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Review article

The role of exosomes in allograft immunity

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ABSTRACT

Extracellular vesicles are emerging as potent vehicles of intercellular communication. In this review, we focus on a subclass of extracellular vesicles called *exosomes*. Previously considered an unimportant catch-all, exosomes have recently been recognized for their role in various diseases and their potential for therapeutic use. We have examined the role of exosomes after human lung transplantation and have delineated the composition of circulating exosomes isolated from lung transplant recipients diagnosed with acute and chronic rejection, primary graft dysfunction, and respiratory viral infection. The presence of lung-associated self-antigens (K-alpha 1 Tubulin and collagen V) and mismatched donor HLA in exosomes isolated from lung transplant recipients signifies that these exosomes originated in the transplanted lungs, and therefore dramatically affect transplant biology and immune pathways. Exosomes released from transplanted organs also carry other proteins, costimulatory molecules, and nucleic acids. Therefore, these molecules may be used as biomarkers for allograft rejection and immunity.

1. Introduction

Extracellular vesicles (EVs) are secreted by cells from multicellular and unicellular organisms. EVs are originated from plasma membranes either ectosomal or endosomal in origin. EVs are released from all type of cells undergoing stress including during transformation, activation, drug treatments, surgeries and infections (bacterial, viral, fungal, etc.). EVs are categorized according to size, and are sorted into groups such as ectosomes, oncosomes, shedding microvesicles (MVs), etc. But this classification varies according to different reports. In this review, we discuss MVs (100–1000 nm in size), apoptotic bodies (> 1000 nm in size), exosomes (40–150 nm in size), and oncosomes (secreted by oncogenic cells; > 1000 nm in size) [1,2]. All the vesicles contain a subset of proteins, lipids, RNA and DNA corresponding to their origin from the parent cell. EVs are thought to be involved in intercellular communication by transferring information via small biological molecules (i.e, lipids, carbohydrates, proteins, small metabolites, and nucleic acids) [3,4]. EVs are being explored for potential use for therapeutic applications, disease prognosis, and biomarker discovery for various diseases. [5]. The EV's have been studied in cancer during the last two decades but study of EVs are limited in the field of transplantation. Since EVs are released by both immune and non-immune cells it is thought to play important roles in the regulation of immunity during disease, chronic illness, infections and solid organ transplants. In this review we will focus on allograft immunity following solid organ transplantation.

Transplantation is the last option for patients with end stage organ disease. Major organ transplants currently performed are kidney, heart, lung, liver, intestine and pancreas either alone or with kidney [6,7]. Numbers of recipients seeking organ transplants are outnumbered as compared to availability of suitable donors. This varies for different organs.

A significant number of patients are on the waiting list for organ transplants which reflects the lack of organ donors. Success rate of the transplant depends on the organ transplanted donor/host compatibility and infections, etc. Transplants often undergo acute and chronic rejection which can be due to either cellular or humoral immune response or a combination of both. In addition, donor factors play a critical role especially during the early period following transplantation. Late allograft loss is most often due to chronic allograft damage resulting in progressive decline of graft function years after transplantation.

Although, in solid organ transplants, several mechanisms underlying acute or chronic rejection and leading to graft dysfunction have been reported which includes cellular immune response to mismatched donor human leucocyte antigens (HLA), development of donor specific antibody against mismatched HLA [8], as well as development of antibody tissue restricted self-antigens (SAgs) [9,10]. Immune responses affecting mainly the small arteries and capillaries can result in cardiac allograft vasculopathy (CAV) [11–13] following heart transplantation, transplant glomerulopathy affecting glomerular basement membrane which histologically recognized as either by duplication, double contouring, or splitting [14,15] following kidney transplantation. The mechanisms leading to chronic rejection of different organ transplants are currently unknown though both humoral and cellular immune

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mechanisms play an important role leading to chronic rejection.

Identification of biomarkers involved in allograft function (stable vs rejection) will assist in identifying transplant recipients who are at risk for developing acute or chronic rejection thereby will allow to develop strategies for early intervention to prevent further damage.

It is our contention that EVs, especially exosomes, play an important role in immune activation or suppression of allograft immunity. Presence of allo-antigens, cell specific antigens, peptides, and costimulatory molecules on the surface of exosomes, as well as the presence of nucleic acids, lipids, small RNAs and transcription factors inside the exosomes are released following transplantation making exosomes as one of the attractive targets towards identifying biomarkers associated with allograft immunity. In the current review we will explain the details of allograft immunity mediated by different kinds of EVs emphasizing exosomes released following transplantation during rejection process.

1.1. Microvesicles (MVs)

Description of MVs were first given by Chargaff and West in 1946 in the context with a factor which is perceptible in platelet free plasma and have potential to generate thrombin leading to blood coagulation. MVs were earlier referred as “platelet dust” due to their origin from platelets in plasma/serum [16,17]. MVs are released by cells that measure between 100 and 1000 nm in size. MVs are released mostly during stress conditions by the budding/blebbing mechanism of the plasma membrane, and are secreted into the cellular milieu. MV’s are vesicles encapsulated by a phospholipid bilayer and their size overlaps with that of bacteria and insoluble immune complexes.

Vesicles secreted by the plasma membrane reflect intercellular communication through the exchange of cellular material, but the exact nature and origin of these MVs, as well as their secretion and ultimate fate, remain largely unknown.

1.2. Apoptotic bodies

“Apoptotic body” term was used by Kerr in 1972 followed by Robert Horvitz et al. Apoptotic bodies are released from cells that undergo apoptosis, and their surface composition consists of phosphatidylserine [18,19]. These apoptotic vesicles are also often referred to as apoptotic bodies. Apoptotic vesicles are reported to be larger than the other secreted vesicles, with their size ranging between 1 and 5 μm. Apoptotic bodies consist of parts of cells which are undergoing death. Apoptotic bodies consist of DNA (damaged and degraded DNA sequences), metabolites, remnants of cellular organelles. Annexin V and phosphatidyl serine and fragmented DNA are the markers of apoptotic bodies.

1.3. Oncosomes

EVs, from tumor cells are termed as oncosomes, they are large vesicles 1–10 μm. Term oncosomes was first described by Janus Rak’s group in 2008 from tumors of the brain [20]. Oncosomes are reported to play a role in the tumor microenvironment by transporting bioactive molecules across tissue spaces and through the blood stream. Oncosomes are capable of carrying microRNA (miRNA), DNA, protein and metabolities. Oncosomes carry mutated DNA and RNA sequences of oncogenes and activated oncoproteins which can lead to horizontal transfer of biological material through intercellular trafficking [21–23]. Cellular uptake of oncogenic cargo through packaged oncosomes induces changes in phenotypes and behavioral pattern of naïve and healthy cells [24]. All the changes in healthy cells are dependent on the contents transferred through oncosomes. Horizontal transfer of mutated nucleic acids and proteins can also lead to resistance to existing therapies. Recent reports have demonstrated the effect on revival of dormant stages of resistant cells in hormone resistant breast cancers [25]. In summary, oncosomes play a critical role in intercellular communication between tumor cells and the tumor microenvironment [26–29].

1.4. Exosomes

Exosomes are membrane-bound vesicles measuring 40–150 nm in size. Exosomes are released by most cells, including mast cells, dendritic cells, B and T lymphocytes, neurons, adipocytes, endothelial cells, and epithelial cells [30–32]. Diseased, unhealthy cells have been noted to secrete more exosomes than healthier cells. The density of exosomes ranges from 1.13 to 1.19 g/mL, and they have been found in many types of bodily fluids, including blood, urine, ascites, breast milk, saliva, amniotic fluid, lymph fluid, and cerebrospinal fluid, from both healthy and unhealthy individuals [33–39]. Exosomes are cup-shaped vesicles, encapsulated by a lipid bilayer, and feature surface proteins, antigens, and specific markers (e.g, CD9, CD81, tetraspanins, Alix, CD63, tumor susceptibility gene 101, heat shock proteins, and specific markers) depending on the cells that release them [40,41].

The composition of exosomes depends on their origin and the clinical condition of the individual (eg, cancer, infection, transplantation) [42]. According to a current exosome content database, Exocarta (Version 4), exosomes from various organisms and cell types have been characterized as containing 4563 proteins, 194 lipids, 1639 mRNAs, and 764 miRNA [43,44]. Exosomes also contain biomolecules, including carbohydrates, proteins, lipids, nucleic acids (ie, DNA and RNA), and metabolites. The lipid and protein composition of exosomes have been studied, and researchers have found that exosomes originating from different types of cells have different lipid and protein compositions depending on the cells’ pathophysiological condition. These structural differences have been studied using Western blotting, fluorescence-activated cell sorting, electron microscopy, and mass spectrometry [45] (Fig. 1).

All the different EV’s ie: MVs, apoptotic bodies, oncosomes and exosomes vary in their structure depending on the type of cytoskeletal proteins, cytoplasmic enzymes, cytokines, chemokines, cell specific antigens, cell signaling molecules, lipids and proteins present on the surface which is described in detail in Table 1. EV’s may be released from same or different cells but possess different functional aspects due to the difference in their architectural constituents and unique signaling molecules depending on the cells from which they are released. To study unique properties of different EV’s their difference in size and distribution have been made use of and differential centrifugation towards purifying EV’s have been employed by different research groups [46–50] (Table 1).

1.4.1. Origin of EVs

The origin and biogenesis of EV’s are different that essentially distinguishes exosomes from MVs, oncosomes and apoptotic bodies. As early as 1980, researchers studying the process of reticulocyte maturation observed extra-vesicular secretion [51]. These extracellular vesicles are subcategorized based on their size, and they are endosomal in origin, due to the inward budding of endosomes. Some large EVs (ie, multivesicular bodies) have been shown to fuse with lysosomes or the plasma membrane, thereby releasing the contents needed for degradation into the extracellular space [52].

Exosomes, the smaller vesicles, are released from MV bodies and deliver functional RNAs (e.g, mRNA and miRNA) to other cells, which assist in intercellular communication [53–56]. The function of exosomes varies depending on their origin. Exosomes derived from antigen presenting cells can express major histocompatibility complex (MHC) class I and II molecules on the cell surface, which enables them to activate $CD8^{\beta}$ and $CD4^{\beta}$ T cells to induce specific immune responses [57–59].

1.4.2. Biology and function of exosomes

The secretion of exosomes into biological fluids contributes to their