Molecular Immunology 93 (2018) 125–132

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Review

Role of extracellular vesicles in rheumatoid arthritis

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ARTICLE INFO

Keywords:
Extracellular vesicle
Rheumatoid arthritis
Signal communication
Pathogenesis
Inflammation
microRNA

ABSTRACT

Cell-derived extracellular vesicles (EVs) are involved in the pathogenesis of rheumatoid arthritis (RA), playing important roles in antigen presentation, inflammation, angiogenesis, cell–cell signal communication, thrombosis, and articular cartilage extracellular matrix degradation. Understanding the pathogenic mechanism of RA is important for developing therapies. The pathogenic indicators of RA, such as submicron-sized EVs, represent promising biomarkers for evaluating RA activity. This review summarizes the recent advances in understanding the pathogenesis of RA, and sheds light on the pathogenic as well as anti-inflammatory or immunosuppressive roles of EVs. We suggest that EVs could be harnessed as tools for drug delivery or targets for RA therapies.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, and systemic autoimmune disease characterized by joint pain, swelling, deformity, and ultimately, disability. T cells mediate the immune disorder in RA, and large amounts of diverse cell types participate in the pathogenesis (Malda et al., 2016); however, the precise pathogenic mechanism is not fully understood. Recently, extracellular vesicles (EVs) have emerged as a crucial player in the pathogenesis of RA, and their multiple biological effects have attracted the attention of many researchers. EVs mediate the communication of diverse cell types in an RA-inflamed joint. A number of pharmacologic intervention drugs for RA have been developed including non-steroidal anti-inflammatory drugs, corticosteroids, and disease-modifying anti-rheumatic drugs (Upchurch and Kay, 2012); nevertheless, none of these therapies are curative. Additionally, many of these drugs have serious side effects or lose effectiveness over time (Kim et al., 2016a). Recently, newer biologics, such as infliximab targeting tumor necrosis factor (TNF)-α (Monaco et al., 2015), have shown marked therapeutic improvements in RA treatment. However, nearly 50% of patients do not respond to anti-TNF-α therapy (Rubbert-Roth and Finckh, 2009). Even though inflammation is well controlled, the destruction of articular cartilage continues to progress (Headland et al., 2015). As these drugs do not repair bone erosions via the formation of new bone, pro-anabolic agents are needed to promote bone formation at the erosion sites. Gene therapy for RA treatment shows promise; however, it is unknown whether gene therapy is safe for treating patients with RA (Yang and Robbins, 2012). Recent studies have revealed that EVs play important roles in both the pathogenic effects and therapeutic treatments of RA.

EVs are membrane-bound vesicles released by almost all cell types during activation or apoptosis. EVs, particularly tumor-derived EVs, have various functions. These heterogeneous structures include exosomes, microvesicles (also known as microparticles), and apoptotic bodies, which can be detected in various biological fluids (Raposo and Stoorvogel, 2013; van der Pol et al., 2012). EV classification or nomenclature is based on a vesicle's size, mechanism of biogenesis, and cell type of origin (Buzas et al., 2014; van der Pol et al., 2012). The formation of exosomes (30–100 nm in diameter) occurs by the invagination of membranes of multivesicular bodies (MVBs), which are then secreted via fusion of the MVB membrane with the cytoplasmic membrane (Denzer et al., 2000; Thery et al., 2002). Microvesicles (100–1000 nm in diameter) are generated directly by outbudding and shedding from the cytoplasmic membrane (Akers et al., 2013; Gyorgy et al., 2011). Apoptotic bodies (1–5 μm in diameter), belonging to apoptotic vesicles (100–5000 nm in diameter), are formed by plasma membrane blebbing of apoptotic cells (Gyorgy et al., 2011). EV

Abbreviations: FLS, fibroblast-like synoviocyte; APC, antigen presenting cell; EV, extracellular vesicle; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; Id1, inhibitor of DNA binding 1; CD13, aminopeptidase N; MCP, monocyte chemoattractant protein; RANK, receptor-activator of nuclear factor kappa beta; RANKL, receptor-activator of nuclear factor kappa beta ligand; TNF, tumor necrosis factor; 15-LO, 15-lipoxygenase; 15-H(p)ETE, 15-hydro(pero)xyeicosatetraenoic acid; TLR, Toll-like receptor; FADD, Fas-associated death domain protein; SASP, salicylazosulfapyridine; MTX, methotrexate; NF-κB, nuclear factor kappa B; IRAK, interleukin-1 receptor-associated kinase; SF, synovial fluid; miRNA, microRNA

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https://doi.org/10.1016/j.molimm.2017.11.016
Received 22 July 2017; Received in revised form 14 November 2017; Accepted 18 November 2017

classification is encumbered by the fact that these multifunctional structures share common features with no specific markers to distinguish them; moreover, different laboratories employ differing terminologies for classification. This review uses the generic term 'EVs' to describe exosomes and microvesicles.

EVs likely mediate cell–cell communication by involving various molecules that are physiologically and pathologically active. EVs function as protective transporters of such mediators, whether they are lipids, proteins, nucleic acids, or adhesion molecules (Beyer and Pisetsky, 2010; Buzas et al., 2014; Thery et al., 2002; van der Pol et al., 2012). Although EVs may be involved in many diseases, this review focuses on their roles in RA.

2. EVs in the pathogenesis of RA

2.1. EV mediation of cell–cell communication

Accumulated evidence suggests that EVs are involved in the pathogenesis of RA. EVs have been identified in the synovial fluid (SF) of patients with RA (Beyer and Pisetsky, 2010; Cloutier et al., 2013; Fourcade et al., 1995), and EVs appear at significantly higher levels in RA cases than in healthy controls (Sellam et al., 2009). EVs isolated from SF or plasma modulated the release of B cell-activating factor (Messer et al., 2009), chemokines, and cytokines from synoviocytes *in vitro* (Berckmans et al., 2005; Vinuela-Berni et al., 2015); however, their effect on pro-inflammatory cytokine release *in vivo* was deficient.

Several studies have suggested that EVs from leukocytes play a role in the pathogenesis of RA (Berckmans et al., 2002; Boilard et al., 2010). Indeed, monocyte- and T cell-derived EVs caused a pro-inflammatory phenotype in synovial fibroblasts (fibroblast-like synoviocytes [FLSs]) via upregulation of cyclooxygenase 2 (COX-2) and microsomal prostaglandin E synthase 1 (mPGES-1). Arachidonic acid, contained in these EVs, was converted to prostaglandin E2 (PGE2) via the catalytic activities of COX-2 and mPGES-1 in synovial fibroblasts (Jungel et al., 2007). Furthermore, the release of matrix metalloproteinases (MMPs; MMP-1, MMP-3, MMP-9, and MMP-13) was enhanced via nuclear factor kappa B (NF-κB)-dependent pathways in synovial fibroblasts by EVs derived from monocytes or T cells. In addition, EVs showed increased production of inflammatory cytokines (Distler et al., 2005), such as interleukin (IL)-6, IL-8, monocyte chemoattractant protein 1/2 (MCP), vascular endothelial growth factor (VEGF) (Berckmans et al., 2005), pro-angiogenic chemokines (Reich et al., 2011), and B cell-activating factor (Messer et al., 2009). These *in vitro* coculture experiments revealed that EVs mediate communication between inflammatory cells and synovial fibroblasts in RA (Fig. 1). Additionally, polymorphonuclear leukocyte-derived EVs induced the release of IL-6 and IL-8 by endothelial cells *in vitro* (these experiments were not performed *in vivo*) (Mesri and Altieri, 1998). In addition to their pro-inflammatory role, monocyte- and granulocyte-derived EVs exert a pro-coagulative function via a factor VII-dependent mechanism, by which they induce thrombin formation *in vitro*. These functions suggest EVs are associated with hypercoagulation and fibrin deposition in joints (Berckmans et al., 2002). As thrombin can induce an inflammatory response (Berckmans et al., 2001; Marin et al., 2001), EVs from leukocytes indirectly contribute to inflammation by inducing thrombin formation in a manner distinct from the direct stimulation of FLSs. However, the role of EVs released from leukocytes requires further *in vivo* studies using an animal model.

Activated platelet-derived EVs (PEVs) are related to the severity of RA (Knijff-Dutmer et al., 2002; Vinuela-Berni et al., 2015); greater numbers of activated PEVs than leukocyte-derived EVs are released in the SF of patients with RA (Berckmans et al., 2002; Boilard et al., 2010). Using the *in vivo* K/BxN serum transfer model of arthritis, platelet-depleted mice displayed a significant reduction in arthritis (Boilard et al., 2010; Semple et al., 1996), suggesting the importance of PEVs in the pathogenesis of RA. Indeed, the generation of PEVs, which express IL-1 on their surface, is triggered by the interaction between collagen and a collagen receptor (glycoprotein VI) on the platelet surface. PEVs stimulate FLSs via the IL-1 receptor and elicit the FLS release of the proinflammatory cytokine IL-6 and the neutrophil chemokine IL-8 (Fig. 2) (Boilard et al., 2010). Furthermore, after entering the inflamed joint, the PEV membrane phospholipids can be metabolized into the pro-inflammatory lipid mediator 12(S)-hydroxyeicosatetranoic acid (12(S)-HETE) by the concerted action of 12-lipoxygenase (12-LO) in PEVs and the secreted phospholipase A2-IIA (sPLA2-IIA) in the inflammatory SF (Duchez et al., 2015). The ligation of 12(S)-HETE and its receptor leukotriene B4 receptor 2 (BLT2) expressed on the surface of neutrophils enhances the internalization of PEVs in neutrophils (Duchez et al., 2015), a process closely related to inflammation (Wright et al., 2014).

Given the significant effect of PEVs in RA pathogenesis, it is important to understand the mechanism and location of platelet activation that leads to the production of PEVs. PEV concentrations in SF are considerably higher than those in the circulation of patients with RA (Biro et al., 2007; Knijff-Dutmer et al., 2002), suggesting that PEVs are likely locally generated in the vasculature of the synovium. PEVs bearing surface components of their original cells (Andaloussi et al., 2013) induce coagulation (Wang et al., 2012; Wolf, 1967), indicating that PEVs might possess functional segments of platelets. Platelets can adhere to the surface of leukocytes (Ehlers et al., 2003; Joseph et al., 2001), which can pass through vascular endothelial cells; therefore, PEVs might also have the ability to bind to leukocytes to enhance the presence of PEVs in SF. Intriguingly, PEVs adhere to the leukocyte surface in the SF of patients with RA (Fig. 1) (Boilard et al., 2010). Platelets adhering to leukocytes release PEVs via glycoprotein VI when encountering collagen of the subendothelial matrix. One hypothesis for the presence of PEVs in RA joints is that PEVs emerge from the endothelial gap in the inflamed synovial vasculature. These gaps allow the efflux of submicron particles when platelets release serotonin. The presence of endothelial gaps was confirmed using an autoimmune arthritic model, and submicron particles could migrate into the joint space (Cloutier et al., 2012). Correspondingly, synovial vasculature displayed fenestrations and enhanced permeability after stimulation (Binstadt et al., 2006), and PEVs reduced the protective role of endotheliocytes (Rautou et al., 2011). The classical role of platelets is to maintain vascular integrity. The contradictory functions of platelets for inducing vascular permeability might be dependent on whether the synovium is inflamed. Additionally, endothelial EVs impair the protective function of endotheliocytes and increase vascular permeability (Rautou et al., 2011).

EVs discharged from both platelets and leukocytes in the SF of patients with RA have a substantial role in inflammation, hypercoagulability, and cartilage extracellular matrix (ECM) degradation by stimulating synovial fibroblasts. EVs are likely the missing link between inflammation and coagulation. Similar to platelets, PEVs can expose tissue factor on their surface and initiate coagulation *in vivo* (Berckmans et al., 2002; Muller et al., 2003). Interestingly, tissue factor is also expressed on the surface of leukocyte-derived EVs, presumably due to the transfer of tissue factor from platelets to leukocytes via PEVs (Celi et al., 1994; Scholz et al., 2002). The increased levels of EVs in plasma from patients with RA might cause cardiovascular diseases, the most common cause of death in patients with RA (Dye et al., 2013).

FLSs (also named synovial fibroblasts) play a central role in the maintenance of joint inflammation and in the destruction of cartilage. Both leukocyte- and platelet-derived EVs can induce pro-inflammatory factor release from FLSs. Leukocyte-derived EVs induce FLSs to release B cell-activated factor involved in B cell activation, proliferation, and resistance to apoptosis (Schneider and Tschopp, 2003). EVs from IL-1β-stimulated FLSs upregulated MMP-13, MMP-3, IL-6, and VEGF expression in chondrocytes *in vitro* and induced angiogenic activities in human umbilical vein endothelial cells (Kato et al., 2014). EVs discharged from activated FLSs contain multiple enzymes for cartilage matrix