The Epithelial Cell Cytoskeleton and Intracellular Trafficking
II. Intestinal epithelial cell exosomes: perspectives on their structure and function

GUILLAUME VAN NIEL AND MARTINE HEYMAN
INSERM EMI0212, Faculté Necker, 75730 Paris, France

Van Niel, Guillaume, and Martine Heyman. The Epithelial Cell Cytoskeleton and Intracellular Trafficking. II. Intestinal epithelial cell exosomes: perspectives on their structure and function. Am J Physiol Gastrointest Liver Physiol 283: G251–G255, 2002; 10.1152/ajpgi.00102.2002.—Intestinal epithelial cells (IEC) are located at the strategic interface between the external environment and the most extensive lymphoid compartment in the body. Besides their central role in the absorption of nutrients, they also provide sample information to the immune system on soluble or particulate antigens present in the intestinal lumen. Like professional antigen-presenting cells, IEC have recently been shown to secrete 30- to 90-nm diameter vesicles named exosomes from their apical and basolateral surfaces. These vesicles carry molecules that are implicated in adhesion and antigen presentation, such as major histocompatibility complex (MHC) class I molecules, MHC class II molecules, CD63, CD26/dipeptidyl-peptidase IV, tetraspan proteins, and A33 antigen. IEC exosomes therefore, constitute a link by which IEC may influence antigen presentation in the mucosal or systemic immune system independent of direct cellular contact with effector cells.

antigen; absorption; antigen presentation; oral tolerance; mucosal immune system

THE INTESTINAL EPITHELIAL layer is classically considered a complex structure primarily regulating absorption of nutrients and ions while excluding the passage of food antigens and infectious agents. Although digestive enzymes degrade most ingested proteins, a small proportion escapes degradation and can be taken up by intestinal epithelial cells (IEC). They gain access to the serosal compartment after processing within late endosomal compartments. Although paracellular diffusion of proteins has been considered a potential route for macromolecules to cross the epithelium, fine control of tight junction permeability prevents diffusion of proteins toward the internal compartment. Thus the interaction of the immune system with antigen is tightly regulated at mucosal surfaces and suggests the transfer of antigenic material by IEC along a transcellular pathway (11). Generally, dietary antigens induce local and systemic tolerance, because one of the major roles of the mucosal immune system is the induction of nonresponsiveness. It is therefore, likely that epithelial-lymphoid interactions are crucial in the regulation of immune responses.

Professional antigen-presenting cells (APC) have recently been shown to release major histocompatibility complex (MHC)-bearing vesicles called exosomes, capable of stimulating T cell responses. Due to their small size and composition, exosomes have been proposed as possible messengers between cells and as vehicles for disseminating information. In the present paper, we discuss the possibility that exosome-like structures released by IEC could be part of the intestinal antigen-presenting pathway.

EXOSOMES FROM VARIOUS CELL TYPES: DIVERSITY IN FUNCTION

Exosomes are small membrane vesicles that were first described in reticulocytes and subsequently in hematopoietic cells. Various cell types have been described to secrete exosomes, including reticulocytes (12), platelets (9), B lymphocytes (16), cytotoxic T lymphocytes (15), dendritic cells (26), and mast cells (17). These 30- to 90-nm vesicles are formed by inward invagination in endosomal compartments named multivesicular bodies (MVBs) that can fuse with plasma membranes releasing the entrapped vesicles into the extracellular medium.

Reticulocytes release exosomes during their maturation into erythrocytes, clearing proteins such as acetylcholinesterase and transferrin receptors in the process. Exosomes are also released from platelets at the sites of vascular injury by the fusion of granules, a storage
site of adhesive glycoproteins, with the plasma membranes (9). They are enriched in CD63, a tetraspan protein implicated in adhesive and costimulatory functions. The cytolytic granules containing perforin and granzyme in secretory lysosomes that are exocytosed through a highly regulated mechanism in cytotoxic T lymphocytes (15) have also been referred to as exosomes (5).

Exosomes have also been described in professional APCs, such as B cells (16) and dendritic cells (26). In these cells, most of the intracellular MHC class II molecules reside in late endosomal compartments and lysosomes, collectively called MHC class II-enriched compartments (MIICs). Multivesicular MIICs, similar to MVBs in other cell types, can fuse with the plasma membrane to release exosomes enriched in MHC class II and class II molecules, CD86 (B7−2), a costimulatory molecule, and in tetraspan proteins (CD87, CD53, CD63, CD81, and CD82), which probably act as adhesion molecules (8). According to the cell from which they originate, exosomes can bear distinct proteins, explaining their functional diversity. B cell-derived exosomes were shown to activate CD4+ T cells in vitro (16) via peptide-MHC class II molecules exposed at their surface; and recently, the binding of B cell exosomes to the plasma membrane of follicular dendritic cells in germinal centers has been underlined. In addition, T cell-derived vesicles bearing MHC class II molecules have recently been shown to transmit antigen from APCs to secondary CD4+ T cells. Indeed, the T cell-MHC class II vesicle complexes were able to activate secondary T cells through an MHC class II-peptide specific process (2).

Dendritic cell-derived exosomes, bearing tumor antigens bound to MHC class I molecules, have been shown to activate cytotoxic CD8+ T cells and to eradicate tumors in vivo in mice (26). This antitumor capacity may present an alternate immunotherapeutic strategy to dendritic cells in humans, as suggested in preclinical models (1). Although mast cells are generally recognized as effector cells in allergic reactions, they too can release exosomes capable of nonspecifically stimulating the activation and the proliferation of B and T cells (21). In concert, these data suggest exosomes might function as intercellular vehicles for peptide presentation, a concept of major interest (Fig. 1).

**ROLE OF INTESTINAL EPITHELIAL CELLS IN ANTIGEN TRANSPORT, PROCESSING, AND PRESENTATION**

Only minute amounts of luminal antigens are endocytosed and transported by epithelial cells. Moreover, the majority of the endocytosed material (90%) is degraded during transepithelial transport. However, a small amount of intact proteins, and peptides of sufficient length to bind restriction molecules, can be transmitted from the IEC to the submucosal layers (22), where they can interact with immune cells throughout the lamina propria or in more distant compartments.

Exosomes were shown to activate CD4+ T cells in vitro to involve different pathways depending on the presence or absence of mucosal inflammation. In addition, due to the polarized nature of IEC, antigen uptake occurs mainly at the apical surface, whereas antigen presentation is only possible at the basolateral surface (10). In vivo, however, direct contact between IEC and CD4+ T cells is limited because of the basement membrane, although IECs can occasionally develop projections through the pores (0.3–3 µm) in this membrane. We recently suggested the hypothesis that IEC, located at a strategic position between the high load of luminal antigens and the intestinal immune system, may use the release of epithelial cell exosomes as a tool for antigen delivery to the underlying immune system (Fig. 2).

**CHARACTERIZATION OF INTESTINAL EPITHELIAL EXOSOMES**

Recently, we showed that the intestinal cell lines HT29–19A and T84-DRB1*0401/CIITA secreted exosome-like vesicles in basal and inflammatory conditions (23). These 30- to 90-nm-diameter vesicles were released from both the apical and basolateral sides and expressed common molecules such as MHC class I molecules, CD26 and CD63 in basal conditions, in
addition to MHC class II molecules in inflammatory conditions. Various other molecules were found expressed by exosomes, including membrane proteins (A33 antigen, epithelial cell surface antigen, syntaxin 3, microsomal dipeptidase) and different cytosolic proteins (Fig. 3). Although CD26 and A33 antigen are molecules found in plasma membranes of epithelial cells, their presence in apical or basolateral exosomes does not rule out that exosomes are organelles distinct from the plasma membrane, because exosomes are vesicles trafficking within the cell and carrying molecules “en route” to the external membrane. The enrichment on exosomes of CD63, a marker of late endosomal compartment, as well as other tetraspan proteins (unpublished data) confirmed that exosomes were not plasma membrane fragments but originated from an endosomal compartment, probably the MIIC compartment. CD26, an exopeptidase known to be highly expressed at the apical membrane of enterocytes, was highly expressed on exosomes. The presence of CD26 on exosomes, mainly apical exosomes, is not really surprising when one considers that this molecule undergoes a complex intracellular traffic. After synthesis, it is addressed both to the basolateral and apical membranes, but basolaterally bound molecules are immediately transcytosed back to the apical surface where they accumulate (4). This intense traffic may explain why CD26 is found in exosomes. Expression of MHC class II molecules in exosomes was detected in inflammatory conditions and may be implicated in their function. Other molecules present in both apical and basolateral exosomes were mainly proteins of the cytoskeleton (actin, tubulin) or enzymes involved in intracellular metabolism or exosome biogenesis (kinases, dehydrogenases, enolase) that may be trapped in the exosomal lumen during their formation. In contrast,
some cytoplasmic or membrane proteins are specifically expressed in either apical or basolateral exosomes, suggesting distinct secretory pathways. Apical exosomes contained proteins usually localized inside or underneath the apical membrane of polarized epithelial cells. Some of these proteins are transported to the apical membrane, where they are inserted (dipeptidyl peptidase IV) or GPI-anchored (microsomal dipeptidase); others may promote the apical addressing of endosomes (syntaxin 3, syntaxin binding protein 2). Distinct proteins, e.g., A33 antigen and the epithelial cell surface antigen, were exclusively found in the basolateral exosomes. Their function is unknown, but they could be implicated in cell-cell or cell-matrix interaction (19).

HYPOTHETICAL FUNCTION OF EPITHELIAL-DERIVED EXOSOMES

Because the intestinal epithelium can be considered a relatively static structure in the body, despite a rapid renewal, it is tempting to hypothesize that exosomes released from this structure may be part of the link between antigens in the intestinal lumen and cells of the immune system in the lamina propria or more distally. In normal conditions, exosomes derived from intestinal epithelial cell lines express low levels of MHC class I and no class II molecules, which are strongly upregulated in inflammatory conditions. Their external position on the vesicles indicates an appropriate orientation for antigen presentation, and the presence of SDS stable MHC class II α/β-dimers strongly suggest that exosomes carry MHC class II molecules loaded with peptide (18). In addition, the presence of CD63 indicates that exosomes are derived from late endosomal compartments where exogenous antigens and MHC class II molecules are colocalized (25). The expression of A33 antigen, a receptor-like molecule of the immunoglobulin superfamily, on basolaterally released exosomes is intriguing, and the implication of this molecule in antigen presentation deserves further investigation. Basolaterally released exosomes may allow antigen presentation through direct interactions with T cells or through their uptake by professional APCs, as hypothesized for dendritic cell-derived exosomes (26). The latter possibility is supported by the observation that MHC class II-positive, B cell-derived exosomes were found attached to the cell surface of follicular dendritic cells (6).

One of the important questions concerns the outcome of this potential antigen presentation through epithelial exosomes. Generally, the intestine is recognized as a tolerogenic environment as far as soluble antigens are concerned. It is important to stress that in the intestinal environment, dendritic cells are normally tolerogenic, due to the downregulation of costimulatory molecules. The concept that exosomes might be tolerogenic is strengthened by studies on liposomes with incorporated MHC II/peptide complexes. In the absence of costimulatory molecules, MHC II-bearing liposomes lead to anergy of CD4+ T lymphocytes, but in the presence of professional APCs providing costimulation, MHC II/liposomes gain the capacity to activate T cells (24). It is therefore, possible that epithelial exosomes, according to their composition (presence of MHC class II molecules but apparent lack of costimulatory molecules) may be involved in this tolerogenic process. Indeed, in a recent study, exosomelike structures named tolerosomes isolated from an in vitro antigen-pulsed rat IEC line were shown to induce antigen-specific tolerance when administered intraperitoneally in naive recipient rats. According to the authors, this tolerance, which was also induced by tolerosomes isolated from rat serum shortly after antigen feeding, may depend on the presence of MHC class II molecules on these structures (13).

Whereas basolaterally released exosomes are likely to interfere with immune cells in the intestinal mucosa, the role of apically released exosomes is more questionable inasmuch as MHC class I and II molecules are present on these vesicles (14, 20). One possibility would be that the apical exosome may serve a pathway of recycling for infective viral particles from the IEC back to the intestinal lumen. Such a possibility does exist, because recent data have shown that Epstein-Barr virus (EBV)-encoded latent membrane protein 1, found on EBV-positive cell lines, is released in exosomes (7). Moreover, most viruses have been shown to be released from the apical side of epithelial cells (3). The presence of syntaxin 3, a molecule necessary for the efficient delivery or secretion of proteins to the apical surface of intestinal cells, on apical exosomes further sustains this hypothesis.

The concept of released cell compartments as functional entities is a new emerging field that deserves further consideration. Future studies will aim to strengthen the characterization of the epithelial-derived exosomes, to better define their biogenesis, and to delineate their role in the modulation of mucosal and systemic immune response.

REFERENCES

nosuppressive effects of EBV-encoded latent membrane protein


