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Role of extracellular vesicles in cell-cell communication and inflammation following exposure to pulmonary toxicants

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Abstract

Extracellular vesicles (EVs) have emerged as key regulators of cell-cell communication during inflammatory responses to lung injury induced by diverse pulmonary toxicants including cigarette smoke, air pollutants, hyperoxia, acids and endotoxin. Many lung cell types, including epithelial cells and endothelial cells, as well as infiltrating macrophages generate EVs. EVs appear to function by transporting cargo to recipient cells that, in most instances, promotes their inflammatory activity. Biologically active cargo transported by EVs include miRNAs, cytokines/chemokines, damage-associated molecular patterns (DAMPs), tissue factor (TF)s and caspases. Findings that EVs are taken up by target cells such as macrophages, and that this leads to increased proinflammatory functioning provide support for their role in the development of pathologies associated with toxicant exposure. Understanding the nature of EVs responding to toxic exposures and their cargo may lead to the development of novel therapeutic approaches to mitigating lung injury.

Keywords

macrophages; inflammation; epithelial cells; ozone; cigarette smoke; LPS

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Conflict of interest

The authors declare no conflicts of interest

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1. Introduction

Because the respiratory track is continuously exposed to the external environment, it is highly sensitive to the adverse effects of inhaled gases, acid aerosols, particles, and pathogens. The lung is also sensitive to systemically administered drugs and other agents, as well as radiation. Exposure to toxic levels of these xenobiotics results in acute lung injury. This can progress to chronic lung disease depending on the nature of the agent, the dose, and duration of exposure. A characteristic response to lung injury is an accumulation of inflammatory cells including neutrophils and macrophages in the tissue. These cells, which are largely derived from blood and bone marrow precursors, function to rid the body of foreign materials and debris and restore normal tissue structure and function. Cell-cell communication between infiltrating neutrophils and macrophages, and parenchymal cells in the lung (e.g., epithelial cells, endothelial cells and fibroblasts) is essential for a successful initiation and resolution of the inflammatory response.

Extracellular vesicles (EVs) have emerged as key players in cell-cell communication during inflammatory responses. EVs are a heterogeneous group of cell-derived membranous structures that are classified as exosomes, microvesicles (MVs), or apoptotic bodies (ABs) according to their mechanism of formation, cargo, and approximate size. Whereas exosomes and MVs are released by healthy cells, apoptotic bodies are generated by cells undergoing programmed cell death. Apoptotic bodies are the largest and most heterogeneous of the EVs, ranging in size from 1–5 μm , and are produced by membrane blebbing during apoptotic disassembly [1]. MVs range in size from 200–500 nm and are formed by the outward budding and fission of the plasma membrane [1–3]. Exosomes are the smallest of the EVs ranging in size from 30 nm–150 nm; they are generated from the endosome and express an evolutionally conserved set of proteins including CD81, CD63, CD9, MHC I and II, Alix, and Tsg101 [4, 5]. The significance of EVs lies in their ability to transfer cargo (e.g., DNA, proteins, lipids, mRNAs and miRNAs) to other cells, thereby influencing recipient cell function. The cargo varies depending on the cellular origin of the EVs and the inflammatory state of the tissue, which changes following exposure to environmental stressors such as cigarette smoke, air pollutants, hyperoxia and other pulmonary toxicants. EVs have been isolated from blood, urine, bronchoalveolar lavage fluid (BAL), and saliva and accumulating evidence suggests that they play a role in both normal physiological processes and in disease pathogenesis by transporting cargo that either exacerbate or attenuate inflammatory responses in recipient cells [6, 7].

Although most cells types within the lung are capable of releasing EVs, the major cellular sources following exposure to pulmonary toxicants are macrophages, epithelial cells, and endothelial cells. Macrophages are located throughout the lung most prominently in the alveolar and interstitial spaces and function as the first line of immune defense against inhaled toxicants. In addition to phagocytizing foreign materials and secreting cytotoxic mediators, resident macrophages play a key role in initiating inflammatory responses in the lung. Inflammatory macrophages have been classified as proinflammatory and anti-inflammatory. These macrophage subpopulations sequentially appear in the lung after exposure to toxicants, consistent with initiation and resolution of inflammation. EVs are

released by both macrophage cell types in response to injury; cargo they carry has been shown to target epithelial and endothelial cells in the lung (Figure 1).

Epithelial cells in the respiratory tract are increasingly recognized to be active effectors contributing to both innate and adaptive immune responses. Together with alveolar macrophages, they function as immune sentinels, responding to inhaled pathogens and other foreign materials by upregulating pattern recognition receptors, initiating defensive signaling cascades, recruiting and activating leukocyte-mediated defenses, killing microbes, and restoring host homeostasis [8]. In the lower respiratory tract, two types of epithelial cells have been identified; type I cells which provide a gas exchange surface and are highly sensitive to inhaled toxicants, and type II cells which synthesize and release surfactants and replace type I cells following lung injury. Evidence suggests that type I epithelial cells are major producers of EVs which mainly contribute to the proinflammatory environment of the lung.

Pulmonary endothelial cells line the blood vasculature. In addition to providing a passive surface for gas exchange, they regulate the synthesis and metabolism of vasoactive compounds such as nitric oxide and endothelin-1, which regulate vascular tone. They also play an active role in hemostasis, as well as inflammation [9]. Damage to endothelial cells during stress and injury results in alterations in hemodynamics, permeability, gas exchange, and intercellular signaling. Endothelial cells have been identified as a source of EVs that influence inflammatory macrophage activity and systemic coagulant activity and are themselves targets of EVs derived from epithelial cells and macrophages.

In this review, we provide an overview of the impact of exposure to pulmonary toxicants on EV release from macrophages, epithelial cells and endothelial cells, focusing on cargo carried by MVs and its impact on target cells and disease pathogenesis.

2. Cigarette Smoke

First-hand cigarette smoke is attributed to approximately five million deaths per year in developed countries, while an additional 600,000 deaths are suspected to be caused by second-hand smoke [10]. Cigarette smoke is a complex mixture containing thousands of different chemical species, many of which are highly reactive including carbonyls, nitrosamines, and quinones [11]. The respiratory system is a major target of cigarette smoke where it is known to cause acute and chronic lung injury including pneumonia, chronic obstructive pulmonary disease, and lung cancer [12, 13]. Cigarette smoke-induced lung injury is thought to be caused by oxidative stress, inflammation, and protease-antiprotease imbalance in the lung [11]. The inflammatory response is caused by activation of epithelial cells, endothelial cells and resident macrophages, which release chemotactic molecules and inflammatory mediators that recruit neutrophils, eosinophils, monocytes and lymphocytes to the site of injury [11, 14, 15]. Accumulating evidence suggests that EVs play a key role in both cigarette smoke-induced epithelial cell activation and inflammation [16].

EVs generated following cigarette smoke exposure are derived from pulmonary epithelial cells and macrophages and, to a lesser extent, endothelial cells. Airway epithelial cells are

the first targets of cigarette smoke and have been shown to release increased numbers of EVs after exposure. The increase has been attributed to thiol-reactive compounds present in cigarette smoke [17]. These EVs are largely directed towards proinflammatory/cytotoxic macrophages causing them to upregulate cell adhesion molecules, promoting their migratory and proliferative activity [18, 19]. Cargo that has been identified in epithelial cell-derived EVs following cigarette smoke exposure includes adhesion molecules (e.g., CD11b, ICAM-1, ELAM-1, etc.), cytokines (e.g., IL-6, IL-8, MCP-1, CCN1 family members (e.g., CYR61, CTGF, NOV family1, CD63, CD81, and tissue factor [7, 20, 21]. These proteins stimulate the release of proinflammatory cytokines from macrophages. CCN1 is also thought to promote inflammation by stimulating IL-8 production [3]. EVs identified as CD63 and CD81 positive contain tissue factor which induces activation of inflammatory macrophages by altering the expression of cell surface thiols. Epithelial cells also release EVs containing miR-210, which inhibits macrophage autophagy and reduces fibroblast differentiation into myofibroblasts [7].

Increased numbers of EVs have been identified in the serum of smokers and are thought to primarily originate from pulmonary epithelial cells [22]. These EVs contain high levels of lipoproteins APOLI, LPA APOB, CETP, CLU, SAA4 and coagulation cascade proteins C1QA, C1Qc, C1S, CFB, CLU, and SERPINA5 [22, 23]. Increases in EVs in plasma and serum of humans can modulate coagulant signaling and cardiovascular function, leading to increased risk of cardiovascular disease in smokers.

Cigarette smoke also modifies the release of EVs from macrophages. Whereas proinflammatory macrophages release increased numbers of EVs after cigarette smoke exposure, EV release from anti-inflammatory macrophages is generally suppressed. For example, cigarette smoke stimulates proinflammatory macrophages to release EVs containing tissue factor Xa, a procoagulant molecule that induces expression of coagulation cascade proteins in red blood cells, endothelial cells and epithelial cells [24]. Macrophages also release EVs containing HMGB1 which promotes proinflammatory activation of macrophages in an autocrine and paracrine manner [19]. Following cigarette smoke exposure, macrophages release 3-fold more MMP-14 containing EVs relative to macrophages from nonexposed subjects. This is important as MMP-14 has gelatinolytic and collagenolytic activity and is involved in tissue remodeling and fibrogenesis [25]. In contrast, the number of anti-inflammatory macrophage-derived EVs containing SOCS1 and SOCS3 decreased after cigarette smoke exposure. Under homeostatic conditions, SOCS1 and SOCS3 transported to epithelial cells in EVs reduce inflammatory cytokine signaling. Suppression of anti-inflammatory macrophage release of SOCS-containing EVs prolongs inflammation [18].

Endothelial cell derived EVs released following cigarette smoke exposure are less well-characterized. Endothelial EVs containing miR-191, miR-126 and miR-125a have been identified which promote proinflammatory macrophage activation, as measured by uptake of apoptotic bodies. Another study showed that cigarette smoke increases endothelial EVs containing high levels of spermine in their outer membranes. Spermine activates extracellular calcium-sensing receptors and promotes smooth muscle constriction, contributing to cigarette induced pulmonary hypertension [26]. Overall, the EV mediated

response of cigarette smoke exposure plays a key role in the development of pulmonary inflammation and chronic disease pathologies.

3. Air Pollution

Exposure to ambient air pollution is a significant public health concern worldwide. It is the ninth leading risk factor for mortality and is responsible for 3.2 million deaths each year [27, 28]. Inhalation of air pollutants contributes to acute and chronic cardiopulmonary diseases including asthma, chronic obstructive pulmonary disease, lung cancer and respiratory infection in healthy and susceptible populations [27]. Air pollution is a complex mixture of particulate matter (PM), metals, carbon monoxide, ozone and sulfur and nitrogen oxides [27]. Of the criteria air pollutants, PM and ozone impart the most widespread cardiopulmonary health effects [27].

PM itself is a complex mixture of organic chemicals, metals, acids and soil or dust particles [29]. The health effects of PM are attributed to the composition and size of particles which are classified as PM₁₀, PM_{2.5}, or PM_{0.1} based on their aerodynamic diameter [30]. It has been suggested that PM₁₀ is more pro-inflammatory due to the presence of more soluble and insoluble components including LPS, whereas PM_{2.5} and PM_{0.1} are less inflammatory but cause greater cardiovascular impairment [31]. Nonetheless, PM exposure causes increased oxidative stress and activation of endothelial cells, macrophages, and epithelial cells in the lung leading to the release of pro-inflammatory mediators and local and systemic inflammation [32, 33].

PM exposure in humans has been reported to be associated with increases in EVs in plasma and serum, which modulates coagulant signaling and cardiovascular function [29, 34–37]. In one report, an increase in endothelial cell-derived EVs was noted in serum following episodic exposure to PM_{2.5}, while another study found increases in platelet and red blood cell-derived EVs in individuals chronically exposed to PM₁₀ [35, 36]. Short-term exposure of overweight/obese subjects to PM₁₀ was associated with increased levels of EVs which were mostly derived from CD14⁺ monocytes/macrophages and CD61⁺ platelets [37, 38]. Increased release of EVs from monocytes, endothelial cells, epithelial cells, and macrophages has also been described after *in vitro* exposure to PM [38–40].

EVs released in response to PM exposure are thought to modulate cardiovascular function by transporting EV-bound tissue factor and miRNAs. PM exposure caused increases in EV-bound tissue factor release from mononuclear and endothelial cells *in vitro* [29]. An analysis of serum from humans exposed to PM₁₀ identified increased circulating tissue factor that was presumably bound to EVs of monocyte origin, but no conclusive evidence was presented [35]. Observational studies have indicated strong associations between PM exposure and EV miRNA content. Thus, chronic PM_{2.5} exposure was positively associated with circulating EVs containing miR-199a/b, miR-223-3p, let-7g-5p, miR-126-3p, miR-130a-3p, miR146a-5p, miR-150-5p, miR-191-5p and miR-23a-3p [41, 42]. A positive association between plasma EVs containing miR-203b, miR-200c and miR-30d was also noted after exposure of steel plant workers to PM, which was attributed to the metallic component, whereas another study reported a significant association with miR-128 and

miR-302c [39, 43]. In obese patients, PM exposure was correlated with a down-regulation of EV miRNAs including let-7c-5p, miR-331-3p, miR-185-5p, miR-106-5p and miR-652-3p [37].

A few studies have mechanistically connected EV miRNAs or EV-bound tissue factor content with phenotypic changes in target cells. One study reported that EVs released from THP-1 macrophages and peripheral blood mononuclear cells (PBMC) following exposure to PM_{2.5} *in vitro* caused increased release of IL-6 and TNF α , but reduced release of IL-8 from co-cultured BEAS-2Bs epithelial cells [40]. Most studies correlated changes in miRNA levels with markers of inflammation, coagulation parameters, or to downstream mRNA targets known to be involved in cardiovascular function. In one report, an association was noted between long-term ambient PM_{2.5} levels and EV-derived miRNA-let-7g-5p, miRNA-126-3p, miRNA-130a-3p, miRNA146a-5p, miRNA-150-5p, miRNA-191-5p and miRNA-23a-3p, each of which regulates genes involved with cardiovascular disease, based on linkage analysis using Ingenuity Pathway Analysis [42]. Additionally, a mediation analysis identified five EV miRNAs (let-7c-5p, miR-331-3p, miR-185-5p, miR-106-5p, miR-652-3p) that were predicted to be associated with elevated fibrinogen levels in overweight/obese subjects exposed to PM₁₀ [37]. Occupational exposure of steel plant workers to PM was also positively associated with changes in miRNA-203b, miRNA-200c and miRNA-30d in EVs which were also related to changes in inflammatory markers and coagulation function [43].

Less is known about the potential role of EVs and EV-miRNAs in ozone-induced lung toxicity. A recent study by our group found that EVs recovered from BAL after ozone treatment of mice, contained increased amounts of miRNA cargo, specifically miR-17, miR-92a, and miR199a [44]. Flow cytometric analysis of these EVs indicated that most originated from CD326+ epithelial cells. Importantly, confocal imaging demonstrated that the EVs were actively taken up by alveolar macrophages. Intratracheal administration of EVs collected from ozone-exposed mice to naïve mice resulted in a significant increase in expression of inducible nitric oxide synthase and IL-6 in lung tissue and CXCL-1, CXCL-2 and IL-1 β in alveolar macrophages. The increase in IL-1 β in alveolar macrophages was attributed to EV-mediated transfer of miR-199a-3p [44]. Taken together, these studies revealed a novel mechanism whereby EVs potentiate ozone-induced proinflammatory signaling by transporting miR-199a-3p from lung epithelial cells to alveolar macrophages promoting their proinflammatory activity.

4. Hyperoxia

Hyperoxic acute lung injury is caused by exposure to high concentrations of oxygen (>50%) for an extended period and may occur in response to administration of supplemental oxygen [45]. Hyperoxia generates reactive oxygen species in the lung that induce lipid peroxidation and mitochondrial damage in epithelial cells resulting in impaired alveolar-capillary membrane integrity and an influx of inflammatory cells [45–48]. The mechanisms of intercellular cross talk involved in the pathogenesis of hyperoxia-induced lung inflammation and injury are not well established, but growing evidence points to a role for EVs [6, 49].

Multiple studies have identified increased numbers of EVs in BAL from mice exposed to hyperoxia the majority of which were found to be MVs [6, 49]. This was in contrast to BAL from air-exposed mice which only contained exosomes [6]. Flow cytometric analyses confirmed that the majority of EVs originated from epithelial cells after hyperoxia exposure, while alveolar macrophages were the primary source of EVs in air-exposed mice [6, 49]. Hyperoxia resulted in enrichment of protein and miRNA cargo within EVs including caspases, miR-92a-3p, miR-320a, miR-33a-5p, miR221-3p, miR-145-5p, miR-342-3p, miR-10a-5p and miR-422a [6, 49].

Functional analyses revealed that inhalation of MVs isolated from hyperoxia-exposed mice caused an increase in macrophages in BAL of naïve mice [6]. These results were confirmed *in vitro* where MVs collected from supernatants of hyperoxia-exposed BEAS-2B epithelial cells promoted the migration of THP-1 macrophages and increased their secretion of TNF α and IL-1 β [6]. Subsequent studies showed that the functional changes in macrophages were due to MV-mediated transport of miR-221 and miR-320a [6].

Caspase-3, an essential endoprotease involved in apoptosis and inflammation, has also been identified in epithelial cell-derived MVs following hyperoxia, along with caspase-1, caspase8 and caspase-12 [49, 50]. Caspase-3 enriched EVs were found to regulate macrophage function and augment a pro-inflammatory response by upregulating rho-associated protein kinase 1 (ROCK1) and macrophage inflammatory protein 2 (MIP-2) [49]. Interestingly, epithelial cell derived EVs have also been detected in serum following hyperoxia exposure suggesting systemic effects [49]. Taken together, these data suggest that epithelial-derived EVs released in response to hyperoxia facilitate macrophage activation and inflammation.

5. Acids

Exposure to acids which can occur when acid gastric contents are aspirated under general anesthesia causes injury to the airways and extensive alveolar epithelial damage (4). This is characterized by increased permeability of the alveolar/capillary barrier resulting in alveolar hemorrhage and edema which initiates a predominantly neutrophilic acute inflammatory response that may progress to fibrosis [51–53]. As with other lung injury models associated with inflammation, there is a growing appreciation for the potential role for EVs in mediating intercellular communication contributing to pathophysiological responses.

Using a hydrochloric acid inhalation-induced lung injury model, Lee et al. [54] demonstrated that most MVs released into BAL were derived from epithelial cells and were readily taken up by alveolar macrophages [54]. The epithelial-derived MVs were enriched with miRNAs including miR-17, miR-92a, miR-221 and miR-320a [54]. Functional analyses revealed that miR-17 and miR-221 upregulated integrin β 1, a key protein responsible for activating genes that stimulate monocyte differentiation and mediate cell adhesion in recipient macrophages [54, 55]. Macrophage uptake of MVs was correlated with increases in TNF α , IL-1 β and IL-6 levels in BAL, suggesting a mechanism of pro-inflammatory activation following acid inhalation [54].

6. Lipopolysaccharide

Pulmonary administration of lipopolysaccharide (LPS), also known as endotoxin, is a widely used experimental model of acute lung injury or its more severe form, acute respiratory distress syndrome. LPS binds to the CD14/TLR4/MD2 receptor complex on epithelial cells, macrophages and neutrophils in the lung thereby initiating inflammatory responses [56]. LPS-induced inflammation leads to multiple organ dysfunction consistent with acute lung injury and is thought to be a consequence of epithelial and endothelial damage, production of proinflammatory and cytotoxic mediators and extensive neutrophilic influx into the lungs [57].

LPS-induced lung injury in mice is associated with increases in EVs in BAL that originate from alveolar macrophages [54]. Functionally, alveolar macrophage-derived EVs promote the recruitment of macrophages to the lung, augment their production of cytokines and inflammatory mediators, upregulate TLR expression and contribute to the development of lung inflammation and injury [54]. EVs isolated from the lungs of LPS treated animals have been reported to consist predominantly of apoptotic bodies containing miR-221 and miR-222 which stimulate lung epithelial cell proliferation by modulating cyclin-dependent kinase inhibitor 1B pathways [58].

LPS-induced lung injury can progress to a systemic hyperinflammatory response resulting in sepsis. Sepsis is the leading cause of death in critically ill patients and treatment protocols are not well-defined [59]. Nair et al. [60] reported an increase in macrophage-derived EVs in the blood of LPS-exposed mice that developed sepsis. These EVs contained high concentrations of macrophage histones, which were postulated to directly bind TLR-4 receptors on circulating immune cells contributing to the development of sepsis [60]. Altogether, these studies highlight the importance of EVs and in particular their cargo in LPS-induced acute lung injury and sepsis.

7. Conclusions

The role of EVs in mediating communication between the different cell types in the lung is increasingly being recognized as key in orchestrating inflammatory responses to diverse pulmonary toxicants. The nature of the cargo carried by EVs varies with their cellular origin, the type of inflammatory insult and the recipient cell. In general, it appears that most EV cargo promotes inflammation, tissue injury, and disease pathogenesis. This suggests that proinflammatory EVs offer a potential new target for the development of therapeutics aimed at mitigating the untoward effects of pulmonary toxicants.

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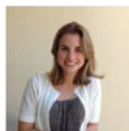
Biography



Jaclynn Andres is currently a graduate student in the Joint Graduate Program in Toxicology at Rutgers University. Prior to beginning her graduate studies, she worked as a technician for the United States Medical Research Institute of Chemical Defense developing medical countermeasures for chemical warfare agents. She also worked as a research technician for Weill Cornell Medicine in Immunology. Her current doctoral research, under the direction of Dr. Debra Laskin, is focused on inflammatory mechanisms of pulmonary injury induced by chemical toxicants.



Ley Cody Smith is a postdoctoral fellow in the Department of Pharmacology and Toxicology in the Ernest Mario School of Pharmacy at Rutgers University. The focus of his research is the role of macrophage subpopulations in chemical-induced lung injury and repair and is performed under the mentorship of Dr. Andrew Gow and Dr. Debra Laskin. Dr. Smith received his master's and doctorate in toxicology from the University of Florida, under the mentorship of Dr. Tara Sabo-Attwood. He is author/co-author of 11 publications. Dr. Smith has served in trainee leadership roles in the Society of Toxicology and the Society of Environmental Toxicology and Chemistry.



Alexa Murray is currently completing her doctoral dissertation in the Joint Graduate Program in Toxicology at Rutgers University. Prior to beginning her graduate studies, she worked as a Research Technician at The Scripps Research Institute in La Jolla, CA in Immunology. Her doctoral research, under the direction of Dr. Debra Laskin, is focused on inflammatory mechanisms of pulmonary injury induced by chemical toxicants. She is active in the Society of Toxicology and has served as student representative for the Immunotoxicology Specialty Section.



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Rita Businaro received her MD and Ph.D. degrees from Sapienza University of Rome with a medical specialization in General Pathology. She completed her postdoctoral training at the Wistar Institute of Anatomy and Biology at the University of Pennsylvania where she studied cancer and immunology. She is currently Professor of Professor of Human Anatomy at the School of Medicine, Sapienza University of Rome and Vice-Chair of I level degree in TSRM, Polo Pontino. She is a member of the Technico-Pedagogical Commission of the Department of Medico-Surgical Sciences and Biotechnologies at Sapienza University, and Vice-President the Italian Fulbright Association. Her research is focused on inflammatory mediators and cellular players in atherosclerosis development and progression, comorbidity of Alzheimer's disease and cerebrovascular disorders, nutraceuticals with anti-inflammatory activity, and the development of new methods for regenerative medicine. She has over 150 publications.



Jeffrey Laskin received his Ph.D. in Experimental Therapeutics from Roswell Park Cancer Institute, the State University of New York at Buffalo, NY. He completed a post-doctoral fellowship at the College of Physicians and Surgeons at Columbia University before joining the faculty of Rutgers University. He is currently a Distinguished Professor in the Rutgers School of Public Health. He is Director of the Division of Toxicology at the Environmental and Occupational Health Sciences Institute (EOHSI) and Deputy Director of the Joint Graduate Program in Toxicology at Rutgers University. He is also Director of the Rutgers University CounterACT Research Center of Excellence, a major research effort to develop the most promising scientific discoveries that lead to improved medical countermeasures to

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Debra Laskin received her Ph.D. degree in Pharmacology and Toxicology from the Medical College of Virginia, Virginia Commonwealth University. She completed her postdoctoral training at the Wistar Institute of Anatomy and Biology at the University of Pennsylvania where she studied carcinogenesis and immunology. She is currently Distinguished Professor and Chair of the Department of Pharmacology and Toxicology and the Roy A. Bowers Endowed Chair at Rutgers University School of Pharmacy. She is Deputy Director of the Rutgers Center for Environmental Exposures and Diseases and a member of the Rutgers Cabinet of the Environmental and Occupational Health Sciences Institute. Her research is focused on nonspecific immunity and inflammation; specifically, the role of activated macrophages and inflammatory mediators in the pathophysiology of xenobiotic-induced tissue injury. She is also studying biochemical and molecular mechanisms underlying macrophage activation and function. She has over 275 publications.

Abbreviations:

ABs	apoptotic bodies
APOB	apolipoprotein B
APOLI	apolipoprotein L1
BAL	bronchoalveolar lavage fluid
C1QA	complement C1q A chain
C1Qc	complement C1q C chain
C1S	complement component
CCN	cellular communication network factor
CETP	cholesterol ester transfer protein
CFB	complement factor B
CLU	clusterin
CTGF	connective tissue growth factor
DAMPs	damage-associated molecular patterns

EVs	extracellular vesicles
HMGB1	high mobility group box 1
ICAM	intracellular adhesion molecule
LPA	lipoprotein A
LPS	lipopolysaccharide
MIP	macrophage inflammatory protein
MMP	matrix metalloproteinase
MVs	microvesicles
NOV	nephroblastoma overexpressed
PBMC	peripheral blood mononuclear cells
PM	particulate matter
ROCK	rho-associated protein kinase
SAA4	serum amyloid A4
SERPINA5	serpin family A member 5
SOCS	suppressor of cytokine signaling
TF	tissue factor
TLR	toll-like receptor
TNF	tumor necrosis factor
Tsg	tumor susceptibility gene

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Highlights

- Extracellular vesicles (EVs) are released from lung macrophages, epithelial cells and endothelial cells
- Exposure to pulmonary toxicants stimulates the release of EVs
- EVs contain miRNA and protein cargo that influences target cell function
- Cell-cell communication via EVs is important in inflammation and tissue injury

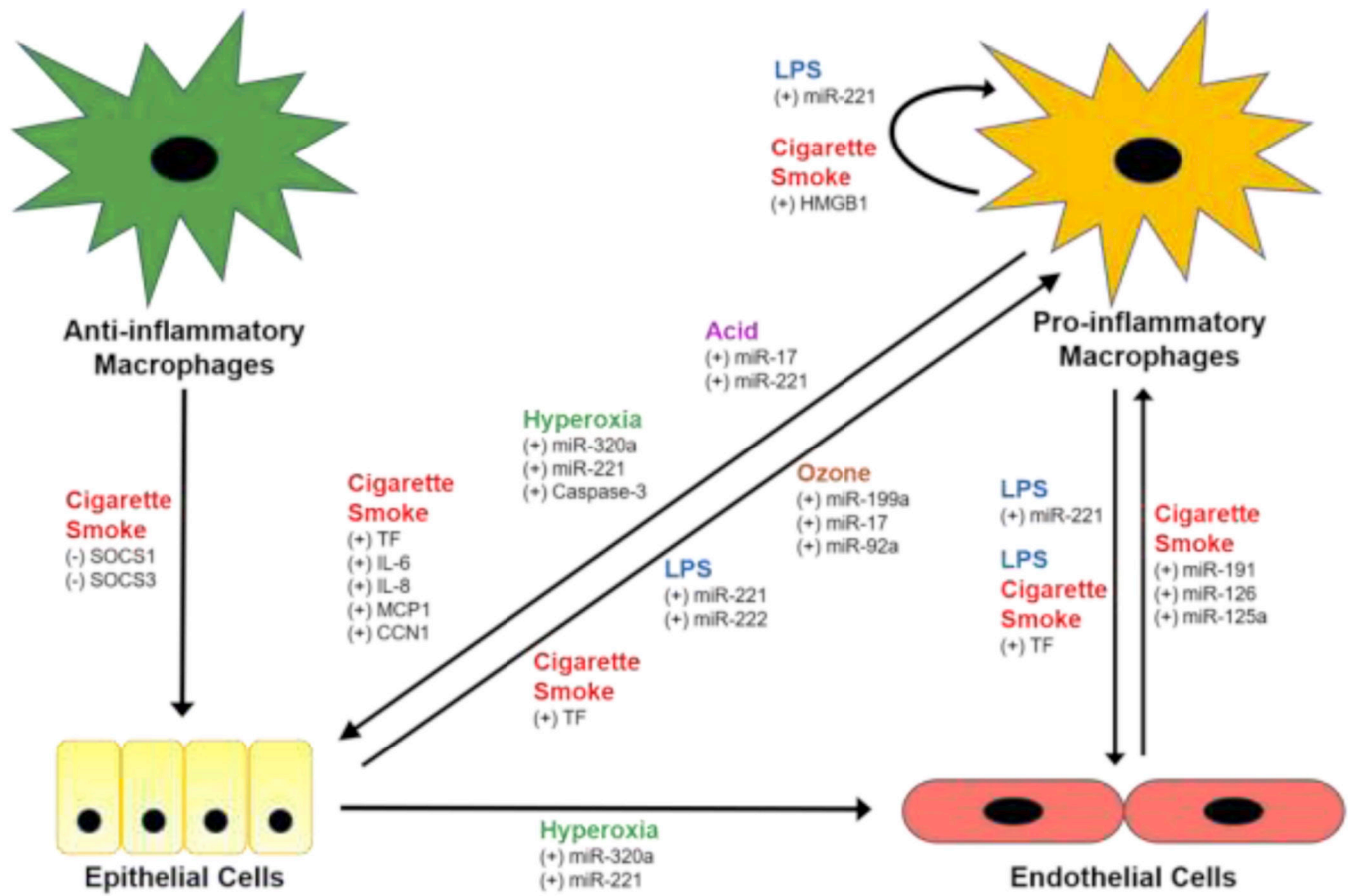


Figure 1.

Cell-cell communication between lung cell types in response to pulmonary toxicants.

Exposure to cigarette smoke, ozone, hyperoxia, acid, and LPS stimulates (+) or inhibits (-) the release of extracellular vesicles (EVs) from anti-inflammatory and pro-inflammatory macrophages, epithelial cells, and endothelial cells in the lung. The EVs transport cargo (e.g., proteins, miRNAs) to recipient cells thereby influencing their function through uni- and bidirectional paracrine and autocrine signaling mechanisms.

Table 1.**Pulmonary Toxicants and Extracellular Vesicles**

ORIGIN	CARGO	TARGET	RESPONSE	REFERENCE
Cigarette Smoke				
Epithelial cells	TF	Proinflammatory macrophages Epithelial cells	Stimulation inflammation	[61]
	CCN1 (CYR61, CTGF, NOV family 1), IL-6, IL-8, MCP-1	Proinflammatory macrophages	Increase IL-8 release and stimulate migration	[20]
Proinflammatory macrophages	TF-Xa	Proinflammatory macrophages	Increase coagulation cascade pathways	[24]
	TF	Proinflammatory macrophages Epithelial cells	Increase inflammation	[61]
Anti-inflammatory macrophages	CCN1 (CYR61, CTGF, NOV family 1), IL-6, IL-8, MCP-1	Proinflammatory macrophages	Increase IL-8 release and stimulate migration	[20]
	SOCS1, SOCS3	Epithelial cells	Decrease cytokine signaling	[18]
Endothelial cells	miR-191, miR-126, miR-125a	Proinflammatory macrophages	Stimulate phagocytosis of Abs	[62]
Air Pollution-PM				
ND	miRNA-203b, miRNA-200c, miRNA-30d	ND	Increase inflammation and coagulation	[43]
Air Pollution - Ozone				
Epithelial cells	miR-199a-3p	Proinflammatory macrophages	Increase IL-1 β release	[44]
Hyperoxia				
Epithelial Cells Proinflammatory macrophages	miR-320a, miR-221	Proinflammatory macrophages Epithelial cells	Increase TNF α and IL-1 β release Increase VEGF release	[6, 49]
	Caspase-3	Proinflammatory macrophages	ROCK1 and MIP-2 dependent activation	
Acid				
Epithelial cells	miR-17, miR-221	Proinflammatory macrophages	Upregulate β 1 and stimulate migration	[54]
LPS				
Epithelial Cells Proinflammatory macrophages	miR-221,	Endothelial cells Proinflammatory macrophages	Stimulate proliferation	[58]

ORIGIN	CARGO	TARGET	RESPONSE	REFERENCE
	miR-222			

Abbreviations. ABs, apoptotic bodies; APOB, apolipoprotein B; BAL, bronchioalveolar lavage fluid; CCN, cellular communication network factor; CTGF, connective tissue growth factor; CYR, cysteine-rich; EVs, extracellular vesicles; HMGB1, high mobility group box 1; IL, interleukin; LPA, lipoprotein A; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; MVs, microvesicles; ND, not determined; NOV, neuroblastoma overexpressed; PM, particulate matter; ROCK, Rho-associated protein kinase; SOCS, suppressor of cytokine signaling; TF, tissue factor; TF-Xa, tissue factor Xa; TLR, toll-like receptor; TNF, tumor necrosis factor

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