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Exosomes: From Functions in Host-Pathogen Interactions and Immunity to Diagnostic and Therapeutic Opportunities

Jessica Carrière, Nicolas Barnich, and Hang Thi Thu Nguyen

Abstract Since their first description in the 1980s, exosomes, small endosomal-derived extracellular vesicles, have been involved in innate and adaptive immunity through modulating immune responses and mediating antigen presentation. Increasing evidence has reported the role of exosomes in host-pathogen interactions and particularly in the activation of antimicrobial immune responses. The growing interest concerning exosomes in infectious diseases, their accessibility in various body fluids, and their capacity to convey a rich content (e.g., proteins, lipids, and nucleic acids) to distant recipient cells led the scientific community to consider the use of exosomes as potential new diagnostic and therapeutic tools. In this review, we summarize current understandings of exosome biogenesis and their composition and highlight the function of exosomes as immunomodulators in pathological states such as in infectious disorders. The potential of using exosomes as diagnostic and therapeutic tools is also discussed.

Keywords Exosomes • Host-pathogen interactions • Immune responses • Infectious diseases

Contents

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Abbreviations

Ag	Antigen
AIEC	Adherent-invasive *Escherichia coli*
APC	Antigen-presenting cells
APOBEC3G	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G
BAT3	HLA-B-associated transcript 3
BCR	B-cell receptor
CD	Crohn's disease
CMV	Cytomegalovirus
CXCL11	C-X-C motif chemokine 11
DC	Dendritic cell
DT	Diphtheria toxoid
EBV	Epstein-Barr virus
EF1α1	Elongation factor 1-alpha 1
EM	Electron microscopy
ESCRT	Endosomal sorting complexes required for transport
EV	Extracellular vesicle
FACS	Fluorescence activated cell sorting
FasL	Fas ligand
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPC1	Glypican-1
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSC	Heat-shock cognate
HSP	Heat-shock protein
HTLV-1	Human T-cell leukemia virus type 1
i.v.	Intravenous
ICAM-1	Intercellular adhesion molecule 1
IEC	Intestinal epithelial cell

IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILV	Intraluminal vesicles
LAM	Lipoarabinomannan
LF	Lethal factor
LMP1	Latent membrane protein 1
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein 1
MFGE8	Milk fat globule-EGF factor 8 protein
MHC	Major histocompatibility complex molecules
miRNA	MicroRNA
MVE	Multivesicular endosome
MyD88	Myeloid differentiation primary response protein 88
NEF	Negative regulatory factor
NF-κB	Nuclear factor-kappa B
NK	Natural killer
NKG2D	Natural killer group 2 member D receptor
OVA	Ovalbumin
PA	Protective antigen
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cells
PfPTP2	Plasmodium falciparum tyrosine phosphatase 2
RANTES	Regulated on activation, normal T cell expressed and secreted
SNARE	Soluble *N*-ethylmaleimide-sensitive fusion attachment protein (SNAP) receptors
TAR	Transactivating response
TGF-β	Tumor growth factor beta
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
tRNA	Transfer RNA
TSG101	Tumor susceptibility gene 101
vtRNA	Vault RNA

1 Introduction

Cell-to-cell communication is crucial for maintaining homeostasis within a multicellular organism. In particular, this communication is fundamental in innate and acquired immunities to trigger well-orchestrated immune responses. Among identified mediators, extracellular vesicles (EVs) have achieved a growing interest and are the subject of an increasing number of studies. Several types of EVs have been described to date that have been given different names throughout literature

such as microvesicles (also called microparticles or ectosomes) to designate EVs directly released from the plasma membrane, membrane particles, microvesicles, nanoparticles, "exosome-like" microvesicles, tolerosomes, prostasomes, or exosomes to refer to EVs released upon fusion of multivesicular endosomes (MVEs) with the plasma membrane. EVs are traditionally classified according to their intracellular origin, their physical properties, or their protein content. Specific isolation tools and techniques to distinguish EVs from different origins in order to establish a reliable classification are lacking. Therefore, Kowal and coauthors have recently compared the protein content of heterogeneous populations of EVs in order to establish a reliable classification (Kowal et al. 2016). According to the authors, EVs can be firstly classified according to their sedimentation speed, and then EV subpopulations can be distinguished according to their floatation density on iodixanol gradient and their protein content (Kowal et al. 2016).

Exosomes are defined as small EVs (30–100 nm in diameter) pelleting at high speed (ultracentrifugation at 100,000 g) and released upon fusion of MVEs with the plasma membrane (Colombo et al. 2014). In the 1980s, P. Sthal's and R. Johnstone's groups originally identified exosomes by their role in elimination of the transferrin receptor via secretion during reticulocyte maturation (Harding et al. 1983; Pan et al. 1985). Since their first description, exosomes have been well-characterized and were shown to be nanovesicles of endocytic origin. Exosomes have been successfully purified from most of body fluids (i.e., serum, saliva, urine, breast milk, etc.) and from cell culture medium (Théry et al. 2006). Analysis of molecular composition of exosomes allowed identification of a rich content with numerous proteins as well as lipids and nucleic acids (Théry et al. 2009). In addition to the molecular composition, numerous groups have been interested in studying the functions of exosomes either in physiological or in pathological states. To date, the most widely documented function of exosomes is their role in immunoregulation. Indeed, exosomes act as crucial regulators in innate immunity since exosomes released from immune cells were shown to be able to stimulate activation, proliferation, and inflammatory responses in various immune recipient cells (Théry et al. 2009). In addition, increasing evidence supports the involvement of exosomes in acquired immunity and particularly in antigen presentation (Théry et al. 2009). The wide range of functions of exosomes in immunoregulation attracts the attention of scientists in fields of research of pathologies such as infectious disorders. As such, exosomes have been shown to be involved in immunoregulation during fungal, parasitic, viral, and bacterial infections, and they can be beneficial either for host defense or for virulence and spread of pathogens. Due to their accessibility in various body fluids and their capacity to convey a complex molecular content even to distant cells, exosomes have been proposed as potential diagnostic, vaccine, and therapeutic tools. However, only a few experiments have been performed to date, in which exosomes were used to diagnose disease, vaccinate, and convey therapeutic molecules.

In this review, we introduce current understandings of biogenesis, secretion, and composition of exosomes. We will then highlight the function of exosomes as immunomodulators in pathological states such as in infectious disorders. The

potential of using exosomes as diagnostic, vaccine, and therapeutic tools will also be discussed. It is worthy to note that in several publications cited in this review, other terms rather than "exosomes" were used, which correspond to a mixture of vesicles from different origins.

2 Exosome Biogenesis and Secretion

Exosomes have been isolated from various body fluids such as urine, saliva, bile, breast milk, or blood (Yáñez-Mó et al. 2015). Exosomes are actively secreted by most cell types, in particular, immune cells such as B cells (Clayton et al. 2005), T cells (Nolte-'t Hoen et al. 2009), dendritic cells (DCs) (Théry et al. 1999; Zitvogel et al. 1998), macrophages (Bhatnagar et al. 2007), platelets (Heijnen et al. 1999), and mast cells (Raposo et al. 1997) and from other cell types such as neurons (Fauré et al. 2006), epithelial (Marzesco et al. 2005), endothelial (Song et al. 2014), and mesenchymal stem cells (Lai et al. 2015).

The unique property of exosomes is attributed to their endocytic origin. During exosome biogenesis, extracellular components and membrane receptors are endocytosed in an early endosome (Fig. 1). Then, early endosomes mature into late endosomes (Stoorvogel et al. 1991) and, during this process, small intraluminal vesicles (ILVs) accumulate into MVEs upon budding of the inner membrane of late endosomes, leading to sequestration of proteins, lipids, and cytosolic components. Although MVEs can subsequently fuse with the lysosome to induce cargo degradation (Woodman and Futter 2008), some MVEs can fuse with the plasma membrane, resulting in the release of ILVs as exosomes (Denzer et al. 2000).

Although exosome biogenesis is still being defined, a well-described mechanism for ILV formation is driven by the endosomal sorting complexes required for transport (ESCRT), which is composed of four ESCRT complexes (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III) with associated proteins (e.g., ALIX, VPS34) (Hanson and Cashikar 2012). Firstly identified in endosomal sorting and degradation of ubiquitinated proteins (Davies et al. 2009; Metcalf and Isaacs 2010), ESCRT proteins have been shown to mediate membrane invagination process and ILV formation (Davies et al. 2009; Hurley 2010; Metcalf and Isaacs 2010). Thus, ESCRT-0 binds ubiquitinated proteins, allowing their delivery to MVEs (Raiborg and Stenmark 2002). Then, ESCRT-0 recruits ESCRT-I, which consequently recruits ESCRT-II and ESCRT-III (Babst et al. 2002; Katzmann et al. 2001). By triggering membrane invagination and scission, ESCRT-III enables ILV formation (Wollert et al. 2009). Several studies support the involvement of ESCRT proteins in exosome biogenesis since knockdown of ESCRT proteins has been shown to abolish ILV formation and exosome secretion (Stuffers et al. 2009; Tamai et al. 2010).

Another ESCRT-independent mechanism in exosome biogenesis has been raised, in which siRNA-mediated silencing of *ESCRT* genes did not abrogate totally exosome release (Stuffers et al. 2009). First, analysis of exosome secretion from

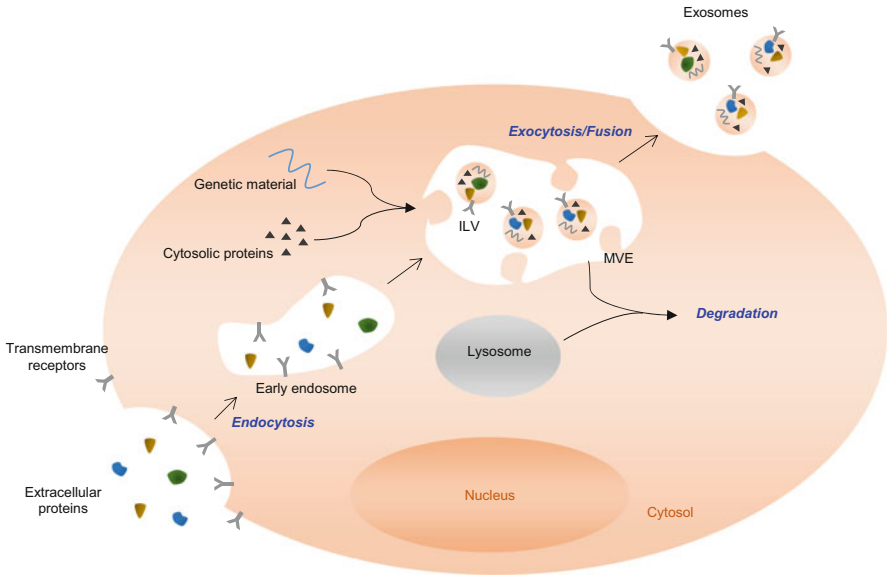

Fig. 1 Exosome biogenesis. Extracellular proteins and transmembrane receptors are endocyted in an early endosome. Early endosomes mature into late endosomes, and small intraluminal vesicles (ILVs) accumulated into their lumen upon budding of the inner membrane of late endosomes, leading to sequestration of cytosolic components and genetic material. Multivesicular endosomes (MVEs) will then fuse with either lysosomes, leading to the degradation of their content, or the plasma membrane, a process involving SNARE proteins and RAB GTPases, leading to the release of ILVs called "exosomes" into extracellular environment

oligodendrocytes, central nervous system cells, showed that exosome secretion requires the sphingolipid ceramide (Trajkovic et al. 2008). Another study reported that tetraspanins might be also involved in exosome biogenesis since depletion of the CD63-coding gene in vitro in melanocytes or in vivo in *cd63*$^{-/-}$ mice led to a reduction of ILV formation (van Niel et al. 2011).

Once MVEs are formed, they can either fuse with the lysosome to mediate cargo degradation (Woodman and Futter 2008) or with plasma membrane, a process mediated by the cytoskeleton, small GTPases, and fusion machinery (Colombo et al. 2014). Among the GTPases involved in ILV exocytosis, several RAB GTPases, which are members of the Ras GTPase superfamily, have been identified in exosomes such as RAB5, RAB11, RAB27, and RAB35. Indeed, RAB11 inhibition by overexpressing a dominant negative mutant in K562 erythroleukemia cells decreased exosome release (Savina et al. 2002). Moreover, inhibition of RAB35 function resulted in an impaired exosome secretion and accumulation of ILVs (Hsu et al. 2010). Furthermore, shRNA-mediated silencing of RAB27A and RAB27B in HeLa cells decreased exosome secretion (Ostrowski et al. 2010). Recently, it was reported that inhibition of RAL-1 GTPase resulted in a hampered fusion of MVEs with the plasma membrane and consequently in a decreased exosome secretion (Hyenne et al. 2015). The fusion machinery, involving soluble *N*-ethylmaleimide-

sensitive fusion attachment protein (SNAP) receptors (SNARE), has been shown to mediate exosome secretion. SNARE proteins form complexes between vesicular v-SNARE (vesicular-associated membrane proteins or VAMP) proteins and cell membrane t-SNARE proteins (Zylbersztejn and Galli 2011). It was reported that overexpression of the SNARE protein VAMP7 in K562 cells led to an impaired exosome secretion (Fader et al. 2009).

3 Exosome Molecular Composition

Exosomes have been shown to contain proteins, lipids, and nucleic acids (Fig. 2). Protein content of exosomes has been extensively analyzed by several techniques including Western blotting, immune-electron microscopy (immuno-EM), fluorescence-activated cell sorting (FACS), and mass spectrometry (Colombo et al. 2014). Exosomal protein content varies depending on the cell type of origin: for example, B-cell-derived exosomes contain the B-cell receptor (BCR), and DC-derived exosomes contain MCH-II, CD86, and ICAM-1 proteins (Théry et al. 2009). Furthermore, exosomes contain some common proteins such as adhesion molecules [milk fat globule-EGF factor 8 (MFGE8), integrins, and tetraspanins (CD63, CD81, and CD9)], chaperones [heat-shock cognate protein 70 (HSC70), heat-shock protein 90 (HSP90)], proteins involved in membrane trafficking (e.g., RAB GTPases, annexins) and in MVE biogenesis (e.g., clathrin, ALIX, TSG101), etc. (Fig. 2) (Colombo et al. 2014). Recently, proteomic analysis of heterogenous populations of small EVs, separated by a combinatorial approach using differential ultracentrifugation, floatation in a density gradient, and immuno-isolation, confirmed that exosomes can be distinguished from other subpopulations as they are co-enriched in CD63, CD9, and CD81 tetraspanins and endosomal markers (Kowal et al. 2016). Interestingly, proteomic analyses revealed that exosomes contain proteins from different cell compartments such as the plasma membrane, cytosol, or endosomes, while proteins from the nucleus, the mitochondria, the endoplasmic reticulum or the Golgi apparatus are almost missing in exosomes (Lundholm et al. 2014; Théry et al. 2009). These data confirm that exosomes arise from specific subcellular compartments and not from cell fragmentation. The identified exosomal proteins are listed in the online databases ExoCarta (http://www.exocarta.org) (Mathivanan et al. 2012) and Vesiclepedia (http://microvesicles.org/).

Exosomal lipid composition has been characterized, mainly using mass spectrometry or high-performance liquid chromatography (Laulagnier et al. 2004a, b; Llorente et al. 2013; Trajkovic et al. 2008; Wubbolts et al. 2003). As such, exosomes have been shown to be enriched in sphingomyelin, phosphatidylserine, cholesterol, and fatty acids, as compared to plasma membrane (Record et al. 2014). Moreover, exosomes are enriched in GM3 ganglioside (Llorente et al. 2013; Wubbolts et al. 2003), ceramide, and derivatives (Laulagnier et al. 2005; Llorente et al. 2013; Trajkovic et al. 2008). However, lysobisphosphatidic acid (LBPA), a lipid enriched in endosomal compartments and thought to be found in ILVs

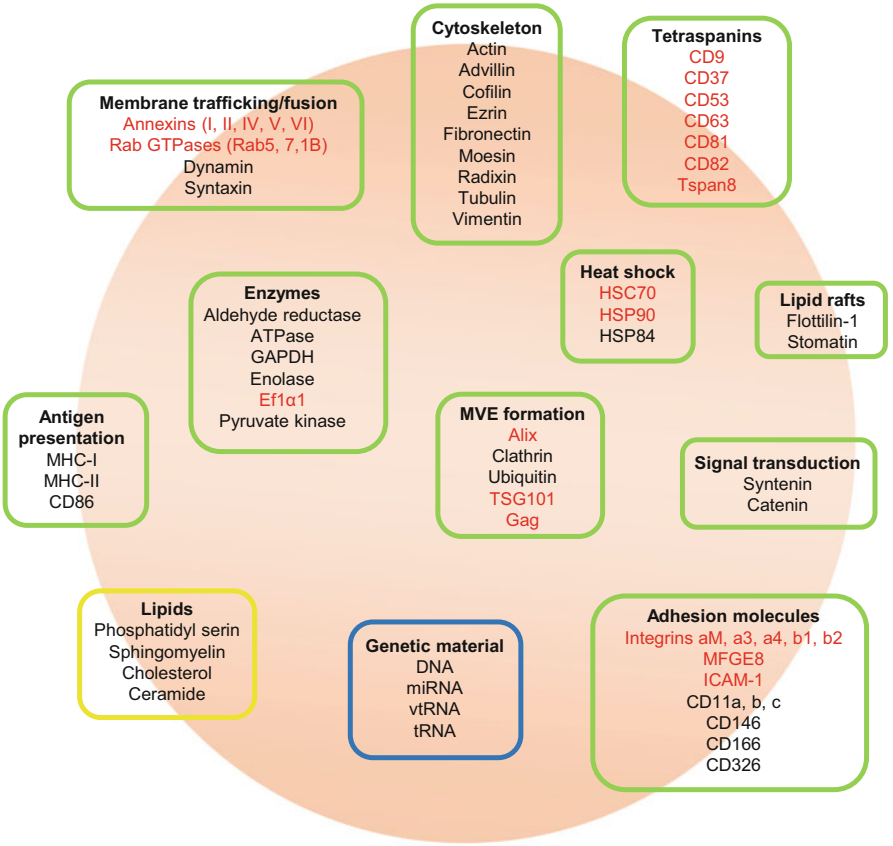

Fig. 2 Molecular composition of a typical exosome. Common composition including genetic material (in *blue box*), proteins (in *green boxes*), and lipids (in *yellow box*) found in a typical exosome is depicted. Proteins shown in *red* have been considered as exosomal markers. *EF1α1* elongation factor 1-alpha 1, *HSC* heat-shock cognate, *HSP* heat-shock protein, *ICAM-1* intercellular adhesion molecule 1, *MFGE8* milk fat globule EGF factor 8 protein, *MHC* major histocompatibility complex molecules, *MVE* multivesicular endosome, *TSG101* tumor susceptibility gene 101

(Matsuo et al. 2004), is not enriched in exosomes (Laulagnier et al. 2004b; Wubbolts et al. 2003). According to these studies, exosomes seem to display a specific lipid composition (enriched in cholesterol, sphingomyelin, and GM3 ganglioside) similar to that of lipid raft microdomains on plasma membranes. Thus, Tan and colleagues suggested and confirmed that lipid rafts are endocytosed into MVEs and released on exosomes (Tan et al. 2013). Interestingly, it has been shown that exosome biogenesis mechanisms evolve during cell maturation since the lipid content of exosomes derived from reticulocytes is similar to that of donor reticulocytes (enriched in ceramide) but is modified in erythrocytes (Carayon et al. 2011).

Exosomal lipids have been included in ExoCarta and Vesiclepedia databases as well.

Numerous groups have analyzed the genetic material in exosomes after the first description of nucleic acids in exosomes by Valadi and colleagues (Valadi et al. 2007). In this pioneer study, exosomes derived from human HMC-1 mast cells and murine MC/9 mast cells were shown to contain multiple and heterogenous RNA species including mRNAs and microRNAs (miRNAs), which were efficiently transferred to recipient cells and biologically active (Valadi et al. 2007). Then, exosomes derived from immune cells have been shown to hold a specific set of miRNAs that can be transferred to recipient cells (Mittelbrunn et al. 2011; Montecalvo et al. 2012). For example, exosomes derived from human THP-1 macrophages convey the miRNA 150, which is handled by recipient endothelial HMEC-1 cells and inhibits the expression of its target gene *c-Myb* (Zhang et al. 2010). Moreover, high-throughput next-generation sequencing techniques have allowed the identification of other small RNAs in exosomes such as small noncoding RNAs [vault RNA (vtRNA), Y-RNA, transfer RNA (tRNA)] but limited amounts of DNA and ribosomal RNA (Nolte-'t Hoen et al. 2012; van den Boorn et al. 2013).

4 Exosome as Immunomodulators

4.1 Exosomes and Innate Immunity

Exosomes have been involved in modulating innate immune responses (Fig. 3). Raposo and colleagues have reported the release of exosomes from B lymphocytes, suggesting the involvement of exosomes in immune responses (Raposo et al. 1996). Natural killer (NK) cells can be activated through the binding to its surface receptor of HLA-B-associated transcript 3 (BAT3), which is expressed on DC-derived exosomes (Simhadri et al. 2008). Exovesicles derived from mature DCs induce a pro-inflammatory response in intestinal epithelial cells which in turn secrete pro-inflammatory cytokines and chemokines [tumor necrosis factor alpha (TNF-α), regulated on activation, normal T cell expressed and secreted (RANTES), interleukin 8 (IL-8), monocyte chemoattractant protein 1 (MCP-1)] in a TNF-α-dependent pathway (Obregon et al. 2009). Although the ultracentrifugation-based purification method used in this study is consistent with exosome purification, an involvement of other types of EVs cannot be excluded. Moreover, exosomes released from mouse DCs express on their surface TNF superfamily members [TNF, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and Fas ligand (FasL)], which directly bind to their receptors on NK cells to enhance their cytotoxic activity (Munich et al. 2012). Intradermal injection of wild-type mice with mouse DC-derived exosomes increased the amount of NK cells in the draining lymph node, and this required activation of

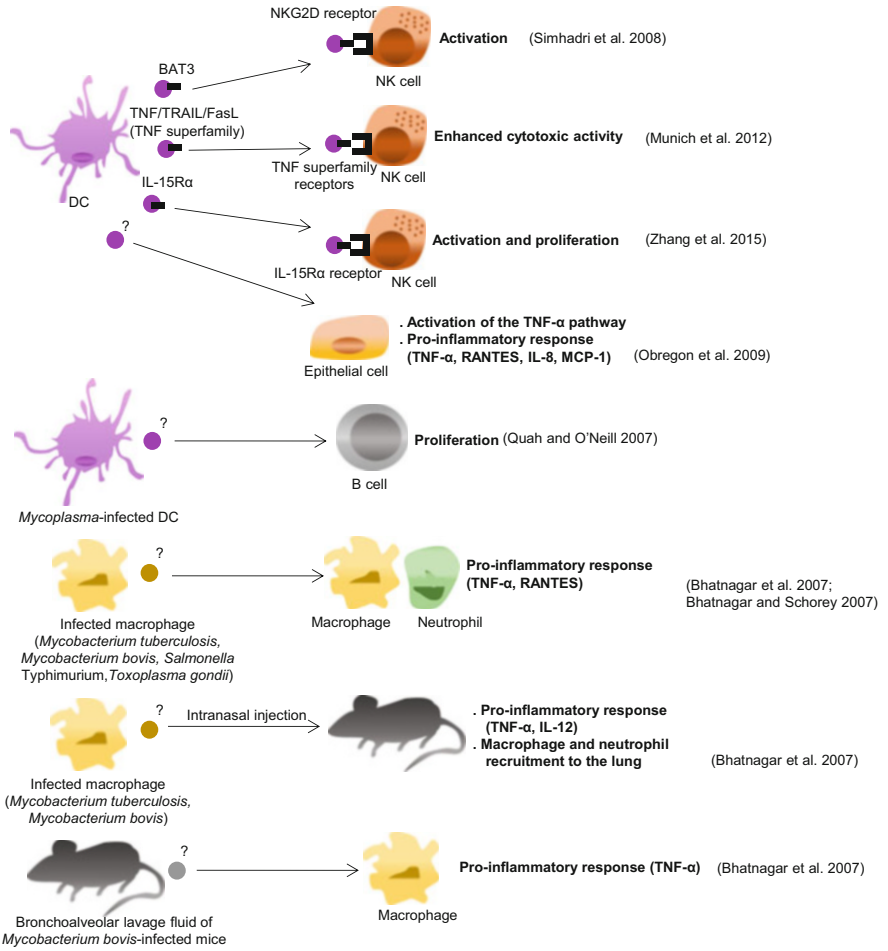

Fig. 3 Exosomes in innate immunity. Here are summarized main functions of exosomes in innate immunity. *BAT3* HLA-B-associated transcript-3, *DC* dendritic cell, *FasL* Fas ligand, *IEC* intestinal epithelial cell, *TNF* tumor necrosis factor, *TRAIL* tumor necrosis factor-related apoptosis-inducing ligand, *NK* natural killer, *NKG2D* natural killer group 2 member D *RANTES* regulated on activation, normal T cell expressed and secreted, *IL* interleukin MCP-1, monocyte chemoattractant protein-1, *?* exosomal content undetermined

the natural killer group 2 member D (NKG2D) receptor on NK cells (Viaud et al. 2009). A recent study reported that exosomes released from mouse DCs carry on their surface IL-15Rα, a NKG2D receptor ligand (Viaud et al. 2009). Upon activation, NKG2D induces activation and proliferation of NK cells (Zhang et al. 2015).

Exosomes released from macrophages infected with intracellular pathogens, when exposed to uninfected macrophages, induce secretion of pro-inflammatory mediators such as TNF-α and RANTES (Bhatnagar et al. 2007; Bhatnagar and

Schorey 2007). Moreover, intranasal injection of mice with exosomes released from mycobacteria-infected macrophages increases secretion of pro-inflammatory mediators (TNF-α and IL-12) and neutrophil and macrophage recruitment in the lung (Bhatnagar et al. 2007). In vitro stimulation of macrophages with exosomes purified from bronchoalveolar lavage fluid of mycobacteria-infected mice provokes an increase of TNF-α secretion (Bhatnagar et al. 2007). It has also been shown that *Mycoplasma*-infected DCs release exosomes that trigger B-cell proliferation, and this is independent of any antigen presentation (Quah and O'Neill 2007).

4.2 Exosomes and Acquired Immunity

Increasing evidence supports the involvement of exosomes in adaptive immune responses and particularly in antigen presentation (Fig. 4). During antigen presentation, antigen-presenting cells (APCs) such as DCs or B lymphocytes present antigen-MHC complexes to T lymphocytes that are consequently activated. Identification of MHC classes I and II and T-cell co-stimulatory molecules on exosomes released from immune cells (Colombo et al. 2014) led scientists to consider exosomes as new mediators of antigen presentation. Moreover, exosomes have been shown to contain antigens. Exosomes derived from tumor cell lines (Napoletano et al. 2009; Wolfers et al. 2001) or ascites from cancer patients (Andre et al. 2002) carry tumor antigens. Furthermore, macrophages infected with *Mycobacterium tuberculosis* or *Mycobacterium bovis* can release exosomes containing bacterial antigens (Giri et al. 2010; Giri and Schorey 2008).

Several works highlighted the capacity of exosomes to perform indirect antigen presentation or cross-presentation (Fig. 4). Antigens conveyed by exosomes are handled by APCs, which complex antigens with their own MHC molecules to present these antigen peptides to T lymphocytes. Stimulation of T lymphocytes with antigen-containing exosomes in the presence of naïve recipient DCs resulted in activation of T cells (Andre et al. 2002; Napoletano et al. 2009; Wolfers et al. 2001). Another study reported that injection of antigen- or peptide-bearing exosomes induced antigen-specific naïve $CD4^+$ T-cell activation in vivo, but in vitro, these exosomes failed to induce antigen-dependent T-cell stimulation unless intermediate DCs were present (Théry et al. 2002). Similarly, exosomes released from mouse mast cells carry antigens and can activate naïve DCs, which in turn activate T cells in vitro (Skokos et al. 2001). Skokos and colleagues injected ovalbumin (OVA)-bearing exosomes released from mast cells, recovered DCs, and showed their ability to activate OVA-specific T-cell hybridomas (Skokos et al. 2003). Moreover, exosomes released from macrophages infected with *Mycobacterium tuberculosis* or *Mycobacterium bovis* carry bacterial antigens that, in the presence of intermediate APCs, can activate $CD4^+$ and $CD8^+$ lymphocytes isolated from mycobacteria-immunosensitized mice (Giri and Schorey 2008).

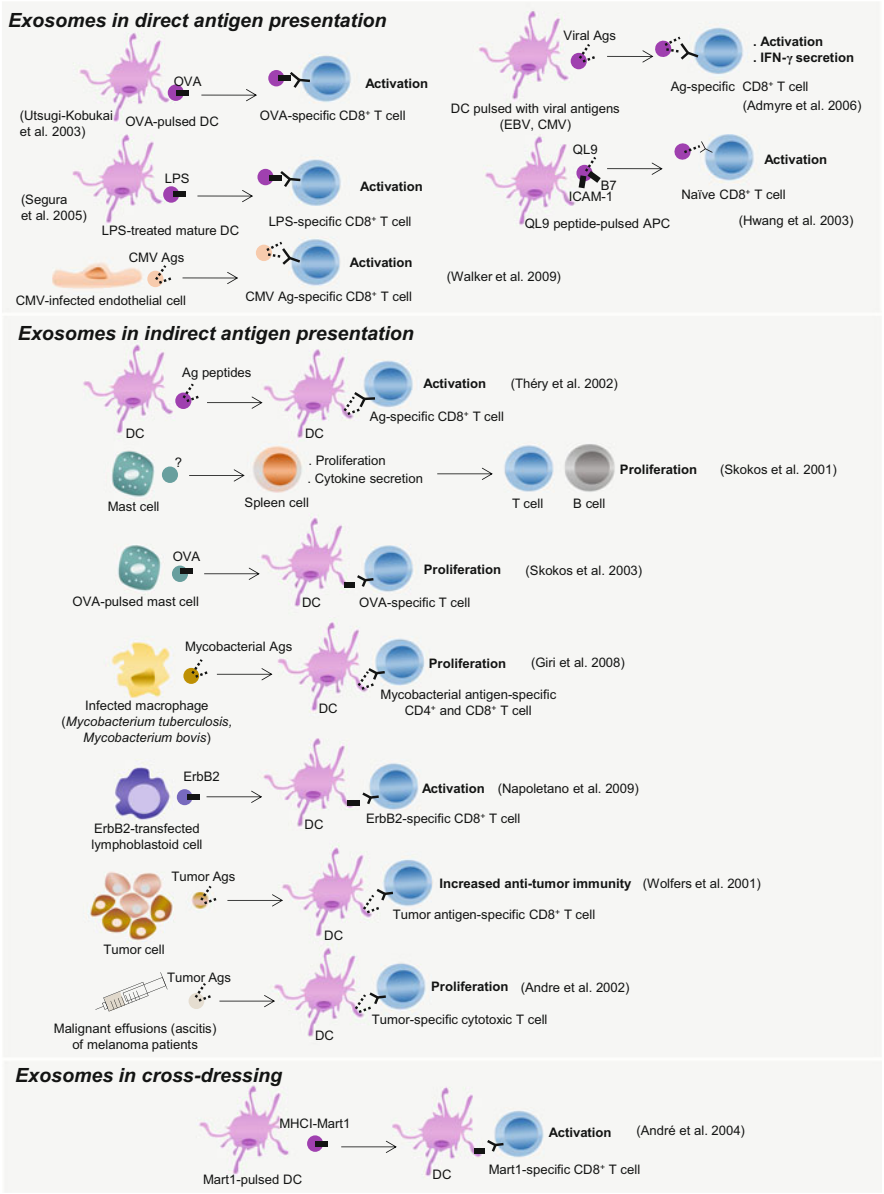

Fig. 4 Exosomes in adaptive immunity. Here are summarized main functions of exosomes in adaptive immunity (i.e., in direct antigen presentation, indirect antigen presentation and in cross-dressing). *Ag* antigen, *APC* antigen-presenting cell, *CMV* cytomegalovirus, *DC* dendritic cell, *EBV* Epstein-Barr virus, *LPS* lipopolysaccharide, *OVA* ovalbumin, *?* exosomal content undetermined.

Increasing evidence has shown that exosomes can be involved in direct antigen presentation (Fig. 4). APC-derived exosome-like microvesicles can directly activate naïve $CD8^+$ T cells in vitro (Hwang et al. 2003). Monocyte-derived DCs secrete exosomes containing viral antigens which can activate T lymphocytes in vitro without the presence of DCs (Admyre et al. 2006). Similarly, OVA-containing exosomes derived from mouse OVA-pulsed DCs can directly activate OVA-specific $CD8^+$ T-cell hybridomas (Utsugi-Kobukai et al. 2003). Another study reported that exosomes derived from lipopolysaccharide (LPS)-treated DCs induced a strong antigen-specific T-cell activation both in vitro and in vivo (Segura et al. 2005).

Some evidence showed that pre-formed antigen-MHC complexes carried in exosomes can be directly handled by APCs and presented to T lymphocytes, in a process named "cross-dressing" (Fig. 4) (Yewdell and Dolan 2011). Montecalvo et al. showed that DCs can secrete exosomes containing antigen-MHC complexes (Montecalvo et al. 2008). These exosomes can be internalized, and the antigen-MHC complexes can be directly presented by DCs to activate $CD8^+$ T lymphocytes (André et al. 2004). However, these results are debatable since some studies have shown the disability of exosomes bearing antigen-MHC complexes to perform "cross-dressing" (Coppieters et al. 2009; Wakim and Bevan 2011).

5 Exosomes in Host-Pathogen Interactions

Exosomes secreted during host responses to infection with several pathogen classes including fungi, parasites, viruses, and bacteria have been isolated and characterized. The content and activity of these exosomes, which can be derived from infected host cells or from pathogens, have been analyzed.

5.1 Exosomes in Fungal Infection

Only few studies concerning the involvement of exosomes in fungal infection are available, and these are limited to the analysis of exosomes derived directly from the fungi but not from fungus-infected cells (Fig. 5). EVs released from the yeast *Cryptococcus neoformans* strain induce cytokine secretion by recipient macrophages as shown by increased TNF-α and tumor growth factor beta (TGF-β) secretion, leading to a restricted fungal infection (Oliveira et al. 2010). It should be noted that the ultracentrifugation-based purification method was used in this study, thus, an involvement of other EVs rather than exosomes cannot be excluded. On another hand, exosomes have been proposed to promote fungal virulence. Indeed, blocking export of exosomes from *C. neoformans* by knocking down *SEC6* (involved in fusion of exocytic vesicles with the plasma membrane) diminished virulence of the yeast in vivo (Panepinto et al. 2009). This decreased

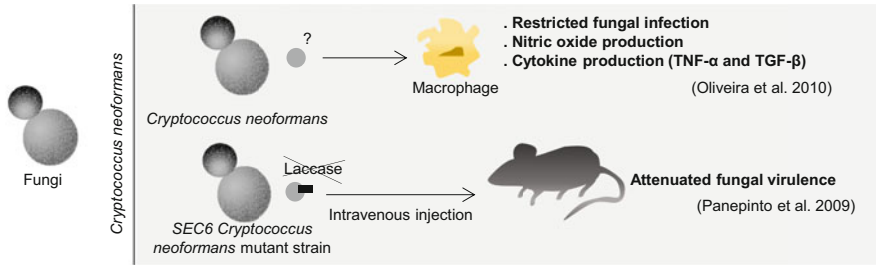

Fig. 5 Exosomes in fungal infection. This figure summarizes the known functions of exosomes in fungal infection. Exosome source and their functional impacts on recipient cells/organisms with underlying mechanism are presented. *TNF* tumor necrosis factor, *TGF-β* tumor growth factor beta, *?* exosomal content undetermined

virulence was shown to be due to the inability of the yeast to export crucial virulence factors such as laccase (Panepinto et al. 2009).

5.2 *Exosomes in Parasitic Infection*

The involvement of exosomes in parasitic infection, including those released from infected host cells and from the parasite, has been analyzed (Fig. 6). The first study of exosomes in parasitic infection is performed by Bhatnagar and colleagues, which showed that exosomes released from macrophages infected with the intracellular protozoan *Toxoplasma gondii* triggered a pro-inflammatory response in naïve macrophages with an increased secretion of TNF-α (Bhatnagar et al. 2007).

Exosomes have also been studied in the context of *Plasmodium* infection. Red cells infected with the malaria-causative parasite *Plasmodium falciparum* release exosome-like vesicles and microvesicles that contain parasite components (Mantel et al. 2013) and particularly the parasitic protein *Plasmodium falciparum* tyrosine phosphatase 2 (PfPTP2), which promotes sexual differentiation of the parasite (Regev-Rudzki et al. 2013). An in vivo study reported that microvesicles isolated from the plasma of malaria-infected mice induce a pro-inflammatory response in macrophages in vitro with increased TNF-α secretion (Couper et al. 2010).

Moreover, infection of blood cells with *Trypanosoma cruzi* provokes the release of microvesicles which, by forming a complex with the complement C3 convertase on the parasite surface, protect the parasite against complement-mediated lysis, resulting in increased parasite survival (Cestari et al. 2012). It was also reported that *T. cruzi* release EVs carrying parasitic small RNAs which confer susceptibility to infection upon uptake by mammalian epithelial cells (Garcia-Silva et al. 2014). In this study, EVs were isolated using ultracentrifugation method, thus, an involvement of exosomes cannot be excluded. Using the same techniques, it has also been shown that small EVs (e.g., exosomes) released from *T. cruzi* display a phosphatase

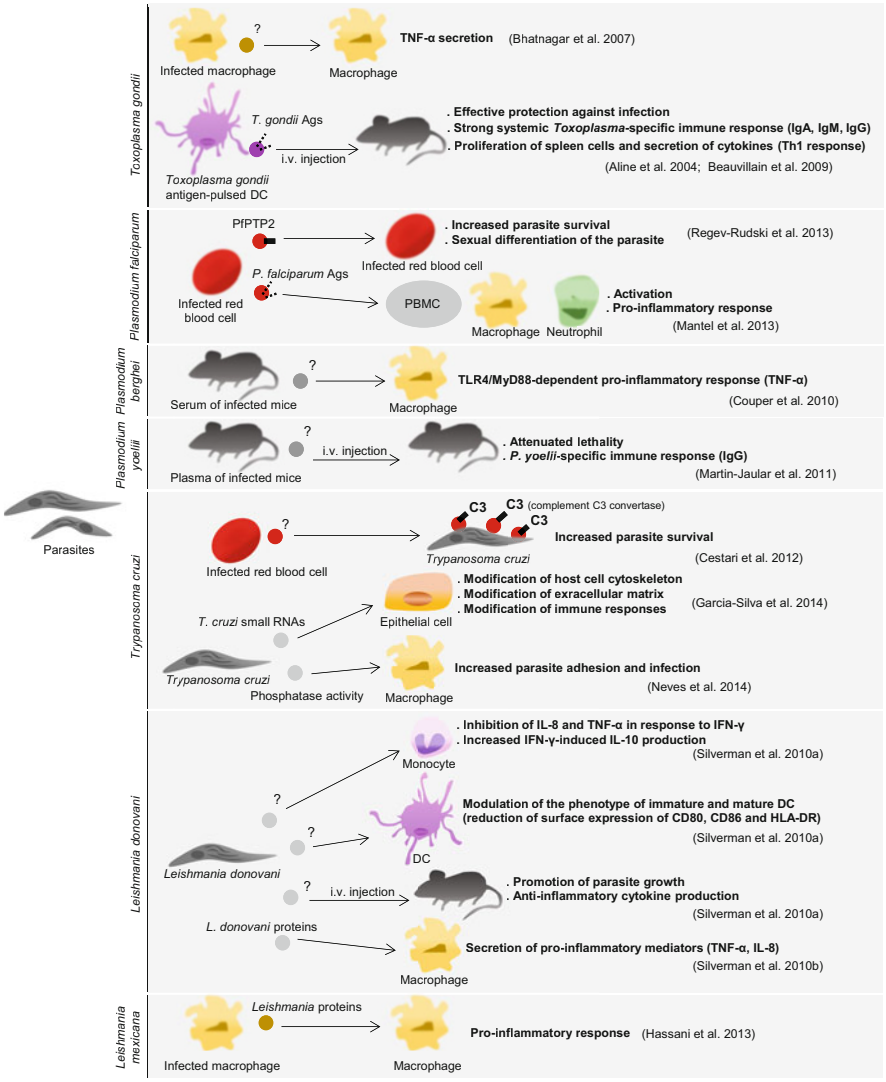

Fig. 6 Exosomes/extracellular vesicles in parasitic infection. Here are summarized known functions of exosomes in parasitic infection. Exosome source and their functional impacts on recipient cells/organisms with underlying mechanism are presented. *DC* dendritic cell, *HLA* human leukocyte antigen, *IFN* interferon, *Ig* immunoglobulin, *i.v.* intravenous, *PBMC* peripheral blood mononuclear cells, *PfPTP2 Plasmodium falciparum* tyrosine phosphatase 2, *?* exosomal content undetermined

activity resulting in increased adhesion and invasion abilities of the parasite in host cells (Neves et al. 2014).

The involvement of exosomes in the context of *Leishmania* infection has also been studied. Exosomes were proposed to mediate the delivery of *Leishmania* into macrophages. Indeed, it has been shown that *Leishmania* spp. release exosomes to

deliver proteins to recipient macrophages, inducing a pro-inflammatory response (Silverman et al. 2010b). Similarly, *Leishmania*-derived exosomes induce secretion of pro-inflammatory cytokines by recipient monocytes (Silverman et al. 2010a). The HSP100 protein has a crucial role in the packaging of *Leishmania*'s proteins into exosomes since its absence resulted in a modification of exosome content and an impaired pro-inflammatory activity in recipient cells (Silverman et al. 2010b). *L. major*- and *L. donovani*-derived exosomes have also been shown to suppress the immune response in vivo since mice pretreated with these exosomes prior to infection showed higher parasite burden compared with untreated mice (Silverman et al. 2010b). Proteomic analyses revealed that exosomes released from *Leishmania mexicana*-infected macrophages contain GP63 protein, an essential virulence factor (Hassani and Olivier 2013).

5.3 Exosomes in Viral Infection

Exosomes in the context of viral infection have been extensively studied (Fig. 7). The hypothesis of an involvement of exosomes in viral infection resulted from several observations. Numerous viruses such as hepatitis B, hepatitis C, and herpesviruses use the ESCRT machinery (Hurley 2010), to leave the infected host cell (Chen and Lamb 2008; Mori et al. 2008). Moreover, Fang and colleagues reported that human immunodeficiency virus (HIV) budding seems to result from a similar pathway to the exosome biogenesis pathway (Fang et al. 2007). This was later supported by another study showing the involvement of TSG101 and ALIX proteins in virus budding, two major proteins acting in exosome biogenesis (Usami et al. 2009).

A role for exosomes released from infected host cells in viral spread and in immunoregulation, which result in an increased infectivity of viruses, has been raised. For instance, exosomes in the context of HIV infection and diffusion has been extensively studied. Several groups reported that CD63 and CD81 tetraspanins, enriched in exosomes, participate in viral budding, viral spread, and in HIV infectivity (Grigorov et al. 2009; Izquierdo-Useros et al. 2009; Jolly and Sattentau 2007; Sato et al. 2008). Particularly, Grigorov and colleagues showed that HIV-1 structural Gag and Env proteins interact with the CD81 tetraspanin in tetraspanin-enriched microdomains on T-cell surface (Grigorov et al. 2009). Furthermore, CD81 expression is crucial for viral replication since shRNA-mediated inhibition of CD81 resulted in an impaired HIV-1 release (Grigorov et al. 2009). It has also been shown that the CD63 tetraspanin is eliminated from the plasma membrane of HIV-1-infected and virion-producing T cells and is embedded on the membrane of released virions (Sato et al. 2008). Interestingly, virion-incorporated CD63 was shown to inhibit HIV-1 infection (Sato et al. 2008). Exosomes have also been shown to convey HIV-1 proteins involved in viral replication cycle such as GAG (Fang et al. 2007) and negative regulatory factor (NEF; (de Carvalho et al. 2014; Lenassi et al. 2010). NEF-containing exosomes

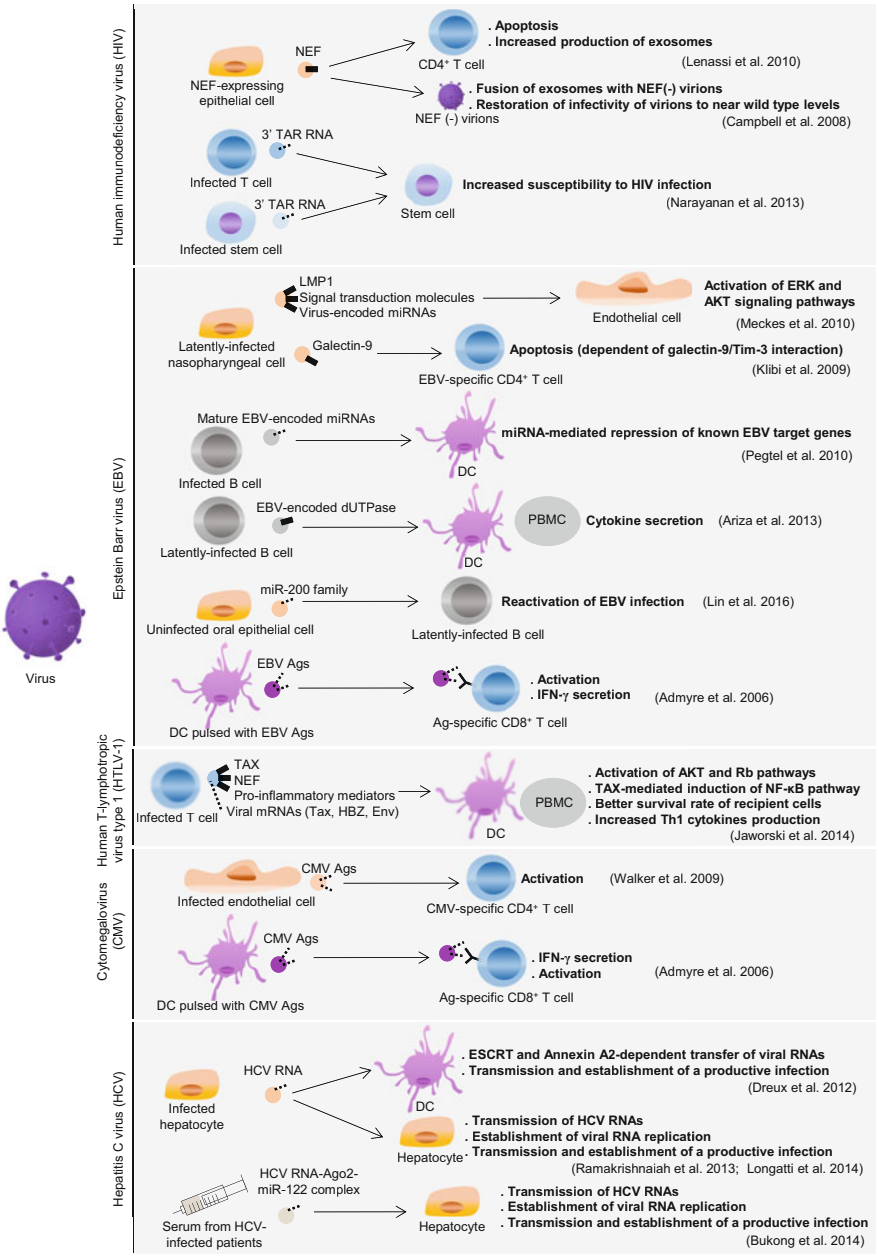

Fig. 7 Exosomes in viral infection. This figure summarizes the known functions of exosomes in viral infection. Exosome source and their functional impacts on recipient cells/organisms with underlying mechanism are presented. *Ag* antigen, *CMV* cytomegalovirus, *DC* dendritic cell, *EBV* Epstein-Barr virus, *ERK* extracellular signal-regulated kinase, *ESCRT* endosomal sorting complexes required for transport, *HCV* hepatitis C virus, *HIV* human immunodeficiency virus, *HTLV-1* human T-lymphotropic virus type 1, *IFN* interferon, *LMP-1* latent membrane protein 1, *NEF* negative regulatory factor, *NF-κB* nuclear factor-kappa B, *PBMC* peripheral blood mononuclear cells, *TAR trans*-activation response element, *?* exosomal content undetermined

induce T-cell apoptosis in vitro, a key feature of HIV infection (Lenassi et al. 2010). Narayanan and colleagues showed that the HIV-1 RNA named transactivating response (TAR) is released into exosomes from infected host cells in vitro and from sera of HIV-infected patients (Narayanan et al. 2013). Moreover, pretreatment of host cells with exosomes derived from HIV-infected cells increased susceptibility of treated cells to HIV infection (Narayanan et al. 2013).

Exosomes have also been studied in the context of infection with other viruses. The latent membrane protein 1 (LMP1), an immunosuppressive protein important in Epstein-Barr virus (EBV) infection (Dukers et al. 2000), was found in exosomes released from EBV-infected B cells, suggesting that exosomes can mediate the immunosuppressive effect of LMP1 during EBV infection (Verweij et al. 2011). Exosomes released from EBV-infected cells also contain the dUTPase enzyme which triggers pro-inflammatory and antiviral responses in recipient DCs and peripheral blood mononuclear cells (PBMCs) (Ariza et al. 2013). Exosomes have also been shown to mediate a functional delivery of viral miRNAs. Indeed, exosomes released from EBV-infected B cells secrete exosomes containing EBV miRNAs which can induce inhibition of known EBV target genes in recipient cells such as C-X-C motif chemokine 11 (*CXCL11*), a cytokine involved in antiviral responses (Pegtel et al. 2010). Moreover, Jaworski et al. reported the incorporation of the human T-cell leukemia virus type 1 (HTLV-1) TAX protein which is crucial for viral replication (Jaworski et al. 2014). Hepatitis C virus (HCV)-infected cells secrete exosomes containing viral RNAs which can be transferred to recipient DCs, inducing DC activation and secretion of interferon-α (IFN-α) (Dreux et al. 2012). It was also reported that the HCV envelop glycoprotein interacts with the CD81 cell membrane protein and that this complex is released within exosomes (Masciopinto et al. 2004). HCV structural proteins have also been identified in exosomes purified from HCV-infected patients' plasma (Masciopinto et al. 2004).

5.4 Exosomes in Bacterial Infection

The involvement of exosomes during bacterial infection has been largely studied (Fig. 8). Particularly, the role of exosomes has been extensively analyzed in the context of mycobacterial infection. Exosomes derived from *Mycobacterium avium*-infected macrophages trigger a pro-inflammatory response in naïve recipient macrophages (Bhatnagar and Schorey 2007). Using antibody-based techniques, these exosomes were shown to contain glycopeptidolipids, a major mycobacterial cell wall constituent (Bhatnagar and Schorey 2007). Wang and colleagues reported that exosomes released from *Mycobacterium avium* subspecies *tuberculosis*-infected macrophages induce an increased release of the pro-inflammatory cytokines IFN-γ and TNF-α in naïve recipient macrophages (Wang et al. 2014). Similarly, *Mycobacterium tuberculosis*- and *Mycobacterium bovis*-infected macrophages release exosomes inducing a pro-inflammatory response in naïve recipient macrophages (Bhatnagar et al. 2007). The authors highlighted the presence of a mycobacterial

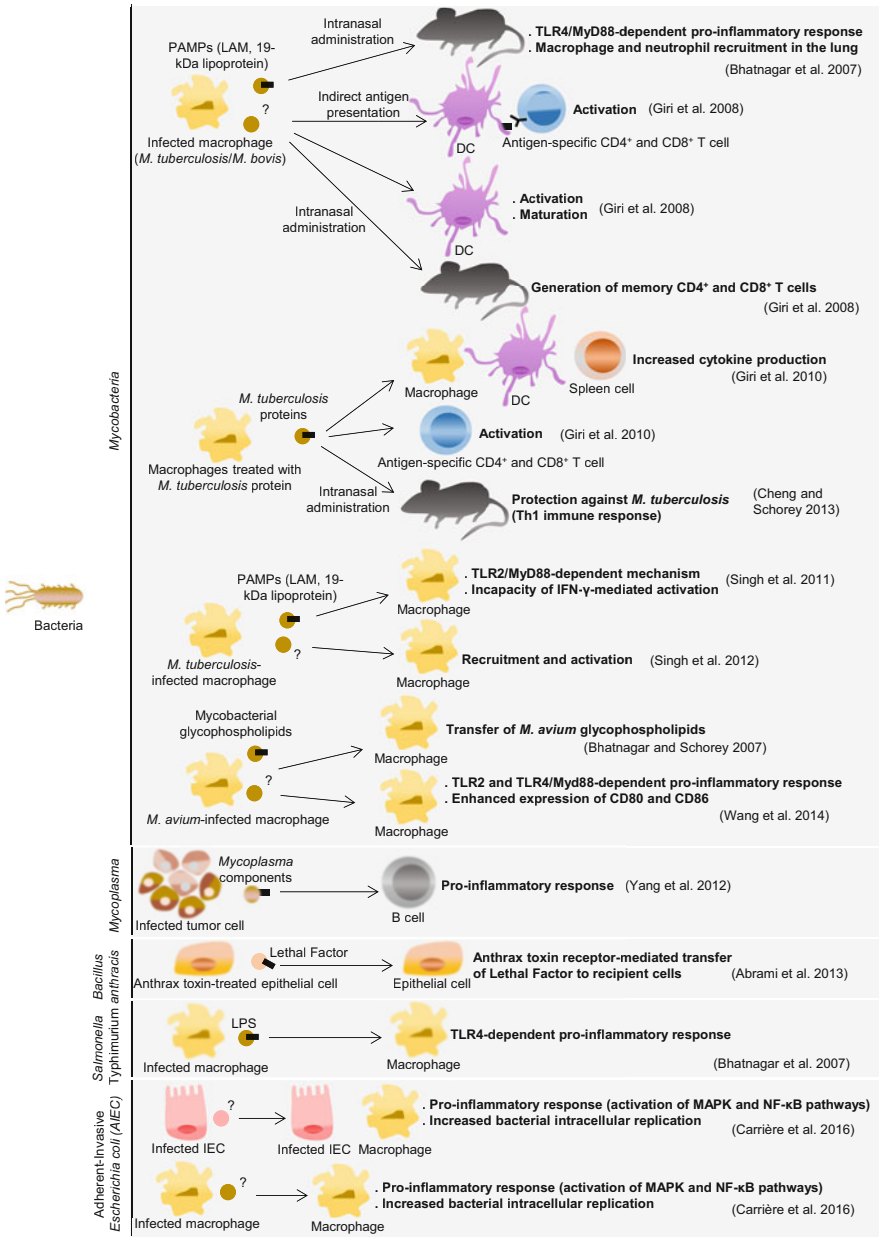

Fig. 8 Exosomes in bacterial infection. This figure summarizes the known functions of exosomes in bacterial infection. Exosome source and their functional impacts on recipient cells/organisms with underlying mechanism are presented. *DC* dendritic cell, *IEC* intestinal epithelial cell, *LAM* lipoarabinomannan, *LPS* lipopolysaccharide, *MAPK* mitogen-activated protein kinase, *MyD88* myeloid differentiation primary response protein 88, *NF-κB* nuclear factor-kappa B, *PAMP* pathogen-associated molecular pattern, *TLR* Toll-like receptor, *?* exosomal content undetermined

lipoprotein mediating this pro-inflammatory message through a Toll-like receptor (TLR)/myeloid differentiation primary response protein 88 (MyD88)-dependent pathway (Bhatnagar and Schorey 2007). These results were confirmed in vivo since exosomes purified from bronchoalveolar lavage fluid of *Mycobacterium bovis*-infected mice contain the mycobacterial components lipoarabinomannan and the 19-kDa lipoprotein and can trigger a pro-inflammatory response in vitro (Bhatnagar et al. 2007). Similarly, exosomes released from *Mycobacterium bovis*- or *Mycobacterium tuberculosis*-infected macrophages in vitro can, when intranasally injected into mice, induce an increased TNF-α and IL-12 secretion as well as neutrophil and macrophage recruitment in the lung (Bhatnagar et al. 2007). Moreover, exosomes released from *Mycobacterium tuberculosis*-infected macrophages can partially inhibit the ability of naïve macrophages to be activated by IFN-γ (Singh et al. 2011), which is crucial in host response to mycobacterial infection since activated macrophages control intracellular bacterial replication (Flynn et al. 1993).

The involvement of exosomes in infection with other bacteria has been also analyzed. It was shown that exosomes released from *Salmonella* Typhimurium-infected macrophages induced a pro-inflammatory response in naïve recipient macrophages (Bhatnagar et al. 2007). The authors showed that the released exosomes contain bacterial LPS (Bhatnagar et al. 2007), which was responsible for this pro-inflammatory response since no inflammatory response was observed in *tlr4*$^{-/-}$ macrophages depleted for the LPS receptor TLR4 (Bhatnagar et al. 2007). Furthermore, *Mycoplasma*-infected cells release exosomes that induced increased IFN-γ secretion in recipient B cells (Yang et al. 2012). Exosomes have also been shown to convey bacterial toxins. In fact, Abrami et al. reported that upon treatment of epithelial cells with the two components of the lethal anthrax toxin, protective antigen (PA) and lethal factor (LF), PA induced the formation of a channel allowing the translocation of LF in the cytosol and in ILVs (Abrami et al. 2013). LF persists in ILVs and is then released in exosomes that can be internalized and consequently delivered in recipient epithelial cells (Abrami et al. 2013).

Our group recently deciphered a previously unknown function of exosomes in the interaction between host cells and Crohn's disease (CD)-associated adherent-invasive *Escherichia coli* (AIEC). Increased abundance of invasive *E. coli* strains in intestinal mucosa of CD patients comparatively to control subjects have been reported (Baumgart et al. 2007; Conte et al. 2006; Darfeuille-Michaud et al. 1998; Martin et al. 2004; Martinez-Medina et al. 2009; Neut et al. 2002; Sasaki et al. 2007; Swidsinski et al. 2002), and these strains were later named AIEC (Darfeuille-Michaud et al. 2004). We showed that AIEC infection induced the release of exosomes by human intestinal epithelial cells and macrophages (Carrière et al. 2016). Characterization of the exosomes released from AIEC-infected cells showed that they are able to trigger a pro-inflammatory response in naïve intestinal epithelial and macrophagic cells with activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways and increased pro-inflammatory cytokine secretion (Carrière et al. 2016). In addition, stimulation of human intestinal epithelial cells and macrophages with exosomes released from

AIEC-infected cells increased bacterial intracellular replication compared with stimulation with exosomes secreted by uninfected cells (Carrière et al. 2016). Our findings suggest that exosomes are involved in the activation of host innate immune responses upon AIEC infection and in bacterial intracellular replication, two key features of host-AIEC interaction.

6 Exosomes in Disease States: Applications in Diagnostic, Vaccine, and Therapeutic Approaches

6.1 Exosomes: Promising Diagnostic Tools

Exosomes have been successfully purified from numerous body fluids such as blood, urine, bronchoalveolar lavage fluid, and saliva (Admyre et al. 2003; Caby et al. 2005; Pisitkun et al. 2004). Due to their easy recovery and their rich content, exosomes have been proposed as a new diagnostic tool in numerous diseases. Thus, the exosomal Fetuin-A, a protein which is synthesized in the liver and secreted into the blood, has been reported as a novel urinary biomarker for detecting acute kidney injury (Zhou et al. 2006). Especially, the content of tumor-derived exosomes has been extensively analyzed and proposed to diagnose cancers. Szajnik et al. reported that the plasma collected from ovarian cancer patients contains higher levels of exosomal proteins compared to control individuals, and that ovarian cancer patients can be distinguished from healthy individuals by the presence of TGF-β1 and MAGE3/6 in plasma-derived exosomes (Szajnik et al. 2013). Some new proteins previously undescribed have been identified in exosomes isolated from malignant pleural effusions of patients suffering from mesothelium, ovarian, breast and non-small cell lung cancers (Bard et al. 2004). Recently, Melo et al. showed using mass spectrometry analysis that exosomes purified from serum of pancreas cancer patients are enriched in glypican-1 (GPC1), a cell surface proteoglycan (Melo et al. 2015). Using flow cytometry, the authors observed that $GPC1^+$ exosomes enabled distinction between cancer patients from healthy individuals, even in early stages of the disease (Melo et al. 2015). Furthermore, another group developed a powerful multiplex detection chip for blood-based diagnosis of ovarian cancer by multiplexed measurement of three exosomal tumor markers CA-125, EpCAM, and CD24 (Zhao et al. 2015).

Exosomal miRNAs have also been proposed as diagnostic biomarkers since altered miRNA expressions have been reported in numerous diseases (Hu et al. 2012). Indeed, modified miRNA and long noncoding RNA profiles have been identified in exosomes isolated from peritoneum lavage fluid and plasma of patients suffering from gastric cancer (Li et al. 2015; Tokuhisa et al. 2015; Zhou et al. 2015; Zöller 2016). Modified miRNA profiles have also been identified in circulating exosomes derived from patients suffering from glioblastoma (Rabinowits et al. 2009), lung cancer (Rabinowits et al. 2009), and ovarian cancer

(Taylor and Gercel-Taylor 2008). The RNA content of exosomes isolated from the blood of patients with dental and neurologic disorders has been analyzed (De Smaele et al. 2010; Miranda et al. 2010; Palanisamy et al. 2010; Rabinowits et al. 2009), and the potential use of exosomal miRNAs as powerful diagnostic biomarkers for Alzheimer's disease has been raised (Van Giau and An 2016).

Finally, exosomes could be used as diagnostic tools in infectious diseases. The amount of exosomes in the serum of *Mycobacterium bovis*-infected mice increased proportionally to the bacterial burden (Singh et al. 2012). Moreover, *Mycobacterium tuberculosis*-infected cells secrete exosomes carrying mycobacterial proteins, suggesting the use of exosomes to diagnose tuberculosis (Kruh-Garcia et al. 2014).

6.2 *Exosome-Based Vaccination: An Encouraging Approach*

With their immunoregulatory property, exosomes have been proposed and tested as vaccines in cancer and in infectious diseases in order to mobilize the immune system against tumor cells or pathogens.

Dai and colleagues genetically modified human colon adenocarcinoma cells with a recombinant adenovirus encoding human IL-18 and showed that exosomes derived from these cells exhibited more potent capability to induce antitumor immunity compared with exosomes derived from nongenetically modified cells, suggesting that modification of exosomes could be an approach to develop exosome-based tumor vaccines (Dai et al. 2006). A phase I clinical trial reported that ascite-derived exosomes in combination with the granulocyte-macrophage colony-stimulating factor (GM-CSF) used as an adjuvant are safe, well tolerated, and induce a specific antitumor immunity in patients with colorectal cancer (Dai et al. 2008). Several studies reported that murine DC-derived exosomes are able to induce antigen-specific $CD4^+$ and $CD8^+$ T-cell responses both in vitro and in vivo and to enhance antitumor immunity in vivo (Damo et al. 2015; Luketic et al. 2007; Näslund et al. 2013a, b; Segura et al. 2005; Théry et al. 2002; Zitvogel et al. 1998). However, several phase I clinical trials using exosomes released from antigen-loaded DCs from cancer patients for treatment of non-small cell lung cancer and melanoma showed that the use of exosomes in vaccination is safe but does not exhibit a significant impact on tumor growth or in cancer regression (Escudier et al. 2005; Morse et al. 2005; Viaud et al. 2010). Nevertheless, a recent clinical trial revealed a modification of the protein and mRNA composition of exosomes released in glioma patients' plasma after receiving antitumor vaccines (Muller et al. 2015). This modification has been shown to be correlated with immunological and clinical responses as well as survival, providing a promising approach to evaluate glioma patients' response to antitumor vaccination (Muller et al. 2015).

Regarding the use of exosomes as vaccines in infectious conditions, it was shown that intravenous injection of exosomes released from DCs infected with the parasite *Leishmania major* conferred vaccinated mice an effective protection against infection, as shown by a decrease in the number of infected cells in draining nymph lodes (Schnitzer et al. 2010). Moreover, exosomes released from macrophages infected with *Mycobacterium bovis* or *Mycobacterium tuberculosis* can activate antigen-specific $CD4^+$ and $CD8^+$ T cells isolated from mycobacteria-immunosensitized mice and promote activation and maturation of DCs (Giri and Schorey 2008). Macrophages treated with *Mycobacterium tuberculosis* proteins released exosomes that, upon intranasal injection into mice, activated DCs and $CD4^+$ and $CD8^+$ T cells isolated from *Mycobacterium tuberculosis*-infected mice (Giri et al. 2010). Furthermore, DCs treated with the highly immunogenic diphtheria toxoid (DT) protein secrete exosomes that, once injected intraperitoneally in mice, stimulate a specific DT IgG response (Colino and Snapper 2006). Exosomes have also been suggested to be used as vaccines in parasitosis. Indeed, upon intravenous injection of exosomes released from DCs pulsed with *Toxoplasma gondii* antigens, anti-*Toxoplasma gondii* IgM antibodies were detected in the serum of mice (Aline et al. 2004). Moreover, mice were subcutaneously vaccinated before pregnancy with exosomes released from DCs pulsed with *Toxoplasma gondii*-derived antigens and infected with the parasite during pregnancy (Beauvillain et al. 2009). The results showed that vaccination resulted in effective protection of pups against congenital infection (Beauvillain et al. 2009). Another study showed that infection of epithelial cells with the protozoan parasite *Cryptosporidium parvum* results in an increased luminal secretion of exosomes (Hu et al. 2013). These exosomes were shown to contain antimicrobial peptides such as cathelicidin-37 and beta-defensin-2 that affect survival of the parasite (Hu et al. 2013). Recently, using proteomic analysis, the parasite *Schistosoma mansoni* has been shown to secrete exosomes carrying potential virulence factors as well as known vaccine candidates (Sotillo et al. 2015). The use of exosomes as vaccine in *Cryptococcus* infection has been proposed since extracellular vesicles of the *Cryptococcus neoformans* yeast strain induce activation of recipient macrophages, improving their abilities to perform phagocytosis and to secrete microbicidal components (Oliveira et al. 2010). Finally, exosomes can constitute a defense mechanism in viral infection. Indeed, Khatua and colleagues identified the secretion in exosomes of the viral cytidine deaminase apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G) (Khatua et al. 2009), a protein known to control the replication of several enteroviruses (Chiu and Greene 2008). The authors reported that APOBEC3G-containing exosomes confer recipient epithelial cells protection against HIV-1 infection (Khatua et al. 2009).

6.3 *Exosomes: Promising New Conveyors of Therapeutic Molecules*

Cells use exosomes as cargos to deliver proteins and genetic material to neighboring or distant recipient cells. In addition to the use of antigen-pulsed DC-derived exosomes to induce antitumor immune responses (Damo et al. 2015; Escudier et al. 2005; Luketic et al. 2007; Morse et al. 2005; Näslund et al. 2013a, b; Segura et al. 2005; Théry et al. 2002; Viaud et al. 2010; Zitvogel et al. 1998), different strategies for delivering therapeutic molecules using exosomes have been proposed and developed. When being directly incubated with exosomes, curcumin, an anti-inflammatory agent, and antitumor agents such as doxorubicin and paclitaxel have been successfully incorporated into exosomes and have been shown to be effective in vitro and in vivo (Sun et al. 2010; Tian et al. 2014; Yang et al. 2015; Zhuang et al. 2011). Indeed, in a mouse model of sepsis, intraperitoneal injection of curcumin-containing exosomes resulted in the protection of mice against a LPS-induced septic shock (Sun et al. 2010). Similarly, in mouse models of brain inflammation, intranasal administration of curcumin-carrying exosomes led to the uptake of exosomes by microglia cells and consequently to an effective curcumin-mediated anti-inflammatory effect (Zhuang et al. 2011). Moreover, administration of doxorubicin or paclitaxel-containing exosomes to tumor-bearing mice or zebra fishes induced antitumor effects (Tian et al. 2014; Yang et al. 2015). As exosomes can convey genetic material, they have been proposed for the delivery of exogenous RNA in disease states. Using electroporation, a siRNA against gluceraldehyde-3 phosphate dehydrogenase was incorporated into DC-derived exosomes and effectively delivered in vivo, leading to the loss of expression of its target gene (Alvarez-Erviti et al. 2011). Some years later, Ohno and colleagues used this technique to deliver miRNAs in breast cancer (Ohno et al. 2013). Breast cancer is associated with an increased expression of the epidermal growth factor receptor (EGFR) in cancer cells (Woodburn 1999). The authors first transfected an EGF-encoding plasmid into human embryonic kidney HEK293 cells and purified secreted exosomes expressing EGF on their surface. By transfecting the antitumor miRNA let-7a in the EGF-expressing exosomes, they were then successful to deliver let-7a miRNA specifically to EGFR-expressing xenograft breast cancer tissue in immunodeficient *rag2*$^{-/-}$ mice (Ohno et al. 2013).

Another strategy based on treating donor cells with drugs has been developed in order to incorporate drugs inside exosomes. This approach enabled the incorporation of antitumor agents such as paclitaxel, etoposide, or carboplatin in HepG2 hepatoma cell line-derived exosomes (Lv et al. 2012). Furthermore, in vitro treatment of NK cells with these exosomes led to an increase of their cytotoxic activity toward cancer cells (Lv et al. 2012).

Finally, two groups have transfected macrophages with plasmids encoding therapeutic proteins such as catalase or glial cell line-derived neurotropic factor (Haney et al. 2013; Zhao et al. 2014). By injecting these macrophages to a mouse model of Parkinson's disease, the authors observed the release of exosomes

carrying modified genetic material and an improvement of motor functions with disease-associated neurodegeneration and neuroinflammation (Haney et al. 2013; Zhao et al. 2014).

7 Conclusion

Although exosomes have become the focus of exponentially growing interest since their first description about 30 years ago, our knowledge of these nanovesicles has only just begun. Working with exosomes remains challenging because of their small size and the fact that other extracellular vesicles (i.e., microvesicles, microparticles, etc.) or biofluid components can be co-extracted with exosomes. Although numerous studies have reported an important role of exosomes in immunoregulation, most of the time, the exosomal component responsible for the functional impact on recipient cells has not been identified. This might be due to the difficulty to identify a relevant candidate among the numerous exosomal proteins, nucleic acids, and lipids. Consequently, the mechanisms underlying the exosome-mediated immune responses observed in recipient cells (i.e., activated or inhibited signaling pathways, etc.) are not always elucidated. Moreover, the role of exosomes as an immunoregulator has been shown only in pathological states, and their functions in homeostasis remain to be elucidated. Finally, although only few experiments and clinical trials have been performed to date, the accessibility of exosomes in various body fluids, the proved safety and feasibility of the use of exosomes in clinical experiments, and the first promising results suggest that exosomes might become a future powerful diagnostic, vaccine, and drug delivery tool for numerous diseases.

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