an elevated disease score, which includes quantification of inflammatory cell infiltrate,[53] providing further clinical relevance of these observations.

In contrast to anticipated pro-inflammatory functions, EVs released by circulating PMNs upon adhesion to vascular endothelium were shown to be enriched with Annexin A1, an anti-inflammatory protein.[112] In this setting, EVs exerted an anti-inflammatory effect by inhibiting PMN adhesion and recruitment to inflamed tissue.[112] Similarly, ICAM-1 released in association with EVs has been suggested to competitively inhibit leukocyte adhesion to endothelium.[136] Endothelial and mesenchymal cell can also release EVs containing miR-206.[137] MiR-206 is significantly elevated in IBD,[138] and acts to suppress NFκB signaling and the release of leukocyte chemoattractants, IL-8, CXCL1 and CXCL2,[139] thus limiting immune cell infiltration. Several other miRNAs were similarly implicated in dampening inflammatory cell recruitment and the resulting tissue injury. For example, miR-141 that is released in EVs was suggested to keep inflammatory cytokine production in check.[140] Downregulation of miR-141 in Crohn's patients and experimental models of colitis resulted in increased CXCL12β production by IECs and enhanced leukocyte infiltration of the intestinal mucosa.[141] miR-146 expression in epithelial cells has been shown to decrease IL-8 and RANTES/CCL5 release,[142] suggesting its role in leukocyte trafficking during colonic inflammation. Following injury, IEC-derived EVs[143] are enriched with hypoxia-induced miRNAs, miR-221 and miR-320a, which induce upregulation and activation of matrix metaloproteinase-9 (MMP-9), a protease known to be involved in macrophage and PMN trafficking by facilitating reorganization of junctional complexes and the extracellular matrix.[49]

Finally, in addition to regulating cytokine expression, EVs can transport various chemokines and lipids, and can locally generate chemotactic gradients for migrating leukocytes. Indeed, in a Trans-well setup, macrophage-derived EVs were shown to induce granulocyte migration.[144] Moreover, IL-8[145] and IL-18[146] encapsulated in EVs can act as chemoattractants of PMNs in various disease settings. Macrophage and DC-derived EVs were

found to contain biologically active enzymes for leukotrienes biosynthesis (LTA4 and LTB4) and promote granulocyte recruitment.[144] Granulocytic peripheral blood cells (RBL-2H3) were shown to release exosomes containing prostaglandins, such as, PGE2,[147] which among other functions can promote calcium and CCR7-dependent migration of monocyte-derived DCs.[148]

Concluding remarks and future perspectives

As our knowledge of EV biogenesis and content expands, the contribution of EVs to intercellular communication and regulation of cellular processes in healthy and inflamed tissue become apparent. Many aspects of EV biology still remain unanswered, including active versus passive release by parent cells, cargo protection, uptake by target cells, content release, and importantly, pro- versus anti-inflammatory function of EVs. However, as we have outlined in this review, particularly in the gut, EVs contribute to the regulation of vascular and epithelial barrier function, wound healing, and function of resident and recruited immune cells (EVs release by various cell types, content and effects on cell function are summarized by schematic representation, Fig. 2). EVs research has already sparked a clinical interest, as EVs were found to be elevated in the serum and tissues of patients with IBD and other multifactorial disorders. Moreover, given that the cargos associated with EVs often reflect diverse healthy and pathogenic states of the releasing cells and tissues, ongoing efforts are dedicated to exploring the possibility in which EVs can be used as biomarkers to diagnose and assess the therapeutic success of complex disorders, including IBD. For example, analysis of serum EV microRNA content in the clinic could be easily achieved by next-generation sequencing or digital PCR techniques, potentially yielding diseases specific gene signatures.[38,39] Furthermore, since EVs are specifically equipped to mediate the transfer of regulatory short RNA molecules between cells, the possibility of exploiting these vesicles for therapeutic purposes is now being investigated. As such, ongoing efforts are being made to develop techniques that encapsulate therapeutic peptides, nucleic acids, and small molecule inhibitors into EVs, and protect them and increase their bio-availability and delivery to disease tissues.[149] This new line of therapy is a great premise for treatment of inflammatory diseases, such as IBDs.

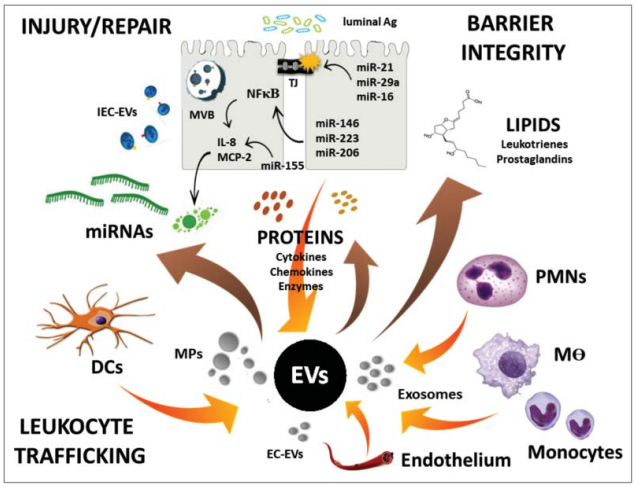

Figure 2. EV content and regulation of intestinal homeostasis. Schematic of EV release by various gut resident and recruited cells, as well as EV-mediated transport of proteins, lipids, and miRNAs to regulate cell function and intestinal homeostasis.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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