TRANSLATIONAL REVIEW

Extracellular Vesicles: New Players in Lung Immunity

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Abstract

Extracellular vesicles (EVs), such as exosomes and microvesicles, play an important autocrine/paracrine role in intercellular communication. Details on the involvement of EVs in the pathogenesis of lung diseases have emerged over the past several years. Moreover, EVs package numerous DNA, proteins, mRNAs, and microRNAs that can regulate immune responses in recipient cells. Almost all respiratory cells release EVs, and these EVs can have protective or detrimental functions, depending on the type of donor cells, type of stimuli, and components. In lung cancer, tumor-derived EVs carry multiple immunoinhibitory signals, disable antitumor immune effector cells, and promote tumor escape from immune control. Furthermore, bacteria- and microbiota-derived EVs can shape the immune system and lead to the development of lung disease. These EVs are capable of maintaining airway homeostasis, inducing proinflammatory effects, and promoting antigen presentation, thus regulating lung inflammation and immune responses. From these viewpoints, we summarize recent findings on EVs in lung biology and immunity. EVs provide a new avenue for understanding the mechanism of inflammatory disease progression and for developing therapeutic approaches for lung immune responses.

Cell–cell communication is crucial for all multicellular organisms and can be mediated through direct cell–cell contact or the transfer of secreted molecules. Currently, novel mechanisms for intercellular communication have emerged that involve the transfer of extracellular vesicles (EVs). In general, EVs are classified on the basis of their approximate size, origin, and cargo. The three major subclasses of EVs include apoptotic bodies, exosomes, and microvesicles (1). Although exosomes and microvesicles are structurally similar, they differ in cellular origin, lipid composition, and size. Exosomes are 50–150 nm in diameter and are generated from the endosome. Exosomes have an evolutionarily conserved set of proteins, including tetraspanins (CD81, CD63, and CD9), major histocompatibility complex (MHC) classes I and II, Alix, and Tsg101. So far, on one hand, it is difficult to identify the origin of the exosomes through the specific protein set of the donor cell because exosomes are derived from the endosomal pathway (2). On the other hand, microvesicles are formed by outward budding and fission of the plasma membrane. Microvesicles, also called microparticles or ectosomes, have a particle diameter of 100–2,000 nm (1). The lipid composition of microvesicles is similar to that of the cell membrane, but it lacks the asymmetric distribution of lipids typically observed across the two leaflets of the plasma membrane. Therefore, the membrane composition of microvesicles reflects that of the donor cell more closely than does the membrane composition of exosomes (2). Although the origins of these vesicles have been defined, current technologies cannot clearly distinguish between the different types of EVs. Isolating specific vesicles with reliable quality and at substantial concentrations is still a major challenge in this field (3, 4).

The significance of EVs lies in their capacity to transfer information to other cells, thereby influencing recipient cell function. A major breakthrough in EV research involved the identification of EVs' nucleic acid contents, such as mRNAs, small noncoding microRNAs (miRs), and long noncoding RNAs, which are transported to recipient cells (5). EVs have been isolated from most bodily fluids, such as blood, urine, BAL fluid (BALF), and saliva, and it is increasingly evident that EVs play a key role not only in the regulation of normal physiological

(Received in original form August 22, 2017; accepted in final form November 8, 2017)

Supported by the Japan Society for the Promotion of Science (Y.F.); GSK Japan research grant 2016 (T.K.); the Satoshi Okamoto Memorial Foundation of Pulmonary Fibrosis (J.A.); the Practical Research Project for Rare/Intractable Diseases, Japan Agency for Medical Research and Development (AMED); and a grant from the Ministry of Health, Labour and Welfare of Japan awarded to the Study Group on Diffuse Pulmonary Disorders, Scientific Research/Research on intractable diseases (K.K.).

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Am J Respir Cell Mol Biol Vol 58, Iss 5, pp 560–565, May 2018

Originally Published in Press as DOI: 10.1165/rcmb.2017-0293TR on November 8, 2017
Internet address: www.atsjournals.org

processes but also in disease pathogenesis, including the regulation of inflammation and immune responses. On the basis of these findings, EVs serve as promising candidates for intercellular communication in the airway microenvironment (6). EVs contribute to the production of various proinflammatory mediators by respiratory cells and immune cells, thus potentially serving as key factors in the airway inflammatory process and lung immunity.

Recently, it was determined that the microbiota is essential for the development of immune responses and airway homeostasis (7). Furthermore, dysbiosis and pathogen colonization have been linked to alterations in immune responses and disease development in the lungs (8). Notably, it was determined that EVs derived from pathogens, including bacteria, transfer their contents to mammalian cells and modulate host innate immunity (9). In this review, we provide an updated overview of the EVs released by host respiratory cells or bacteria, highlighting their potential for immune regulation in various lung inflammatory diseases.

EVs in Lung Inflammation and Immunity

It was first reported in 2003 that lung-derived exosomes are present in healthy human BALF and express CD86, intercellular adhesion molecule 1, and MHC classes I and II molecules (10). The presence of MHCs and costimulatory molecules suggests that exosomes can be released from antigen-presenting cells and that they have the potential for immunomodulatory effects. EVs form a heterogeneous population in BALF because various types of respiratory cells, such as epithelial cells, fibroblasts, endothelial cells, cancer cells, stem cells, and various resident immune cells, in the lungs release EVs (Figure 1). Among them, lung epithelial cells and alveolar macrophages are the key cellular players that are responsible for immune and inflammatory responses at the alveolar surface (11, 12). To date, some evidence has suggested that lung-derived EVs, especially those derived from lung epithelial cells or alveolar macrophages, appear to play a key role in intercellular communication within the airway and lung parenchyma. Indeed, Kulshreshtha and colleagues reported that lung epithelial cells are the main source of exosomes in an asthmatic lung model after IL-13 induction (13). It is important to consider that the main sources of lung-derived EVs can differ in healthy or inflammatory conditions.

Lung epithelial cell–derived exosomes contain a range of surface-protective proteins, including epithelial mucins (14). These structures may play a role in innate defense against respiratory pathogens that bind α-2,6–linked sialic acid, such as human influenza virus. Kesimer and colleagues demonstrated that exosomes derived from lung epithelial cells contain membrane-tethered mucins of various sizes that alter the physical properties of the structures and affect their measured size, conformation, and charges (15). Mucins on exosomes modulate specific interactions between vesicles and inhaled insults and/or host cells. Therefore, lung epithelial cell–derived exosomes may be involved in diverse physiological processes in lung immunomodulation and inflammation. Bourdonnay and colleagues reported that alveolar macrophages secrete suppressor of cytokine signaling 1 (SOCS1)-containing exosomes and SOCS3-containing microvesicles that are taken up by alveolar epithelial cells to mediate the inhibition of cytokine-induced signal transducer and activator of transcription activation (16). SOCS proteins are critical brakes in intracellular cytokine signaling for the control of inflammatory and immune responses. Notably, SOCS secretion was diminished in BALF in normal humans and mice exposed to cigarette smoke. These results suggest an important protective role for EV SOCS proteins in lung homeostasis. Another study demonstrated that microvesicles derived from alveolar macrophages also regulate the airway microenvironment through the transfer of exosomal miR-223 to various respiratory cells, including lung epithelial cells, to modulate cellular homeostasis and differentiation (17). These data suggest that lung-derived EVs regulate protective cellular functions and homeostasis of the lungs through cell–cell communications.

In general, environmental and endogenous stressors, such as smoke exposure, DNA damage, infection, and hyperoxia, have been identified as regulators of EV release that can modify EV composition in the lungs. EVs serve as cargo carriers that transmit a variety of stress signals and detrimental function

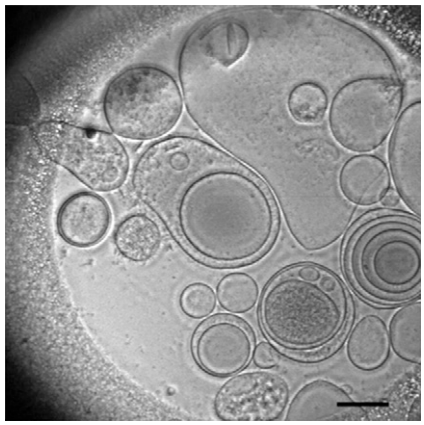

Figure 1. Morphology of purified extracellular vesicles (EVs) from human BAL fluid. A representative phase-contrast transmission electron microscopic image is presented. EVs of various sizes and shapes are observed. These EVs were isolated using a conventional ultracentrifugation method. Scale bar: 200 nm.

in the stress response in the airway microenvironment (18). Stress-induced EVs have the potential to initiate and propagate inflammation, given their ability to transfer proinflammatory molecules in the lungs. Indeed, Soni and colleagues reported that alveolar macrophage and epithelial cells rapidly released microvesicles into alveoli after LPS stimulation (19). These microvesicles carried substantive amounts of TNF. LPS-stimulated microvesicles released within the alveolar space play an important role in initiating lung inflammation during the early phase of acute lung injury (19). Moon and colleagues reported that hyperoxia-associated oxidative stress stimulates EV generation in lung epithelial cells and that epithelial cell–derived EVs prompted the proinflammatory activation of macrophages (20). These effects subsequently resulted in neutrophil infiltration, inflammatory cytokine bursts, and lung inflammation. The EVs encapsulating caspase-3 play a crucial role in mediating inflammatory lung responses and regulate macrophage activation through the Rho-associated coiled-coil kinase 1 pathway (20). Lee and colleagues demonstrated that hyperoxia-associated oxidative stress produced numerous EVs in BALF and culture medium of lung epithelial cells (21). In addition, lung epithelial cell–derived microvesicles containing miR-221/miR-320a triggered alveolar macrophage–mediated proinflammatory effects under hypoxic conditions (21).

On the basis of these findings, stress-induced EVs may be a detrimental signal in triggering the immune response, eventually resulting in the pathogenesis of chronic lung inflammatory diseases (Figure 2). Indeed, researchers in many studies have reported that lung-derived EVs are associated with chronic obstructive pulmonary disease (COPD) and asthma pathogenesis (22, 23). For instance, our recent studies demonstrated that cigarette smoke extract–induced human bronchial epithelial cell–derived EVs can promote airway fibrosis in COPD pathogenesis through exosomal miR-210–mediated insufficient autophagy (24). Furthermore, Haj-Salem and colleagues reported that exosomes released from bronchial fibroblasts derived from patients with severe eosinophilic asthma promoted epithelial cell proliferation, which is an established feature of epithelium remodeling in severe asthma (25). Taken together, stress-induced, lung-derived EVs are associated with detrimental immune responses and the pathogenesis of chronic lung inflammatory diseases.

Recently, mesenchymal stem cell (MSC)-derived EVs have become an attractive tool for treating various types of acute inflammatory diseases and organ injury models (26). MSC-derived EVs have special functions, including antiapoptotic and antiinflammatory responses, and MSC-based therapy for acute respiratory distress syndrome is currently being evaluated in phase 1 clinical trials (27). To date, some groups have demonstrated the therapeutic effects of MSC-derived EVs in acute lung injury (28–30), pulmonary artery hypertension (31, 32), and asthma (33). Although further investigations are required to explore the therapeutic mechanisms of action of MSC-derived EVs, EVs represent an attractive field of research for treating acute inflammatory lung diseases and are capable of recapitulating the therapeutic effects of MSC-based cell therapy.

EVs in Lung Cancer Immunology

Lung cancer cells secrete EVs that are involved in various cancer malignant phenotypes (34). To date, several lines of evidence indicate that EVs mediate cross-talk between cancer cells and immune cells. An earlier study demonstrated that dendritic cell–derived exosomes prime specific cytotoxic T lymphocytes (CTLs) and activate antitumor immune responses (35). The presence of MHC-I and MHC-II molecules on the surface of dendritic cell–derived exosomes facilitates the direct stimulation of CTLs and $CD4^+$ T cells. On one hand, growing evidence suggests that dendritic cell–derived exosomes are a useful tool for tumor antigen–specific immunity and may exhibit potential utility in cancer immunotherapy (36). On the other hand, recent studies suggested that cancer cells can evade the host immune system by transferring EV nucleic acids and proteins to immune cells. Indeed, cancer cell–derived EVs can carry multiple immunoinhibitory signals, such as Fas ligand, TNF-related apoptosis-inducing ligand, and galactin-9; disable antitumor immune effector cells; and promote immune cell apoptosis or aberrant surveillance (37, 38).

These data suggest that immunologically active lung cancer cell–derived EVs are critical for cancer immunological communication in the tumor microenvironment. Furthermore, recent evidence indicates that specific molecules and signaling pathways in cancer cells regulate antitumor immunity by altering the composition of their EVs. Exosomes derived from Rab27a-overexpressing lung cancer cells elicited more potent antitumor immune effects (39). The improved antitumor efficacy may be due to the enrichment of molecules that contribute to the induction of immune activation, such as Hsp70, Hsp90, and tumor antigens. Gobbo and colleagues reported that Hsp70-enriched exosomes derived from various cancers, including lung cancer, can interact with Toll-like receptor 2 on myeloid-derived suppressive cells, thereby activating these cells and suppressing tumor immunity (40). Moroishi and colleagues reported that EVs from Hippo pathway kinases LATS1/2 (large tumor suppressor 1 and 2)-null tumors induce an antitumor immune response (41). LATS1/2-deficient tumor-derived EVs contain increased amounts of nucleic acids, which stimulate the host Toll-like receptor MYD88/Toll/IL-1 receptor domain–containing adapter-inducing IFN-β nucleic acid–sensing pathways, provoking the type I IFN response to establish robust antitumor immunity. Inhibition of LATS1/2 may enhance tumor immunity in lung cancer through nucleic acid–enriched EV transfer. Cancer immunotherapy represents a new approach to the treatment of patients with non–small cell lung cancer. Therefore, lung cancer cell–derived EVs and their components may constitute crucial targets for cancer immunotherapy.

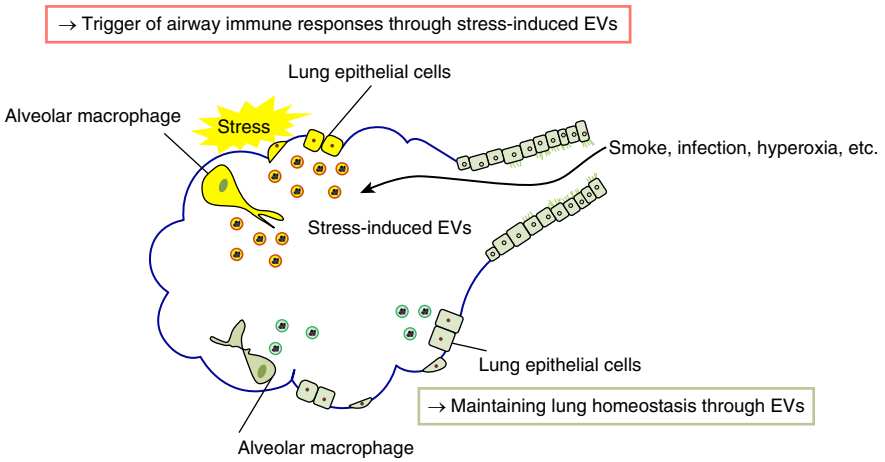

Figure 2. Lung-derived EVs under normal or stress conditions. EVs derived from respiratory cells, especially lung epithelial cells or alveolar macrophages, regulate lung physiological cellular function and homeostasis through EV transfer. By contrast, stress-induced EVs derived from these cells may be a crucial signal in the triggering of the immune response, resulting in the development of chronic obstructive pulmonary disease and asthma pathogenesis.

EVs Mediate the Interaction between Microbiota and the Host Lung Immune Response

EVs derived from bacteria can transmit virulence factors into host cells and enhance the immune dysfunction and lung

inflammation caused by the bacteria (42). Gram-negative bacteria–derived EVs, named *outer membrane vesicles* (OMVs), contain a variety of molecules, including LPS from the bacterial outer membrane, proteins mediating adhesion and invasion to host cells, immunomodulatory factors, and nucleic acids (43). To date, the EVs produced by bacteria have been shown to induce neutrophilic pulmonary inflammation, which is involved in COPD pathogenesis (44). Park and colleagues reported that the entry of EVs derived from *Escherichia coli* into the bloodstream induced systemic inflammation mimicking sepsis (45). The same group reported that *Staphylococcus aureus*–derived EVs were associated with the pathogenesis of neutrophilic pulmonary inflammation (46). In addition, the inhalation of indoor dust EVs, especially those derived from gram-negative bacteria, induces both T-helper cell type 1 (Th1)- and Th17-cell responses and neutrophilic inflammation in the lung, which lead to the pathogenesis of inflammatory airway diseases such as neutrophilic asthma and COPD (47). To better understand the role of EVs produced by bacteria in lung inflammation, the group also clarified that EVs derived from gram-negative bacteria (*E. coli*) induce pulmonary emphysema primarily by IL-17A–mediated neutrophilic inflammation, which is involved in COPD pathogenesis (48). These findings suggest that bacteria-derived EVs may be a novel causative agent of inflammatory lung diseases, but specific contributing components remain largely unknown.

The importance of balance between the different subsets of the microbiota has been described in the development of numerous diseases. Recent studies demonstrated that the gut microbiota affects lung immune responses and disease development (49). Emerging evidence indicates the potential of manipulating the gut microbiota in the treatment of lung diseases (8). In this aspect, gut microbiota–derived EVs might provide new insights into immune responses, given that these EVs move to distant sites and activate immune cells and epithelial cells (50). Kang and colleagues reported that EVs derived from the gut microbiota, especially from *Akkermansia muciniphila*, protect against the progression of dextran sulfate sodium–induced colitis (51). Some studies have demonstrated that normal gut microbiota–derived EVs or OMVs regulate immune effects and intestinal homeostasis in the gut (52). Furthermore, gut microbiota–derived EVs enter the systemic circulation and can be delivered to different organs, resulting in a variety of immunological and metabolic responses. For instance, EVs derived from the gut microbiota, especially those from *Pseudomonas panacis*, induce insulin resistance, thereby impairing glucose metabolism in skeletal muscle (53). Although the gut–lung axis is only beginning to be understood, these data suggest that gut microbiota–derived EVs may be key communication messengers between the gut microbiota and host lung immune responses through lateral EV component transfer.

However, the lung microbiota can shape airway immune responses, resulting in the modulation of lung homeostasis and inflammation. Culture-independent techniques have demonstrated that a large number of microorganisms coexist in the lung (54). Notably, the presence of lung microbial dysbiosis in inflammatory lung diseases such as COPD and asthma suggests that the lung microbiota composition might orchestrate the immune system and promote disease development (49). EVs may be the key players that link the lung microbiota to inflammatory lung diseases by regulating immune responses. Kim and colleagues analyzed the lung microbiota from COPD lung tissue and EVs derived from COPD lung tissue (55). Bacterially derived EVs exhibited distinctive characteristics in the lungs of nonsmokers, healthy smokers, and patients with COPD. Furthermore, the EV microbiota was distinct from the entire lung tissue microbiota in terms of operational taxonomic units, biodiversity, principal component analysis clustering, and dominant organisms. The type of specimen (lung tissue or lung EVs) used for microbiota analyses had a greater effect on principal component analysis proximity than the patient group (55). Although the underlying functions of lung microbiota–derived EVs are only beginning to be unraveled, these vesicles might regulate airway immune response and inflammatory lung disease pathogenesis (56). On the basis of the findings we report, EVs produced by lung and gut microbes may activate lung epithelial and immune cells. EVs produced by commensal bacteria can benefit the host

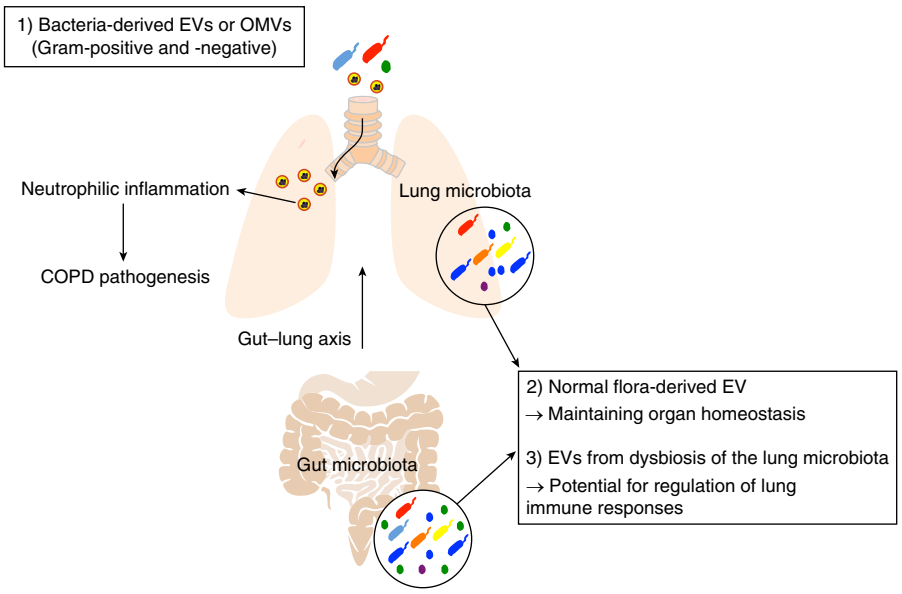

Figure 3. Microbiota-derived EVs and the host lung immune response. We propose that the interaction between the microbiota and the host lung immune responses mediated by EVs has the potential to increase understanding of fundamental lung immune responses. *1*) Bacteria-derived EVs or outer membrane vesicles (OMVs) induce neutrophilic inflammation and the development of chronic obstructive pulmonary disease (COPD). Furthermore, EVs produced by lung and gut microbes may activate lung immune responses. *2*) EVs produced by normal flora promote mucosal tolerance and protect against the onset of inflammatory diseases. *3*) Conversely, EVs derived from dysbiosis of the microbiota have the potential to activate lung immune responses.

by promoting mucosal tolerance and protecting against the onset of lung diseases (56). In contrast, EVs derived from dysbiosis of the lung microbiota can induce activated lung immune responses and inflammatory lung disease pathogenesis. This field will be expanding in the near future (Figure 3).

Conclusions

Emerging evidence suggests that EVs are central to the biological mechanisms underlying the development of lung inflammatory diseases. Lung-derived EVs regulate protective cellular function and homeostasis of the lungs through EV transfer. Various stress-induced EVs, especially those derived from lung epithelial cells and alveolar macrophages, may be a detrimental signal in triggering the immune response and may be involved in the development of COPD and asthma. In addition, understanding how EV communication enables cancer immune escape may provide a potential breakthrough in immunotherapy against lung cancer. We further report that the interaction between bacteria and host lung immune responses through EVs has the potential to increase understanding of fundamental inflammatory processes in the lungs. Further studies of lung-derived or bacteria-derived EVs and their functions in lung immunity are warranted. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol* 2013;200:373–383.
2. Andaloussi SEL, Mäger I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discov* 2013;12:347–357.
3. Gould SJ, Raposo G. As we wait: coping with an imperfect nomenclature for extracellular vesicles. *J Extracell Vesicles* 2013; 2:20389.
4. Yáñez-Mó M, Siljander PR, Andreu Z, Zavec AB, Borràs FE, Buzas EI, *et al*. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 2015;4:27066.
5. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007;9:654–659.
6. Fujita Y, Kosaka N, Araya J, Kuwano K, Ochiya T. Extracellular vesicles in lung microenvironment and pathogenesis. *Trends Mol Med* 2015;21:533–542.
7. Dickson RP, Erb-Downward JR, Huffnagle GB. Homeostasis and its disruption in the lung microbiome. *Am J Physiol Lung Cell Mol Physiol* 2015;309:L1047–L1055.
8. Budden KF, Gellatly SL, Wood DL, Cooper MA, Morrison M, Hugenholtz P, *et al*. Emerging pathogenic links between microbiota and the gut–lung axis. *Nat Rev Microbiol* 2017;15:55–63.
9. Schorey JS, Cheng Y, Singh PP, Smith VL. Exosomes and other extracellular vesicles in host–pathogen interactions. *EMBO Rep* 2015;16:24–43.
10. Admyre C, Grunewald J, Thyberg J, Gripenbäck S, Tornling G, Eklund A, *et al*. Exosomes with major histocompatibility complex class II and co-stimulatory molecules are present in human BAL fluid. *Eur Respir J* 2003;22:578–583.
11. Hiemstra PS, McCray PB Jr, Bals R. The innate immune function of airway epithelial cells in inflammatory lung disease. *Eur Respir J* 2015;45:1150–1162.
12. Whitsett JA, Alenghat T. Respiratory epithelial cells orchestrate pulmonary innate immunity. *Nat Immunol* 2015;16:27–35.
13. Kulshreshtha A, Ahmad T, Agrawal A, Ghosh B. Proinflammatory role of epithelial cell-derived exosomes in allergic airway inflammation. *J Allergy Clin Immunol* 2013;131:1194–1203.e14.
14. Kesimer M, Scull M, Brighton B, DeMaria G, Burns K, O'Neal W, *et al*. Characterization of exosome-like vesicles released from human tracheobronchial ciliated epithelium: a possible role in innate defense. *FASEB J* 2009;23:1858–1868.
15. Kesimer M, Gupta R. Physical characterization and profiling of airway epithelial derived exosomes using light scattering. *Methods* 2015; 87:59–63.
16. Bourdonnay E, Zasłona Z, Penke LR, Speth JM, Schneider DJ, Przybranowski S, *et al*. Transcellular delivery of vesicular SOCS proteins from macrophages to epithelial cells blunts inflammatory signaling. *J Exp Med* 2015;212:729–742.
17. Ismail N, Wang Y, Dakhlallah D, Moldovan L, Agarwal K, Batte K, *et al*. Macrophage microvesicles induce macrophage differentiation and miR-223 transfer. *Blood* 2013;121:984–995.
18. Beninson LA, Fleshner M. Exosomes: an emerging factor in stress-induced immunomodulation. *Semin Immunol* 2014;26:394–401.
19. Soni S, Wilson MR, O'Dea KP, Yoshida M, Katbeh U, Woods SJ, *et al*. Alveolar macrophage-derived microvesicles mediate acute lung injury. *Thorax* 2016;71:1020–1029.
20. Moon HG, Cao Y, Yang J, Lee JH, Choi HS, Jin Y. Lung epithelial cell-derived extracellular vesicles activate macrophage-mediated inflammatory responses via ROCK1 pathway. *Cell Death Dis* 2015;6: e2016.
21. Lee H, Zhang D, Zhu Z, Dela Cruz CS, Jin Y. Epithelial cell-derived microvesicles activate macrophages and promote inflammation via microvesicle-containing microRNAs. *Sci Rep* 2016;6:35250.
22. Fujita Y, Yoshioka Y, Ito S, Araya J, Kuwano K, Ochiya T. Intercellular communication by extracellular vesicles and their microRNAs in asthma. *Clin Ther* 2014;36:873–881.
23. Kadota T, Fujita Y, Yoshioka Y, Araya J, Kuwano K, Ochiya T. Extracellular vesicles in chronic obstructive pulmonary disease. *Int J Mol Sci* 2016;17:1801.
24. Fujita Y, Araya J, Ito S, Kobayashi K, Kosaka N, Yoshioka Y, *et al*. Suppression of autophagy by extracellular vesicles promotes myofibroblast differentiation in COPD pathogenesis. *J Extracell Vesicles* 2015;4:28388.
25. Haj-Salem I, Plante S, Gounni AS, Rouabhia M, Chakir J. Fibroblast-derived exosomes promote epithelial cell proliferation through TGF-β2 signalling pathway in severe asthma. *Allergy* 2018;73: 178–186.
26. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. *Mol Ther* 2015;23:812–823.
27. Wilson JG, Liu KD, Zhuo H, Caballero L, McMillan M, Fang X, *et al*. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. *Lancet Respir Med* 2015;3:24–32.
28. Zhu YG, Feng XM, Abbott J, Fang XH, Hao Q, Monsel A, *et al*. Human mesenchymal stem cell microvesicles for treatment of *Escherichia coli* endotoxin-induced acute lung injury in mice. *Stem Cells* 2014;32: 116–125.
29. Monsel A, Zhu YG, Gennai S, Hao Q, Hu S, Rouby JJ, *et al*. Therapeutic effects of human mesenchymal stem cell-derived microvesicles in severe pneumonia in mice. *Am J Respir Crit Care Med* 2015;192:324–336.
30. Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, *et al*. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat Commun* 2015;6:8472.
31. Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, *et al*. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. *Circulation* 2012; 126:2601–2611.
32. Chen JY, An R, Liu ZJ, Wang JJ, Chen SZ, Hong MM, *et al*. Therapeutic effects of mesenchymal stem cell-derived microvesicles on pulmonary arterial hypertension in rats. *Acta Pharmacol Sin* 2014; 35:1121–1128.

33. Cruz FF, Borg ZD, Goodwin M, Sokocevic D, Wagner DE, Coffey A, *et al*. Systemic administration of human bone marrow-derived mesenchymal stromal cell extracellular vesicles ameliorates *Aspergillus* hyphal extract-induced allergic airway inflammation in immunocompetent mice. *Stem Cells Transl Med* 2015;4: 1302–1316.
34. Fujita Y, Kuwano K, Ochiya T, Takeshita F. The impact of extracellular vesicle-encapsulated circulating microRNAs in lung cancer research. *Biomed Res Int* 2014;2014:486413.
35. Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, *et al*. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med* 1998; 4:594–600.
36. Pitt JM, André F, Amigorena S, Soria JC, Eggermont A, Kroemer G, *et al*. Dendritic cell-derived exosomes for cancer therapy. *J Clin Invest* 2016;126:1224–1232.
37. Taylor DD, Gerçel-Taylor C, Lyons KS, Stanson J, Whiteside TL. T-cell apoptosis and suppression of T-cell receptor/CD3-ζ by Fas ligand-containing membrane vesicles shed from ovarian tumors. *Clin Cancer Res* 2003;9:5113–5119.
38. Kosaka N, Yoshioka Y, Fujita Y, Ochiya T. Versatile roles of extracellular vesicles in cancer. *J Clin Invest* 2016;126:1163–1172.
39. Li W, Mu D, Tian F, Hu Y, Jiang T, Han Y, *et al*. Exosomes derived from Rab27a-overexpressing tumor cells elicit efficient induction of antitumor immunity. *Mol Med Rep* 2013;8:1876–1882.
40. Gobbo J, Marcion G, Cordonnier M, Dias AMM, Pernet N, Hammann A, *et al*. Restoring anticancer immune response by targeting tumor-derived exosomes with a HSP70 peptide aptamer. *J Natl Cancer Inst* 2015;108:djv330.
41. Moroishi T, Hayashi T, Pan WW, Fujita Y, Holt MV, *et al*. The Hippo pathway kinases LATS1/2 suppress cancer immunity. *Cell* 2016;167: 1525–1539.e17.
42. Kaparakis-Liaskos M, Ferrero RL. Immune modulation by bacterial outer membrane vesicles. *Nat Rev Immunol* 2015;15:375–387.
43. Lee EY, Bang JY, Park GW, Choi DS, Kang JS, Kim HJ, *et al*. Global proteomic profiling of native outer membrane vesicles derived from *Escherichia coli*. *Proteomics* 2007;7:3143–3153.
44. Kim JH, Lee J, Park J, Gho YS. Gram-negative and Gram-positive bacterial extracellular vesicles. *Semin Cell Dev Biol* 2015;40:97–104.
45. Park KS, Choi KH, Kim YS, Hong BS, Kim OY, Kim JH, *et al*. Outer membrane vesicles derived from *Escherichia coli* induce systemic inflammatory response syndrome. *PLoS One* 2010;5:e11334.
46. Kim MR, Hong SW, Choi EB, Lee WH, Kim YS, Jeon SG, *et al*. *Staphylococcus aureus*-derived extracellular vesicles induce neutrophilic pulmonary inflammation via both Th1 and Th17 cell responses. *Allergy* 2012;67:1271–1281.
47. Kim YS, Choi EJ, Lee WH, Choi SJ, Roh TY, Park J, *et al*. Extracellular vesicles, especially derived from Gram-negative bacteria, in indoor dust induce neutrophilic pulmonary inflammation associated with both Th1 and Th17 cell responses. *Clin Exp Allergy* 2013;43: 443–454.
48. Kim YS, Lee WH, Choi EJ, Choi JP, Heo YJ, Gho YS, *et al*. Extracellular vesicles derived from Gram-negative bacteria, such as *Escherichia coli*, induce emphysema mainly via IL-17A-mediated neutrophilic inflammation. *J Immunol* 2015;194:3361–3368.
49. Shukla SD, Budden KF, Neal R, Hansbro PM. Microbiome effects on immunity, health and disease in the lung. *Clin Transl Immunology* 2017;6:e133.
50. Romano M. Gut microbiota as a trigger of accelerated directional adaptive evolution: acquisition of herbivory in the context of extracellular vesicles, microRNAs and inter-kingdom crosstalk. *Front Microbiol* 2017;8:721.
51. Kang CS, Ban M, Choi EJ, Moon HG, Jeon JS, Kim DK, *et al*. Extracellular vesicles derived from gut microbiota, especially *Akkermansia muciniphila*, protect the progression of dextran sulfate sodium-induced colitis. *PLoS One* 2013;8:e76520.
52. Muraca M, Putignani L, Fierabracci A, Teti A, Perilongo G. Gut microbiota-derived outer membrane vesicles: under-recognized major players in health and disease? *Discov Med* 2015;19: 343–348.
53. Choi Y, Kwon Y, Kim DK, Jeon J, Jang SC, Wang T, *et al*. Gut microbe-derived extracellular vesicles induce insulin resistance, thereby impairing glucose metabolism in skeletal muscle. *Sci Rep* 2015;5:15878.
54. Faner R, Sibila O, Agustí A, Bernasconi E, Chalmers JD, Huffnagle GB, *et al*. The microbiome in respiratory medicine: current challenges and future perspectives. *Eur Respir J* 2017;49:1602086.
55. Kim HJ, Kim YS, Kim KH, Choi JP, Kim YK, Yun S, *et al*. The microbiome of the lung and its extracellular vesicles in nonsmokers, healthy smokers and COPD patients. *Exp Mol Med* 2017;49:e316.
56. Choi Y, Park H, Park HS, Kim YK. Extracellular vesicles, a key mediator to link environmental microbiota to airway immunity. *Allergy Asthma Immunol Res* 2017;9:101–106.