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Review article

Exosomes and nanotubes: Control of immune cell communication

Kessler McCoy-Simandle[a], Samer J. Hanna[a], Dianne Cox[a,b,c,*]

[a] Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, 1300 Morris Park Ave, Gruss MRRC 306, Bronx, NY 10461, USA
[b] Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, 1300 Morris Park Ave, Gruss MRRC 306, Bronx, NY 10461, USA
[c] Gruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, 1300 Morris Park Ave, Gruss MRRC 306, Bronx, NY 10461, USA

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ABSTRACT

Cell–cell communication is critical to coordinate the activity and behavior of a multicellular organism. The cells of the immune system not only must communicate with similar cells, but also with many other cell types in the body. Therefore, the cells of the immune system have evolved multiple ways to communicate. Exosomes and tunneling nanotubes (TNTs) are two means of communication used by immune cells that contribute to immune functions. Exosomes are small membrane vesicles secreted by most cell types that can mediate intercellular communication and in the immune system they are proposed to play a role in antigen presentation and modulation of gene expression. TNTs are membranous structures that mediate direct cell-cell contact over several cell diameters in length (and possibly longer) and facilitate the interaction and/or the transfer of signals, material and other cellular organelles between connected cells. Recent studies have revealed additional, but sometimes conflicting, structural and functional features of both exosomes and TNTs. Despite the new and exciting information in exosome and TNT composition, origin and in vitro function, biologically significant functions are still being investigated and determined. In this review, we discuss the current field regarding exosomes and TNTs in immune cells providing evaluation and perspectives of the current literature.

Contents

Abbreviations: APC, Antigen presenting cell; Cdc42, Cell division control protein 42; DC, Dendritic cell; EBV, Epstein-Barr virus; ESCRT, Endosomal sorting complex required for transport; HIV, Human immunodeficiency virus; ICAM-1, Intercellular adhesion molecule 1; IgA, Immunoglobulin A; IgG2, Immunoglobulin G2; IL-2, Interleukin 2; LAMP1, Lysosomal-associated membrane protein-1; LAMP2, Lysosomal-associated membrane protein-2; LFA-1, Leukocyte-function associated antigen 1; LST1, Leukocyte specific transcript 1; MHC, Major histocompatibility complex; miRNA, MicroRNA; MVB, Multivesicular body; NK, Natural Killer; NKG2D, Natural-killer group 2, member D; N-WASP, Neuronal Wiskott-Aldrich syndrome protein; Rac1, Ras-related C3 botulinum toxin substrate 1; RalA, Ras-related protein Ral-A; TCR, T-cell receptor; TGF, Transforming growth factor; TNFaip2, Tumor necrosis factor –α-induced protein; TNTs, Tunneling nanotubes; WGA, Wheat germ agglutinin.

* Corresponding author. Tel.: +1 718 430 4005.
E-mail addresses: kessler.mccoy-simandle@einstein.yu.edu (K. McCoy-Simandle), samer.hanna@phd.einstein.yu.edu (S.J. Hanna), dianne.cox@einstein.yu.edu (D. Cox).

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1. Introduction

Everyday humans use a variety of means of communication that are critical for our survival and our ability to function as a community. Analogous to this, a similar practice also occurs on the microscale of the organism, where cells must communicate with each other to function as an intact organism. Cell-cell communication is important in every aspect of the human body whether it is between neurons of the brain, synchronous beating of heart cells, or uniform contraction of muscles. Our complex immune system also relies on communication for crucial immune cell functions including cell survival, maturation, migration, signaling, and most importantly, coordinating inflammatory responses. In the course of any infection, cells of the immune system must interact with each other for the proper immune response to be activated. Even in the absence of infection, immune cells must communicate to perform normal maintenance functions. Historically, research in communication between immune cells has focused on soluble means of communications (e.g. cytokines). This term refers to a cell secreting a factor, which another cell recognizes and then responds. Soluble factors allow for global communication between cells and for gradients of signal to form. For example, the generation of a fever is a response to soluble communication, as is migration or chemotaxis of neutrophils to a site of injury or infection. The characteristics of soluble factors are extremely important and lead to effective cellular communication throughout the body. However, not all communication can be attributed to soluble means. Another means of cell-cell communication occurs when cells are in physical contact with each other and molecules on the surface of each cell interact leading to a response in one or both of the cells. A well-studied example of communication by direct cell contact is the immune synapse, which forms by direct contact between a T cell and an antigen presenting cell (Davis and Dustin, 2004), or the interaction that occurs when immune cells must leave the circulation and migrate across the endothelium of the blood vessel (Nourshargh and Alon, 2014). Gap junctions also contribute to contact-dependent communication allowing for trafficking of small molecules between cells (Saez et al., 2003). Yet these types of interaction are usually of short range. Between contact dependent communication and secreted factors both short range direct and long range global communication patterns are met. However, there are additional alternative means of communication that allow for some aspects of direct cell communication to span longer distances. The focus of this review will be on two alternative means of communication between immune cells: exosomes and tunneling nanotubes (TNTs).

2. Exosomes

In addition to classical cytokine and chemokine-based signaling, immune cells are known to communicate through secreted extracellular vesicles and, in particular, endosomally-derived exosomes. These are small membrane bound vesicles secreted into the extracellular environment, which can carry a variety of different molecules (Johnstone et al., 1987, Ratajczak et al., 2006, Tian et al., 2010). Immune cells can generate exosomes as well as take up exosomes produced by other cell types (Mittelbrunn et al., 2011). The last decade has revealed a plethora of functions for exosomes in the immune system, nervous system, stem cells and cancer cells.

2.1. Structure

Exosomes were first identified by several groups using pulse-chase and electron microscopy experiments in reticulocytes (Harding et al., 1983, Johnstone et al., 1987, Pan et al., 1985). In general, exosomes are small round shaped membrane-bound vesicles with sizes ranging between 40 and 100 nm in diameter (Conde-Vancells et al., 2008, Raposo et al., 1996, Raposo and Stoorvogel, 2013). Because of their small size, exosome structure is not readily observed using the light microscope but when examined by electron microscopy, exosomes appear as flattened spheres surrounded by a lipid bi-layer (Fig. 1). These characteristics are consistent with the sizes and morphology of internal vesicles in the multivesicular endocytic compartment, or MVB, from which exosomes originate (Raposo et al., 1996). Secreted exosomes also float on sucrose gradients with a density ranging between 1.13 g/ml to 1.19 g/ml (Escola et al., 1998, Raposo et al., 1996, Thery et al., 1999). This provides criterion to differentiate exosomes from membrane vesicles and protein aggregates released by apoptotic cells in addition to their structure and protein composition (Thery et al., 2001). Secretion of exosomes is constitutive in many cell types including EBV-transformed B cells (Raposo et al., 1996) and immature dendritic cells (DCs) (Thery et al., 1999). However, exosome secretion is a regulated process in other hematopoietic cell types including mast cells and T-cells (Raposo et al., 1997). When regulated, MVBs fuse with the plasma membrane following activation in a Ca^{2+}-dependent manner (Blott and Griffiths, 2002). Interestingly, T-cells seem to switch between constitutive release and regulated release depending on stimulation or activation (Blanchard et al., 2002).

Exosomes have been isolated from various sources, in large part from tissue culture media *in vitro* but also *in vivo* from circulation. The common method utilized by most groups to purify exosomes is through a series of centrifugation steps to remove cellular organelles and other debris, followed by ultracentrifugation to pellet exosomes (Davis et al., 1986, Raposo et al., 1996, Thery et al., 2006). Sucrose gradients are then used to separate proteins from lipid-containing membrane vesicles (Escola et al., 1998, Raposo et al., 1996, Thery et al., 2006, Thery et al., 2009). More recently, polymer-based or immuno-capture methods have been used as fast and simple procedures for exosome purification that do not require ultracentrifugation. Despite the purification method, purified exosomes are further confirmed using multiple techniques including western blot, microscopy and proteomic analysis to characterize their morphology, composition and physical features. Commonly used markers for exosome purification in protein detection methods include tetraspanins CD9, and CD63, which are found to be associated and enriched in intracellular vesicles within MVBs (Escola et al., 1998). Recently, the International Society for Extracellular Vesicles (ISEV) has proposed a series of criteria to define minimal characterization of extracellular vesicles, particularly exosomes. Based on the ISEV categories, three or more specific proteins should be present on vesicles to be properly referred to as exosomes including tetraspanins, integrins, adhesion molecules and others (Lotvall et al., 2014). However, a detailed comparison is still needed to determine if the different methods purification precipitate different amounts or types of vesicles. Differences in these methods may contribute to potential variations between studies.

2.2. Biogenesis/formation

Based on proteomic analyses, exosomes were surprisingly found to lack proteins from the nucleus, mitochondria, endoplasmic reticulum or the golgi apparatus (Raposo et al., 1996, Thery et al., 2001, Thery et al., 1999). Several studies on exosomes from immune cells, including DCs, T-cells, and B-cells support the fact that exosomes are not derived from plasma membrane fragments (Blanchard et al., 2002, Clayton et al., 2001, Raposo et al., 1996, Thery et al., 2001). The presence of MVB markers including CD63 and major histocompatibility complex (MHC) class II support the endosomal origin of exosomes (Kleijmeer et al., 1996, Thery et al., 2001). Extensive protein analyses of exosomes secreted by DCs, lymphocytes, and other