MEDICAL REVIEW

Exosomes: Novel Organelles Implicated in Immunomodulation and Apoptosis

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INTRODUCTION

Vesicular traffic and transport is a major part of cellular function. Most of what we know from this field involves intracellular traffic: the secretory pathway, the endocytic pathway, the transcytotic pathway, the recycling pathways, and the protein sorting pathways, to name a few. Over the last few years, however, a novel form of vesicular traffic has been noted to occur in cells of the hematopoetic system, particularly cells of immune function. What characterizes this phenomenon is a newly studied form of intercellular trafficking, in which the secreted product of one cell is actually variable cargo on small vesicular membranes termed “exosomes.”

Exosomes were initially described as 50 to 90 nm vesicles released by reticulocytes upon their final maturation into erythrocytes [1]. By electron microscopy, these structures were seen to be found as vesicles within multivesicular bodies (MVBs) [2], their biogenesis being thought to occur as invaginations from the MVB limiting membrane into its lumen [3]. Upon direct fusion of the MVB limiting membrane with the plasma membrane, these vesicles may be released into the extracellular milieu. The released vesicles are then termed exosomes. The principle function of exosomes was initially thought to be the elimination of plasma membrane proteins no longer needed in the mature erythrocyte, such as the transferrin receptor [4].

The putative role of the exosome has expanded recently, however, with the initial observation that similar vesicles are secreted from Epstein-Barr Virus transformed B lymphocytes [5]. A hallmark of these B cell derived exosomes was that they were enriched in MHC class II molecules that could stimulate CD4+ T cell clones in vitro [5]. Since then, several papers published in the last few years have begun to delineate the ever increasing functions of exosomes in immune cell function. This review will focus on some of these recent observations.

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b Abbreviations: MVB, multivesicular bodies; APC, antigen presenting cells; CTL, cytotoxic T cell; HSP, Heat shock proteins; AICD, activation-induced cell death; TNF, tumor necrosis factor; MBP, myelin basic protein; EAE, experimental autoimmune encephalomyelitis.
EXOSOMES IN CANCER IMMUNOLOGY

One very interesting potential role of exosomes is in modulation of the immune system to fight cancer. Dendritic cells, which are one of the immune system’s most powerful professional antigen presenting cells (APCs), have been noted to be involved in cytotoxic T cell (CTL) induction following various immunotherapy protocols against tumor antigens [6]. Dendritic cells have been shown to produce exosomes bearing both MHC class I and class II glycoproteins [7]. The exosomes isolated from in vitro cultured dendritic cells pulsed with acid eluted tumor peptides were able to exert remarkable antitumor effects in selected mice bearing the tumors, similar to the effects of intact dendritic cells [7]. The effect was achieved with only a few micrograms of intradermally injected material. These effects were not seen with saline injection, nor with exosomes from dendritic cells pulsed with acid eluted peptides from the normal spleen [7]. T cells isolated from the immunized mice bore cytotoxic activity against the tumor cells in vitro, showing that the exosomes had induced a cytotoxic T cell response in the host [7]. Moreover, nude mice (with no functional immune system) did not exhibit the antitumor effect of exosome treatment, and neither did exosomes bearing allogeneic MHC molecules, further substantiating an immune mediated antitumor response [7].

The finding of MHC class I molecules on exosomes was surprising, and may postulate a role in the immunomodulating ability of these vesicles. One naïve possibility is that the exosome itself is presenting tumor peptide conjugated to MHC class I to CTLs and initiating induction of the cytotoxic response. This would presume the presence of the appropriate costimulatory proteins on the exosomal membrane. Accordingly, enrichment of CD86 (B7.2) has been found on exosomes [7]. In effect, the exosomes could serve as long-distance transducers of a specific immune response. This would explain how poorly immunogenic tumors, which have been shown to cause downregulation of costimulatory molecules from the APC cell surface, could now be subject to immune attack [7]. Exogenous MHC class I coupled to tumor peptides could be providing the necessary T cell inducing activity, for example. One argument against the direct induction of CTLs by exosomes, however, is the fact that in vitro, exosomes are not as potent as dendritic cells themselves in T cell induction [7]. T cell stimulation by exosomes becomes much more efficient in the presence of dendritic cells, making an indirect mode of action more likely [7].

Recent work has begun to shed some light as to the mechanism of action of the exosomes in the antitumor response. Biochemical characterization of the exosomes has revealed an interesting accumulation of certain proteins both on the exosome membrane as well as within its lumen [4, 8]. Specifically, hsc73, a heat shock protein known to have antitumor activity via an immune response, is highly enriched potentially within the lumen of the exosome [4].

Heat shock proteins (HSPs), like hsc73, are stress-induced chaperonins which bind to a large complement of endogenous peptides [9]. HSPs isolated from tumor cells can induce a potent antitumor rejection response in tumor-bearing mice [9]. Peptides associated with hsc73 are very efficiently loaded onto MHC class I and II molecules of APCs [9], and there is electron microscopic evidence that HSPs can undergo receptor-mediated endocytosis within APCs [10], helping to account for the highly efficient in vivo antitumor effect with nanogram amounts of these molecules [10]. Interestingly,
hsc73, in the absence of any peptide, has been shown to produce macrophage-induced T cell stimulation as well [11]. This implies that HSPs may have immuno- 
genic or adjuvant qualities by themselves, and perhaps the presence of certain pep- 
tides bound to the HSPs could help in the specificity of a potent immune response. 
Exosomal hsc73 may be involved in transfer of peptides for loading onto MHC mol- 
eules, stimulation of a generalized immune response, or in some other as yet unexpected phenomenon. Thus, although potentially very interesting, determining the specific role for hsc73 in the immuno-
biology of the exosome requires ongoing investigation.

Furthermore, MFG-E8, a soluble membrane-associated integrin binding protein, as well as several members of the tetraspan integral membrane proteins involved in cell-cell contact, were enriched on exosomes [4, 12]. Interes-
tingly, macrophages and immature dendritic cells express integrins that bind MFG-E8, raising the possibility that the exosomes may be targeted to these cells [4]. This may also explain some of the evi-
dence implicating exosomes in indirect T cell stimulation, as well as how exosomes may serve as long-distance immunomodu-
lators. A certain dendritic cell, which is bearing useful immunogenic peptides on its MHC glycoproteins could now, via its exosomes, endow several other antigen-presenting cells with the given peptide in the context of the appropriate MHC/cos-
timulatory/cell adhesion molecules. This could increase the efficiency of the immune response and is one explanation for how small amounts of exosomal material are able to induce a potent antitumor response in mice [7].

Of note, it was found that immature dendritic cells, rather than mature dendrit- 
ic cells found in secondary lymphoid tis-
sue, are the ones selectively producing exosomes [4]. That these immature den-
dritic cells are found in peripheral tissues argues that exosomes may be more involved in sensitization of other dendritic cells rather than direct T cell activation [4]. This is also supported by the data dis-
cussed earlier, where exosomes in vitro were relatively poor at T cell stimulation unless dendritic cells were also present [7]. Recently, the source of MHC class II molecules on the surface of follicular dendritic cells has been shown to be of exoso-
mal origin, rather than having been expressed by the follicular dendritic cell itself and presented at the cell surface [13]. Thus, follicular dendritic cells may be the in vivo target of exosomes, providing these cells with MHC class II molecules that may then be exploited in the immune response [13]. Therefore, whether by indirect priming of APCs with antigen-loaded MHC I/II, direct CTL induction via anti-
gen loaded MHC class I, immunostimula-
tion and antigen loading by hsc73, or some other undiscovered mechanism, exosomes are potentially exciting new immunomod-
ulating factors that may be exploited in novel antitumor therapeutics.

Interestingly, a recent report has shown that tumor cells themselves may be an important source of exosomes [14]. Exosomal vesicles containing MHC mol- 
eules and tumor-derived antigens were noted to be present in several different mouse and human tumor cells [14]. Although these exosomes carried MHC class I molecules, they were unable to induce a cytotoxic T cell response in vitro; however, they were able to induce a potent T cell response when loaded onto human dendritic cells [14]. Furthermore, the tumor antigens present on the exosomes were able to be transferred to the dendritic cells and subsequently to generate a CD 4+ mediated cross-response as well [14]. Therefore, it appears as though tumor-
derived exosomes may serve as an impor-
tant source of tumor antigens that may trigger a protective immune mediated anti-tumor response. Moreover, the tumor antigens present on these exosomes seemed to be shared by several different tumor cell types, raising the exciting possibility that such exosomal antigens may be used for cross vaccination against many different tumor types [14].

**EXOSOMES IN APOPTOSIS**

Another potentially very interesting role of immune cell exosomes is in induction of apoptosis. After a cellular immune response, most of the activated T cells are destroyed by activation-induced cell death (AICD) to protect against damaging autoimmune effects [15]. AICD of many T cell lines is potentiayed by FasL and APO2L, two membrane-bound proteins that are members of the tumor necrosis factor (TNF) superfamily [15]. Upon binding to their respective receptors, these transmembrane ligands induce apoptosis in the appropriate receptor-bearing cells, which frequently occurs as an autocrine or paracrine response [15].

Previously, it has been shown that supernatants from activated Jurkat cell cultures or FasL transfected COS cells have apoptotic activity against Fas expressing cells [16, 17]. This activity was found to be attributed to a soluble form of FasL, formed by Zn$^{2+}$-dependent metalloproteinases, but with much lower toxicity than the nonproteolyzed molecule [15]. Afterward, it was shown that intact FasL and APO2L released into culture supernatant were associated with a particulate fraction, and that the release was mediated by stimulation of the T cells [15]. Release of the toxic molecules was inhibited by cytochalasin B, an inhibitor of actin cytoskeletal reorganization necessary for microvesicle generation in certain systems [15]. Furthermore, the stimulation dependent presence of 100 to 200 nm microvesicles in the culture supernatants was confirmed by electron microscopy [15]. The microvesicle associated intact FasL was a much more potent toxic agent than the soluble form, indicating that the intact molecule is the functional apoptotic inducer [15]. Indeed, other work has correlated the presence of soluble FasL with downregulation of apoptosis [18].

What is the benefit of microvesicle associated apoptotic signals? One clear benefit is preservation of the membrane bound form of the ligand, which has the greater activity. Another is the potentially greater surface area for ligand expression compared with the plasma membrane [15]. Moreover, for reasons that are not entirely clear, FasL and APOL associated with microvesicles have a much higher cross-linking efficiency when compared with the plasma membrane associated molecules [15]. In addition, microvesicles may potentiate paracrine/autocrine responses by incurring less mobility to the molecules compared with their soluble forms, thereby achieving high local concentrations [15].

A potentially intriguing possibility, by analogy to the dendritic cell-derived exosomes, would be the specific targeting of apoptotic transducers and receptors, via cell-cell contact molecules, to particular subsets of cells. This could serve as a new modulator for toxic immunomodulation. In light of this, some tumor cell lines have been shown to constitutively secrete Fas and FasL on microvesicles [19]. It is tempting to speculate that this could serve either as a tumor specific self destruct mechanism, or more ominously, as an immunosuppressing activity. Future work in the field should help further elucidate the role of exosomes in apoptosis.

**EXOSOMES AND TOLERANCE**

Another role for exosomes that has emerged recently is in T cell-mediated peripheral tolerance. It has been shown
that T cell expression of MHC class II molecules may play an important role in maintaining tolerance to peripheral self antigens [20]. T cells can express MHC class II molecules by two known methods: The first is expression of endogenously synthesized molecules, seen in blastogenic T cells, and the second is through acquisition of exogenous molecules from professional APCs [20]. This exogenous acquisition is thought to occur through specifically targeted exosomes, via an antigen-specific interaction [20]. Moreover, once acquired, these T cells can now confer the same antigen/MHC class II complex to other responder cells, providing evidence for an active intercellular exchange of antigen/MHC class II among APCs and antigen-specific T cells via exosomes [20].

T cells expressing MHC class II molecules, known as T-APCs, pulsed with antigenic peptides have been shown to be able to transfer these antigen bound MHC molecules to activated responder T cells in a T cell receptor-dependent fashion [20]. Three observations support the fact that responder cells have acquired intact antigen/MHC from a donor cell. The first is that the acquisition was not blocked by cyclohexamide, a potent inhibitor of protein synthesis [20]. Second, mitogenic stimulation of the T cells, which is not dependent on T cell receptor activation, endowed those cells with the ability to express allogeneic MHC molecules present on respective APCs [20]. And finally, lipophilic dye from APCs was able to be transferred to activated responder T cells coincident with the surface expression of either syngeneic or allogeneic MHC molecules, suggesting exosomal transfer [20].

What utility would this exosomal transfer of antigen/MHC class II from a T cell APC to a responder T cell serve? As stated earlier, expression of MHC class II on the surface of T cells may be important in mediating peripheral tolerance to self antigens [20]. Positive selection within the thymus enriches for T cells, which are able to weakly bind self antigen/MHC [20]. This partial agonist activity has been shown to correlate with acquisition of MHC class II proteins on the T cell surface, and the long-term expression of these proteins is also dependent on this weak interaction [20]. It is thought that T cells bearing self antigens coupled to MHC class II glycoproteins are involved in maintaining tolerance to self antigens by interacting with T cells through high-affinity T cell receptors, but in the absence of costimulatory molecules necessary for generating an immune response [20]. Indeed, antigen presentation by T-APCs has been shown to induce apoptosis or anergy in responder cells [21].

Thus, whether a T cell generates an immune response or becomes anergic depends on a highly specific interaction between the T cell receptor and the antigen/MHC complex, as well as the presence or absence, respectively, of costimulatory molecules [20]. Exosomal transfer of self antigen/MHC complex to other potentially reactive T cells could help in the maintenance of peripheral self-tolerance by rendering these cells now anergic, and by propagation of the tolerogenic phenotype through generation of additional T-APCs [20]. This was shown through injection of T-APCs pulsed with myelin basic protein (MBP) into mice with experimental autoimmune encephalomyelitis (EAE), an autoimmune demyelinating disease with MBP reactive T cells. In treated mice, the severity of the disease was significantly diminished and corresponded with the rapid acquisition of MHC class II expression on the surface of reactive clones [20]. It would be interesting to see whether these responder T cells with the newly acquired antigen/MHC class II surface expression could propagate the disease-modulating activity to other syngeneic animals. Exosomal transfer of such tolerogenic activity could potentially provide
very useful and efficient therapeutics against autoimmune diseases and could revolutionize transplant immunomodulation. Further studies should continue to elucidate the function of exosomes in maintenance of self-tolerance.

PERSPECTIVE

In this review, the exciting relatively recent discoveries of the role of exosomes in immune function have been explored. More and more, it is becoming clear that, rather than being an epiphenomenon, exosomes are playing a fundamental role in immunobiology. Furthermore, exosomes have shown a diversity that poises them as an interesting organelle to study from a variety of standpoints. Depending on what proteins constitute the exosomes, they may have cytotoxic effects, immunomodulating effects, apoptotic activity, and tolerogenic activity.

These remarkable attributes have a lot of potential for pharmacotherapeutic roles. Moreover, the fact that they are small, potent, and non-living makes them highly attractive bioactive molecules on which to focus therapeutics. Theoretically, if the many constitutive features of exosomes could be identified, then one could envision creating them in vitro for potential manipulative purposes.

Thus, exosomes have begun to redefine and expand the role of immune cell function, particularly with respect to the fundamental principles of antigen presentation. Recent identification of exosomes in intestinal epithelial cells and in mast cells involved in B and T cell stimulation is a harbinger of other as yet unidentified sources of these organelles involved in modulating the immune response [22, 23]. Exosome biology is only at its inchoate stages, and future studies will hopefully continue to provide more information on the many functions of these versatile organelles in immunology.

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