Microparticles as immune regulators in infectious disease – an opinion

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Despite their clear relationship to immunology, few existing studies have examined the potential role of microparticles (MP) in infectious disease. MP have a different size range from exosomes and apoptotic bodies, with which they are often grouped and arise by different mechanisms in association with inflammatory cytokine action or stress on the source cell. Infection with pathogens usually leads to the expression of a range of inflammatory cytokines and chemokines, as well as significant stress in both infected and uninfected cells. It is thus reasonable to infer that infection-associated inflammation also leads to MP production. MP are produced by most of the major cell types in the immune system, and appear to be involved at both innate and adaptive levels, potentially serving different functions in each. Thus, they do not appear to have a universal function; instead their functions are source- or stimulus-dependent, although likely to be primarily either pro- or anti-inflammatory. We argue that in infectious diseases, MP may be able to deliver antigen, derived from the biological cargo acquired from their cells of origin, to antigen-presenting cells. Another potential benefit of MP would be to transfer and/or disseminate phenotype and function to target cells. However, MP may also potentially be manipulated, particularly by intracellular pathogens, for survival advantage.

Keywords: microparticles, microbial immunity, cell–cell communication

INTRODUCTION

The paradigm of intercellular cell signaling mechanisms has until recently been assumed to be limited to direct cell–cell contact and via local soluble factor release. However, there has been increasing interest in the role of plasma membrane-derived small lipid/protein vesicles in cell–cell communication in the last decade. Microparticles (MP) are one such type of vesicles gaining more recognition as their biological roles are being explored. Although there is no standardized terminology for these small extracellular vesicles, the term, microparticle, usually refers to a subset of extracellular, cell membrane-derived vesicles ranging from 0.1 to 1 μm in diameter, shed directly from the plasma membrane through the process of ectocytosis (Gasser and Schifferli, 2004; Cocucci et al., 2009; Pap et al., 2009). MP are also referred to as "microvesicles" or "ectosomes," depending on the context of the study. Although MP are shed from steady-state cells, elevated levels are observed in actively proliferating cells and cells activated by a wide range of stimuli, from mitogens and inflammatory mediators, to antigenically foreign compounds, such as pathogen components, as well as various other stress-inducing challenges in both *in vitro* and *in vivo* models (Ratajczak et al., 2006; Ardoin et al., 2007). *In vivo*, MP are most commonly found in blood, however, they are also observed in other bodily fluids, such as synovial fluid (Berckmans et al., 2005; Distler et al., 2005b) and urine (Smalley et al., 2008).

Microparticles are often compared with exosomes and apoptotic bodies (**Table 1**), however, while superficial structural similarities exist between them, both the mechanism of MP formation and their subsequent composition are fundamentally

different (Théry et al., 2009; Mause and Weber, 2010; György et al., 2011). MP shedding is a multi-enzyme-regulated process. Under normal conditions, the asymmetric composition of the plasma membrane is maintained by three principal translocase enzyme families of flippases, floppases, and scramblases, which actively maintain phosphatidylcholine (PC) and sphingomyelin (SM) on the external leaf, while phosphatidylserine (PS) and phosphatidylethanolamine (PE) are kept on the internal leaf. On cellular activation, these enzymes are inhibited following an influx of Ca^{2+} into the cytoplasm, and consequently the normal phospholipid asymmetry is disrupted (Coltel et al., 2006; Piccin et al., 2007; Pap et al., 2009). This is accompanied by the loss of actin–spectrin anchorages and the degradation of actin filaments by calpain. The disruption of the cytoskeleton allows the plasma membrane to protrude outward. These protrusions are pinched off as individual MP in a Rho-associated kinase I (ROCK-I)-dependent manner from the cell membrane, with PS and PE exposed on the outer surfaces. In contrast, exosomes fall into the 50–100 nm range and are formed by inward budding into the endosomal lumen of small intra-luminal vesicles (ILV) that aggregate to become multivesicular bodies (MVB). MVB subsequently fuse with the plasma membrane to release the ILV into the extracellular space as exosomes (Théry et al., 2009). Exosomes are enriched in CD63, and major histocompatibility complex (MHC) I and II molecules, but tend to have low levels of PS and procoagulation factors on the surface (Denzer et al., 2000; Théry et al., 2009; Mause and Weber, 2010; György et al., 2011). Apoptotic bodies, yet another type of subcellular vesicle, are much larger

Table 1 | Comparison between microparticles, exosomes, and apoptotic bodies.

	Microparticles	Exosomes	Apoptotic bodies
Size	100–1000 nm	50–100 nm	1000–4000 nm (Théry et al., 2009; Mause and Weber, 2010; György et al., 2011)
Biogenesis	Cell surface ectocytosis	Exocytosis from MVB/internal compartments	Blebbing and fragmentation (Théry et al., 2009; Mause and Weber, 2010; György et al., 2011)
Markers	Tissue factor (De Rop et al., 2011; Zwicker et al., 2011), cell surface markers of cell origin, PS	CD63, CD81, CD9, LAMP1 high MHC-I/II (Denzer et al., 2000)	PS, genomic DNA, histones (Mause and Weber, 2010)
Functions	Pro- and anti-inflammatory, antigen presentation (Distler et al., 2005a; Huber et al., 2007; Couper et al., 2010; Ramachandra et al., 2010; Sadallah et al., 2011b), coagulation (Owens and Mackman, 2011; Zwicker et al., 2011)	Pro- and anti-inflammatory, antigen presentation (Aline et al., 2004; Kim et al., 2007; Schorey and Bhatnagar, 2008; Ramachandra et al., 2010)	Pro- and anti-inflammatory (Savill et al., 2002), antigen presentation (Climent et al., 2011)

(1–4 μm) and are essentially fragments of cells produced in late stage apoptosis (**Table 1**).

The process by which MP are produced results in their possessing many of the characteristics of the cell from which they originated. This includes many of the surface molecules, such as integrins, receptor ligands, co-stimulatory molecules, as well as cytoplasmic contents, such as activated signaling molecules and even genetic materials e.g., mRNA (Baj-Krzyworzeka et al., 2006; Aliotta et al., 2010) and miRNA (Yuan et al., 2009). MP either attach and fuse to their target cell (Del Conde et al., 2005), or are internalized and processed by pinocytic or phagocytic mechanisms (Dasgupta et al., 2009; Faille et al., 2009; Abdel-Monem et al., 2010). Since MP carry biological molecules from their cells of origin, they may induce associated responses in their target cells, depending on the source of MP, the cell type on which they act and the route by which they are incorporated.

Given that MP are produced in response to many stress conditions and are involved in several immunological processes, it seems likely that MP are involved in the regulation of the immune response against invading pathogens, however, their potential role in infectious diseases has not been extensively studied. This review aims to consolidate current evidence on their biological role and venture opinion on their potential impact in infectious disease.

MP IN INFLAMMATORY CONDITIONS

One of the hallmarks of bacterial, viral, fungal, or parasitic infection is the formation of an inflammatory milieu around infected or affected cells. Pathogen-associated molecular pattern (PAMP) recognition receptors (PRR), such as Toll-like receptors, certain classes of RNA helicases, like the retinoic acid inducible gene-I (RIG-I), and NOD-like receptors (NLR), specifically detect components of the invading pathogen and trigger a cascade of intracellular signaling events that lead to the activation of transcription to initiate the first wave of innate immune responses (Creagh and O'Neill, 2006; Seth et al., 2006). Whether the initial detection of the pathogen alone can induce MP shedding is unclear. However, endotoxins, produced by bacteria during infection, or the sheer load of replicating intracellular virus would undoubtedly cause stress in affected cells. Indeed, generation of MP may be closely associated with the initiation of apoptosis and necrosis (Ardoin et al., 2007). Whether stress-inducible proteins, such as

hypoxia-inducible factor 1α (HIF-1α), heat-shock factor (HSF-1), heat-shock protein (Hsp) family, nuclear factor κB (NF-κB), Jun NH2-terminal kinase (JNK), and p53 (Ohiro et al., 2003; Razorenova et al., 2005; Thompson and Locarnini, 2007; Zinkernagel et al., 2007; De Maio, 2011; Li et al., 2011; Rawat and Mitra, 2011; Zhang et al., 2011) and MHC class I polypeptide-related sequence A and B (MICA, MICB; Stern-Ginossar and Mandelboim, 2009) are involved, remains to be determined. Nevertheless, a broad array of pro-inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, and type 1 interferons (IFN-I) are produced in response to infection (Medzhitov and Horng, 2009), and many of these induce MP shedding in the absence of infection (Combes et al., 1999; Sheremata et al., 2006; Penet et al., 2008). Not surprisingly, therefore, both stimulation *in vitro* with lipopolysaccharide (LPS; Aharon et al., 2008; Bernimoulin et al., 2009) and intravenous injection of bacterial endotoxin *in vivo* (Zubairova et al., 2006; Wang et al., 2009) induce MP shedding. Furthermore, elevated blood MP levels are also observed in septic patients (Nieuwland et al., 2000; Mostefai et al., 2008), in human immunodeficiency virus (HIV) patients (Aupeix et al., 1997) and Ebola Virus-infected macaques (Geisbert et al., 2003), as well as malaria patients and Plasmodium-inoculated laboratory animals (Combes et al., 2004, 2005; Penet et al., 2008; Couper et al., 2010; Pankoui Mfonkeu et al., 2010). Thus elevated circulating MP are associated with systemic infection by a range of organisms.

Many activated immune cells not necessarily infected by pathogens, such as monocytes, macrophages, dendritic cells (DC), and lymphocytes, produce MP when activated by relevant stimuli. MP have been shown to be produced by monocytes and macrophages stimulated with A23187 (Cerri et al., 2006), LPS (Satta et al., 1994; Obregon et al., 2006; Aharon et al., 2008), and starvation (Aharon et al., 2008), by DC stimulated with LPS (Obregon et al., 2006, 2009), by T cells stimulated with agonistic CD3 antibodies, Fas ligand, heat, actinomycin-D, staurosporine, PHA, PMA (Scanu et al., 2008), and by neutrophils stimulated with fMLP and C5a (Gasser and Schifferli, 2004). In addition, MP are produced by cells isolated from patients with autoimmune disease, such as diabetes (Martin et al., 2004), as well as vascular disease (Tesse et al., 2005). The wide variety of possible stimuli producing MP in the absence of infection makes it difficult to predict pathways leading to MP production in infectious disease, or predict

their function in this context and few experimental models utilize infectious pathogens. Nevertheless, these studies provide valuable clues to what possible responses might occur in infection.

REGULATION OF IMMUNE RESPONSES

As mentioned above, elevated blood MP levels have been reported in models of sepsis, viral infection, and cerebral malaria. Because of the generalized inflammatory conditions that such events generally create, it seems reasonable to propose this would be the case for most, if not all infections accompanied by significant hematogenous spread, i.e., during parasitemia, bacteremia, viremia or fungemia. Furthermore, due to the propensity of MP to carry contents derived from their cells of origin, it is likely that they contain antigens, either present or induced in infected cells. Plasmodium parasite antigens were detected in the MP purified from the plasma of infected animals (Couper et al., 2010). This is unlikely to be unique to parasitic infections, as infecting viruses inevitably become an integral part of the cell, inducing a range of virus-specified proteins crucial for their replication and these could become incorporated into the MP contents, either specifically or non-specifically. Thus, MP could remotely convey viral antigens via the blood stream to professional antigen-presenting cells (APC), such as macrophages and DC residing centrally in the spleen and liver without the necessity for their direct interaction with infected cells. Both organs normally have high throughputs from the systemic circulation, increasing the likelihood that MP will interact with APC. From a host point of view, this maintains a desirable separation between the peripheral site of infection and the site of immune induction. Additionally, TNF-stimulated, endothelial cell-derived MP have the capacity to induce activation and maturation of plasmacytoid DC, although not conventional DC (Angelot et al., 2009). Together, such remote interactions could increase the probability of detecting infection early, enabling prompt initiation of innate and adaptive responses by APC responding to MP in the blood. Indeed, it has been shown that MP produced by *Mycobacterium*-infected macrophages were able to activate and present antigen to naive T cells (Ramachandra et al., 2010), thus obviating the necessity for direct APC and T cell contact to generate a T cell response. In these experiments, MP were better at inducing T cell proliferation than exosomes. The study by Couper et al. (2010) also demonstrated the activation of bone marrow-derived macrophages, with upregulation of CD40 and TNF expression. This raises the further possibility that MP may facilitate the mobilization of subsets recruited from the bone marrow during infection (King et al., 2007; Getts et al., 2008).
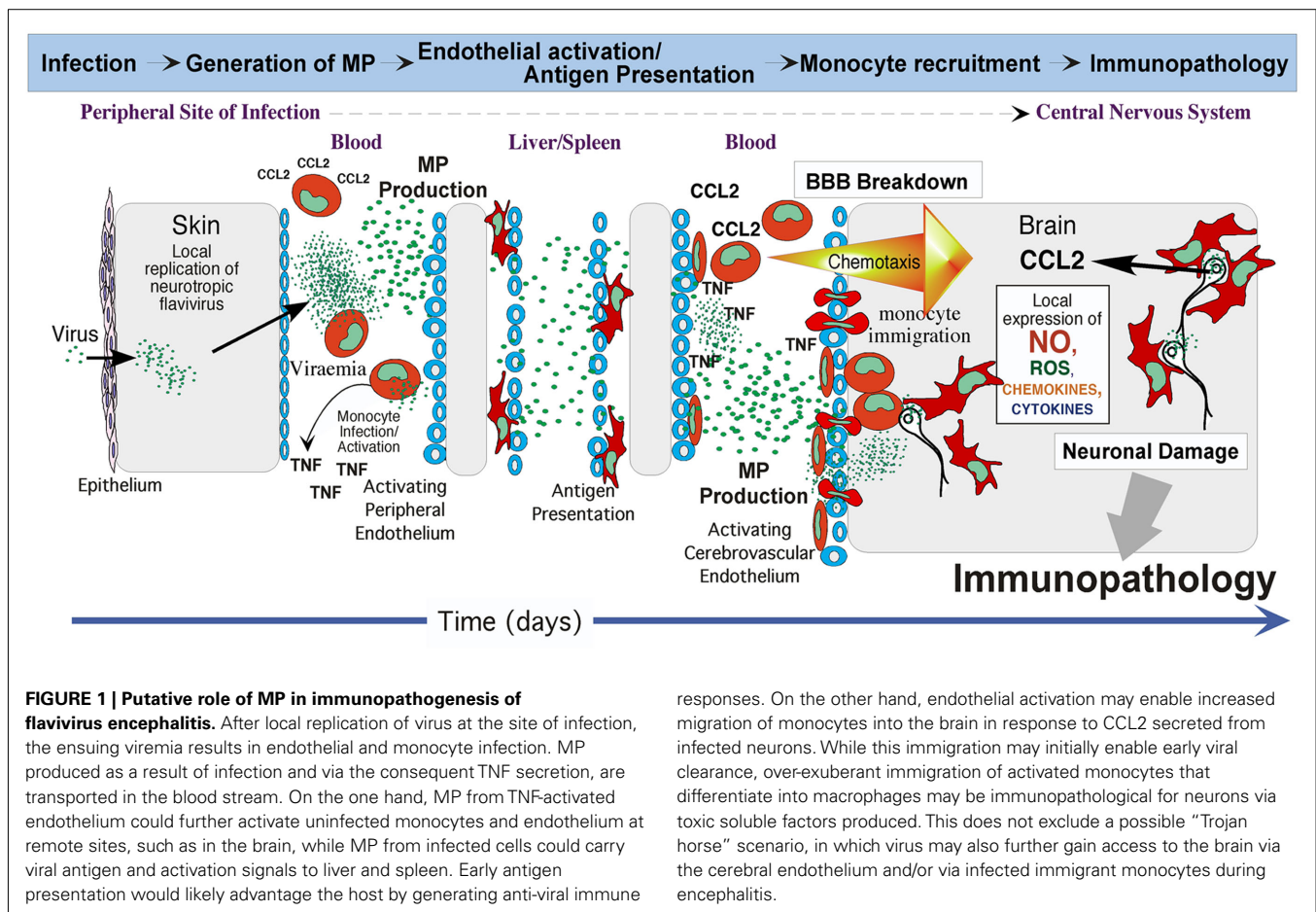
As well as accepting MP by fusion, DC also produce MP following activation. LPS-induced MP from DC can transfer antigen to recipient DC, which can present this to T cells (Obregon et al., 2006, 2009; Pizzirani et al., 2007). Other cellular membrane function can be transferred by MP from activated to naïve T cells via the tetraspanin, CD81, important for adhesion and lymphocyte maturation (Fritzsching et al., 2002; Quast et al., 2011). Furthermore, CD154^{+} platelet-derived MP introduced into CD154^{−/−} mice triggers CD4 T cell-dependent production of antigen-specific IgG and germinal center formation, suggesting that with the right surface molecules MP can fully activate the adaptive immune response (Sprague et al., 2008). Indeed, MP transfer could explain the rapid exchange of T cell receptors (TCR) observed in the early stages of T cell activation to expand antigen-specific T cell clones (Chaudhri et al., 2009). Irrespective, it seems likely that MP containing pathogen antigens along with the necessary co-stimulatory molecules can activate T cells, bypassing cell–cell contact, thus enabling a wider, more efficient initiation, amplification and/or modulation of early adaptive immune responses.

Microparticles may also contain pro-inflammatory cytokines, such as IL-1β, providing an alternative or additional pathway for cytokine secretion into the extracellular space to modulate local cytokine/chemokine signaling (Mackenzie et al., 2001; Pizzirani et al., 2007). Depending on their mode of manufacture and cellular release, it seems highly likely that other cytokines/chemokines, may make use of this pathway (Garcia et al., 2005; Dean et al., 2009). Certainly, T cell-derived MP have been shown to activate monocytes (Distler et al., 2005a; Huber et al., 2007; Scanu et al., 2008) and monocytes activated with MP from PHA- or PMA-stimulated blood T cells produce TNF, IL-1β, and soluble interleukin-1 receptor antagonist (sIL-1Ra; Scanu et al., 2008). MP derived from T cells and monocytes also induce NF-κB-dependent expression of matrix metalloproteinases (MMP; Distler et al., 2005b), as well as some pro-inflammatory cytokines and chemokines such as IL-6, IL-8, macrophage chemoattractant protein (MCP)-1, MCP-2, and regulated upon activation, normal T cell expressed and secreted (RANTES) in synovial fibroblasts (Berckmans et al., 2005; Distler et al., 2005b). Monocyte/macrophage-derived MP also upregulate inflammatory mediator synthesis by epithelial cells (Cerri et al., 2006).

Not surprisingly, under certain conditions MP can also induce anti-inflammatory IL-10 expression in monocytes (Köppler et al., 2006) and transforming growth factor β1 (TGF-β1) in macrophages (Sadallah et al., 2011a). In some cases, MP can even be pro-apoptotic, especially in macrophages, which can undergo apoptosis upon uptake (Distler et al., 2005a; Huber et al., 2007). This activity presumably is due to differences in the source of MP. For example, neutrophil-derived MP specifically upregulate the expression of TGF-β1, which helps to suppress the inflammatory response (Distler et al., 2005a; Huber et al., 2007). The MP used in these models induce macrophage apoptosis, possibly because the MP were generated from apoptotic cells, which might have transferred the apoptotic signals to the macrophages (Böing et al., 2008). This is similar to the behavior of macrophages to dampen inflammation after ingesting apoptotic bodies, to prevent over-reaction (Savill et al., 2002). However, these studies did not use pathogens and macrophages activated by Plasmodium were in contrast highly pro-inflammatory (Couper et al., 2010).

On the other hand, MP might contain factors induced or expressed by the infecting organism that could impede or abrogate the early induction of an effective immune response. For example, Influenza A NS1 protein expressed intracellularly has the capacity to suppress IFN-I production and inhibit apoptosis to enable better viral replication (Pauli et al., 2008; Wang et al., 2010). Similarly, several flavivirus subunits interfere with IFN-signaling at various levels and in different ways (reviewed in Daffis et al., 2009; Suthar et al., 2009). Such molecules, carried into cells yet to be infected could arguably give the infecting organism a survival advantage, enabling greater levels of replication in the

FIGURE 1 | Putative role of MP in immunopathogenesis of flavivirus encephalitis. After local replication of virus at the site of infection, the ensuing viremia results in endothelial and monocyte infection. MP produced as a result of infection and via the consequent TNF secretion, are transported in the blood stream. On the one hand, MP from TNF-activated endothelium could further activate uninfected monocytes and endothelium at remote sites, such as in the brain, while MP from infected cells could carry viral antigen and activation signals to liver and spleen. Early antigen presentation would likely advantage the host by generating anti-viral immune responses. On the other hand, endothelial activation may enable increased migration of monocytes into the brain in response to CCL2 secreted from infected neurons. While this immigration may initially enable early viral clearance, over-exuberant immigration of activated monocytes that differentiate into macrophages may be immunopathological for neurons via toxic soluble factors produced. This does not exclude a possible "Trojan horse" scenario, in which virus may also further gain access to the brain via the cerebral endothelium and/or via infected immigrant monocytes during encephalitis.

host for longer periods of time, thus increasing the probability of transmission to vectors and/or to the next host. In the case of arboviruses, considering the relatively short times that organisms spend in the blood stream, even small increases in concentration and time spent in the blood stream could potentially subtend a significant survival advantage.

IMPACT ON DISEASE

The inflammatory conditions created by MP could in principle play important roles in controlling infection and pathogen clearance. How crucial these roles are in the immune system remain unclear, but as one of many factors that regulate the immune response against invading pathogens, MP could significantly influence the outcome for the host, both during the initial "knockdown" phase of controlling pathogen and in the ongoing adaptive response to it. Pro-inflammatory cytokines induced by MP would act to manage and contain the infection, while chemoattractant proteins would send signals to recruit leukocytes to the site of infection to eliminate the pathogen. Indeed, a further interesting characteristic of MP is their ability to upregulate adhesion molecules such as intercellular adhesion molecule-1 (ICAM-I; Berckmans et al., 2005) in target cells and promote monocyte recruitment (Mause et al., 2005). Along with ability of MP to induce MMP expression and TNF release, the endothelium becomes more permeable for immune active proteins and leukocytes to access the site of infection. Although this is an important

step to enable pathogen clearance, it can be a double-edged sword in certain infectious diseases. In cases of lethal flavivirus encephalitis, in which immunopathology is one of the prominent features of pathogenesis, excessive monocyte infiltration of the central nervous system (CNS), and the accompanying pro-inflammatory cytokine spike are a major cause of mortality (King et al., 2007; Getts et al., 2008). We hypothesize that during viremia preceding encephalitis, flavivirus infecting endothelium, and blood monocytes induce MP, both as a consequence of infection and via TNF released by infected cells (Cheng et al., 2004). TNF independently enhances breakdown of the blood–brain barrier, potentially enabling virus access to the CNS (Wang et al., 2004). MP from TNF-activated endothelium could further activate uninfected monocytes and endothelium, while MP from infected cells could carry viral antigen, as well as activation signals to the liver and spleen. Early antigen presentation would likely advantage the host in generating anti-viral immune responses. However, WNV-infected CNS neurons recruit activated (CCR2^{+}) monocytes from the bone marrow to the brain via CCL2 (Getts et al., 2008). Here, TNF- and MP-mediated activation of CNS endothelium would facilitate macrophage margination, adhesion, and diapedesis in response to the chemokine gradient, thus potentially increasing numbers of monocytes recruited to the brain and exacerbating immunopathology (**Figure 1**).

Exaggerated immune responses also compromise the integrity of the blood–brain barrier in cerebral malaria via monocyte, TNF,

and MP activities (Combes et al., 2010). Moreover, in mice deficient in the floppase, ATP-binding cassette transporter A1, which modulates PS distribution on the outer plasma membrane leaflet, MP production is reduced in cerebral malaria, abrogating neurological signs in this model, and presenting the possibility for pharmacological modulation of MP to ameliorate immunopathology (Combes et al., 2005; Penet et al., 2008).

Immunological aspects aside, MP could potentially also be used directly as transport by viruses, including flavivirus, Influenza, and herpes simplex virus (HSV) that propagate via a process of ectocytosis from the plasma membrane of an infected cell. In a model of HIV-1, the glycome profile and the budding signal of the virion were similar to that of the MP secreted in conjunction, suggesting that the mechanism for viral propagation was similar to that of the formation of MP (Krishnamoorthy et al., 2009; Gan and Gould, 2011). Even if MP formation selectively excludes budding virions, the MP may still potentially contain unpackaged viral genome and other viral proteins necessary for replication in the target cell. In either scenario, MP may be used by viruses as "Trojan Horses" to spread to other cells. Indeed, HIV-1 induces MP that "express" CD45, CD80, CD86, as well as MHC-I and II (Esser et al., 2001), thus, potentially masking themselves as being of "host" origin. In addition, HIV also induces CCR5-containing MP and can transfer this receptor to other peripheral blood mononuclear cells (PBMC) deficient of endogenous CCR5, enabling infection of these cells (Mack et al., 2000). However, the success of allogeneic transplantation of $\Delta 32/\Delta 32$ CCR5 stem cells in keeping the patient HIV free, indicates that much more remains to be understood about these processes (Hütter et al., 2009).

In conclusion, the study of MP is an exciting emerging field. As with all emerging areas, a number of technical issues remain to be fully resolved. For example, the accurate determination of MP size and phenotype, their clear distinction from and relationship to, other membrane vesicles, and their pure separation for functional experimental purposes, remain a significant challenge (György et al., 2011). While MP clearly have likely roles in homeostasis and inflammation, few studies have to date directly addressed their involvement in infectious disease. Thus, many questions remain to be answered, perhaps most important among which are, whether the production of MP as a programmatic response to infection confers an overall species survival advantage. At the mechanistic level, also, how cellular decision-making occurs to produce MP, what they should contain, and whether production of the full range of membranous vesicles is required and/or produced kinetically to accommodate host requirements from initial infection to disease resolution, remains to be elucidated. As with most defenses against invading pathogens, on the one hand, the transfer of phenotype and function by MP may be of considerable benefit to the host, in the early warning and initiating of immune defenses at both innate and adaptive levels. On the other, however, this may exacerbate immune responses leading to immune pathology, or be commandeered by the invading pathogen for its own survival.

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