Occasional review

Effects of cigarette smoke on epithelial cells of the respiratory tract

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Chronic inhalation of cigarette smoke is associated with mucus hypersecretion, mucus pooling, pulmonary connective tissue damage, and chronic airflow obstruction.

Cigarette smoke has therefore been causally linked to the development of chronic obstructive pulmonary disease (either chronic bronchitis or emphysema),[1] increased airway responsiveness,[2] exacerbations of asthma,[3] impaired pulmonary immune function,[4] and increased pulmonary infections.[5] Cigarette smoke has also been established as an important risk factor for lung,[6] laryngeal,[7] and nasal neoplasia.[8] Pathogenetic mechanisms related to smoke-induced respiratory perturbations, however, are not fully understood.

One cell type in the lung that may play a major part in the pathogenesis of cigarette smoke-induced lesions is the airway epithelial cell. These cells line the lumen of the airways, and thus are in a unique position to interact directly with inhaled cigarette smoke. Most research involving cigarette smoke and airway epithelial cells has focused on the "target" cell responses of these cells in relation to their relatively simple roles in barrier and mucociliary clearance functions. Depending in part on the chronicity of exposure, certain functions may be altered – for example, ciliary beating, mucus secretion. It has recently become apparent, however, that airway epithelial cells may also act as "effector" cells, playing pivotal roles in regulation of airway reflexes, immunological and inflammatory responses, and maintenance of bronchodilation. As part of their overall response to chronic insult these cells are capable of producing and/or releasing a number of inflammatory mediators, or undergoing alterations in expression of cell adhesion molecules – processes that may initiate or perpetuate airway inflammation.[9] To date the influence of cigarette smoke on effector functions of epithelial cells has yet to be investigated in detail.

Much of the information presented herein is based on acute in vitro cigarette smoke exposures of epithelial cell cultures or airway explants, and on relatively acute human or laboratory animal exposures. Thus, non-neoplastic and non-emphysematous end points of respiratory disease have been emphasised. Nevertheless, early events in the response to cigarette smoke or its components may be critical, and certainly an understanding of these events may help to elucidate the pathogenetic mechanisms of many chronic respiratory diseases.

Components of cigarette smoke

Chemical analytical studies have identified over 3800 compounds in tobacco smoke.[10] Mainstream cigarette smoke is composed of a complex mixture of gases and condensed tar particles. In experimental studies cigarette smoke is often separated into two phases by a glass fibre filter that retains nearly all particulate matter greater than 0·1 μm in diameter. The retained particulate matter is commonly referred to as the "tar" phase, while the material passing through the filter is referred to as the "gas" phase. Known toxins and carcinogens have been identified in both the gaseous and particulate phases.[11] Sidestream smoke (smoke emitted from the burning tip of the cigarette) is the major constituent of environmental tobacco smoke. The chemical composition and gas-to-particle associations of environmental tobacco smoke may be different from that of mainstream smoke, owing to prolonged time and cooling in the air.[12] Sidestream cigarette smoke emissions contain carbon monoxide, ammonia, formaldehyde, benzene, nicotine, acrolein, various gases and particles, and an assortment of potentially genotoxic and/or carcinogenic organic compounds.[13] Increased pulmonary particulate burden due to cigarette smoke may also play a part in respiratory disease.[14] Recent epidemiological findings have indicated adverse effects of particulate air pollutants at concentrations below currently permissible levels.[15] Respirable suspended particles in indoor air in homes may increase from approximately 30 μg/m^3 to greater than 60 μg/m^3 due to accumulation of environmental tobacco smoke.[16]

The reported effects of "cigarette smoke" may include that of mainstream smoke, variably aged environmental tobacco smoke/sidestream and exhaled smoke, gaseous phase components only, particulate phase components only, or individual chemical compounds such as acrolein,[17] acetaldehyde,[18] or formaldehyde.[19] Some studies have used aqueous extracts of cigarette smoke obtained by bubbling the smoke through a buffer, with or without filtering to remove suspended particulates,[20] while other researchers have focused on free radical production arising from chemical reactions within the cigarette smoke.[21]

Overall, owing to the variability in experimental methodologies (including the type of "cigarette smoke") used, interstudy comparisons may be difficult to interpret. It is well to keep this in mind when reading this review.

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Component cells of airway epithelium

The airway epithelium is a mosaic of at least eight principal cell types, the exact composition of which may vary depending on the level of the airway and the species of interest. Ciliated epithelial cells and goblet cells form most of the luminal surface of the larger airways, and they are joined by tight junctions to form a relatively impermeable barrier to material from the luminal surface.[22] The tight junctions are also important for polarised secretion and the selective permeability exhibited by airway epithelium. Other epithelial cell types (dependent on the species and airway level) include basal cells, neuroendocrine cells, Clara cells, serous cells, small mucous granule cells, brush cells, and a variety of intermediate cell types.[23] The nasal airways comprise a similar spectrum of cells together with several unique types required for olfaction (olfactory sensory and basal cells and the sustentacular cell).[23] Although the effects of cigarette smoke on nasal airways have been less extensively studied, significant exposure may result from nasal exhalation. Moreover, nasal epithelium is the first respiratory epithelial surface exposed to environmental tobacco smoke.

Airway epithelial surfaces are covered by a thin layer of mucus consisting of sol and gel fractions. In addition, mast cells or occasional lymphocytes are found within the airway epithelium or lumen, although more commonly in the submucosal layer.[22] The epithelium also contains an abundance of nerve fibres whose receptors (irritant receptors, stretch receptors) run circumferentially around the luminal cells and respond to stimuli such as gases, smoke, or antigens.[22] This review will focus on the epithelial cells that line the luminal surfaces of the conducting (non-gas exchange) airways. It should be kept in mind, however, that cigarette smoke-induced alterations in these cells may result in altered function of other airway epithelial components. For example, the cigarette smoke-induced increase in mucosal permeability may also allow increased access of the epithelial nerve receptors and mast cell populations to irritant or antigenic stimuli.[22]

Histological changes in response to cigarette smoke

Laboratory animals exposed to cigarette smoke develop histological changes in both the upper and lower airways (table 1). Hyperplasia and squamous metaplasia of the respiratory epithelium of the dorsal nasal turbinates was observed in rats following exposure to sidestream cigar-

Table 1 Histological changes of the respiratory tract associated with exposure to cigarette smoke

Airway epithelial hyperplasia
Nasal respiratory epithelial hyperplasia
Airway mucous cell hyperplasia
Airway submucosal gland hypertrophy
Intraluminal, mucosal, and parenchymal inflammation
Emphysema
Hyperplasia of pulmonary neuroendocrine cells
Reduction of pulmonary lymphoid cells (for example, BALT)
Squamous metaplasia of airway and nasal epithelium
Preneoplastic and neoplastic lesions

ette smoke for 90 days.[24] Mice inhaling cigarette smoke developed basal cell hyperplasia and squamous metaplasia of the nasal cavity, as well as hypertrophy of Clara cells.[25] In a cigarette smoke-induced model of "bronchitis" exposure of Sprague-Dawley rats to smoke (25 cigarettes/day for 14 days) increased epithelial thickness at three of the four airway levels assessed due to a 100–400% increase in the number of secretory cells.[26] Initial changes included basal cell proliferation accompanied by mucous metaplasia of surface epithelial serous cells, and proliferation of the newly formed mucous cells. The acidic glycoprotein-containing secretory cells were dramatically increased in number, while the neutral glycoprotein-containing secretory cells were decreased. Histological changes in airways of patients with chronic bronchitis also included mucous cell hyperplasia and hypertrophy of submucosal glands with a shift to acidic, sialidase resistant, intracellular glycoproteins.

When non-steroidal anti-inflammatory drugs or corticosteroids were given concurrently with smoke exposure in a rat model of bronchitis, smoke-induced mucous cell hyperplasia was variably inhibited, depending on the drug and level of the airways examined.[27] Additional studies found that after cigarette smoke exposure in the rats the time required for secretory cell hyperplasia to resolve was shorter in larger than smaller airways – for example, trachea nine days; distal bronchioles up to 84 days.[28] Treatment with non-steroidal anti-inflammatory drugs during the recovery period hastened the return to normal epithelial architecture in the smaller airways (to 4–9 days) but had no effect on tracheal recovery.[29] Treatment of rats with 1% N-acetylcysteine during the recovery period also reduced the time for secretory cell numbers to return to normal.[30] Furthermore, concurrent administration of N-acetylcysteine with cigarette smoke exposure inhibited the initial increase in the number of acidic glycoprotein-containing secretory cells, but had little effect on numbers of cells containing neutral glycoproteins.[26]

Balansky et al[31] exposed Sprague-Dawley rats to cigarette smoke once daily for 40 days and observed dramatic histological changes in terminal airways, including severe neutrophilic infiltration of the mucosa, hyperplastic and metaplastic lesions, and micropapillomatous foci. Concurrent administration of N-acetylcysteine effectively prevented these changes. Studies in humans have also shown significantly increased numbers of total inflammatory cells and polymorphonuclear leucocytes in walls of membranous bronchioles in smokers. This response does not subside following smoking cessation as both current and ex-smokers had similar numbers and types of inflammatory cells in their airway walls.[32]

Inhaled cigarette smoke also induces preneoplastic changes in rat tracheal epithelial cells, lesions influenced by dietary deficiencies. In vitamin A (retinoic acid) deficient rats cigarette smoke caused epidermoid metaplasia characterised by stratified, keratinised, and squamous epithelial changes,[33] and focal areas of

squamous metaplasia including extensive squamous metaplasia of the larynx.[34] Conversely, when neonatal rat tracheas in organ culture were treated simultaneously with an experimental retinoid compound (Ro 15-0778), lesions associated with cigarette smoke condensate (hyperplasia, metaplasia, loss of secretory activity, and ciliary function) were prevented.[35] To our knowledge similar studies have not been performed in humans, although several investigators are evaluating a potential use for retinoids in preventing or reversing metaplastic changes in airway epithelium at the molecular level. Much of this work has been done with in vitro models.

Effects of cigarette smoke on epithelial barrier function

In airway epithelia tight cell–cell and cell–matrix contact allows for maintenance of a relatively impermeable (or selectively permeable) barrier between the airway lumen and underlying submucosal and vascular components of the respiratory system. Owing in part to this barrier, specific transport mechanisms of airway epithelia can regulate chloride secretion or sodium absorption and precisely maintain the amount and composition of respiratory tract lining fluid. Alterations in this fluid occur when normal airway epithelial function is disrupted by disease states such as cystic fibrosis, or exogenously derived perturbation such as cigarette smoking. Although not specific for cigarette smoking, processes that alter integrity of the airway epithelium (especially that of the alveolar spaces) are associated with an increased rate of ^{99m}Tc-DTPA clearance. Using ^{99m}Tc-DTPA thoracic scintigraphy has confirmed that lungs of smokers are more permeable than those of non-smokers.[36] Increased epithelial permeability occurs at an early stage in asymptomatic or neophyte smokers.[37] Rapid clearance is promoted by increased lung volumes and decreased surfactant secretion; however, the exact mechanism(s) for increased ^{99m}Tc-DTPA clearance in these states is unknown.[38]

Cigarette smoke-induced changes in ^{99m}Tc-DTPA clearance could reflect alterations in permeability of pulmonary vasculature,[39] alveolar permeability, and/or altered integrity of the conducting airways. Cigarette smoke increases endothelial permeability by its effects on cytoskeletal elements as shown in porcine pulmonary artery preparations,[40] tachykinin release in rat nasal passageways,[40] and cation influx in rat airway vasculature.[41] Following cigarette smoke exposure in guinea pigs changes in alveolar permeability were associated with type I cell injury.[42] In rabbits acute cigarette smoke exposure was associated with increased ^{99m}Tc-DPTA clearance, histological focal alveolar oedema and haemorrhage, but no ultrastructural changes in the alveolar capillary membrane.[43] Increased ^{99m}Tc-DPTA clearance occurred in rats exposed to cigarette smoke, but light microscopically detectable abnormalities were not seen in the lung parenchyma.[44]

Increased permeability of the airway epithelium to large molecular weight molecules (for example, dextran[45]) has been demonstrated in guinea pigs following inhalation of cigarette smoke, and appeared to be linked to damage to epithelial tight junctions.[46] Bronchial epithelial cells exhibited increased detachment following application of proteases to their apical surface when cultures were first exposed to cigarette smoke extract (also on their apical surface).[47] These results suggest that an intact airway epithelium is an important barrier against proteases released from intraluminal inflammatory cells.

Exposure of primary hamster tracheal epithelial cells to non-toxic concentrations of cigarette smoke extract inhibited intercellular communication across gap junctions.[48] In primary chick embryo hepatocytes cigarette smoke condensate induced a greater than 60% reduction in gap junction numbers.[49] Furthermore, the number of gap junctions and the rate of gap junction communication was inhibited by cigarette smoke condensate from tobacco-burning, but not tobacco-heated, cigarettes in cultured rat hepatic epithelial cells and human fibroblasts.[50]

Taken together, these studies indicate that cigarette smoke and/or many of its components can have a number of deleterious effects on intercellular communication and epithelial permeability. These alterations can play a part in the development of chronic respiratory diseases.

Alterations in airway secretions and ciliary clearance apparatus

It is beyond the scope of this report to review the structure and synthesis of mucins and structure and function of cilia. Readers are referred to several excellent review articles in this area.[51] Briefly, mucociliary clearance is dependent on the coordinated activities of (a) normal ciliary structure and function, (b) appropriate quantities and physicochemical properties of mucus, and (c) appropriate quantity (depth) of periciliary lining fluid. Alteration or dysfunction of any one of these components may result in reduced efficiency of the entire mucociliary clearance escalator.[52]

Cigarette smoke has profound effects on mucociliary function but the underlying mechanisms have not been elucidated. Studies have been hampered by: (a) complex interrelationships between various components of the mucociliary apparatus; (b) the complexity of the cigarette smoke mixture; (c) the fact that studies on the time course of particle clearance depend, not only on mucociliary velocity, but on initial particle distribution and deposition patterns;[53] and (d) the fact that acute effects of cigarette smoke in naively exposed subjects do not necessarily reflect those found in chronically exposed subjects.[53] Effects of cigarette smoke on mucociliary function have been reviewed previously.[52]

Environmental cigarette smoke exposure has been implicated increasingly in the development of upper respiratory tract infections and otitis media, especially in children.[54] Cigarette smoke and other indoor air pollutants have been shown to alter upper airway mucociliary func-

tion. Nasal mucociliary clearance in asymptomatic smokers was longer than in lifelong non-smokers, although no differences were detected in the mean nasal ciliary beat frequency.[55] Conversely, acute exposure to cigarette smoke in the rabbit was associated with increased mucociliary activity of the maxillary sinuses. These effects, however, were mediated reflexly via NK1 receptor stimulation by tachykinins released secondarily to irritant effects of cigarette smoke on sensory afferent nerves in the upper airways.[56]

Tracheal mucus from asymptomatic smokers is increased in both volume and water content, with a lower total solid content than mucus from non-smokers. This mucus caused increased mucociliary "clearability" in frog palate transport assays.[57] Significant elevations of a macrophage-derived mucus secretagogue (MMS-68) have been detected in the bronchoalveolar lavage fluid of cigarette smokers.[58] Tracheal potential differences, a measurement of transepithelial electrical potential difference used to assess ion permeability and ion transport across respiratory epithelia, were reduced in chronic cigarette smokers.[59]

Under similar airway deposition patterns inner lung zone clearance of particles (as assessed by gamma camera imaging) was significantly slower in asymptomatic smokers than in age matched non-smoking controls.[60] Alveolar deposition after 24 hours, however, was reduced, suggesting that smokers have a greater proportion of small conducting airways capable of mucociliary clearance.[61] These findings may be related to smoking-induced changes in bronchiolar secretory cells where secretion of cross-linked mucus glycoproteins is enhanced, increasing the efficiency of mucus–cilia coupling. Non-smokers have poorer mucociliary defence capabilities in their more distal peripheral airways, which would not be necessary unless chronic insults arose. In these asymptomatic subjects cigarette smoke-associated alterations in mucus may be beneficial. Inhalation of smoke from a single cigarette acutely (and reversibly) decreased the electrical potential difference across canine tracheal epithelium.[62] Both in vivo and in vitro acute exposure of cigarette smoke in the dog inhibited active ion transport (chloride secretion) by tracheal epithelium.[62] Chronic smoke exposure in the dog (10 cigarettes/day for 10 months) caused persistently increased tracheal mucus flux.[63]

In vivo and in situ animal studies have been used to dissect the reflex secretory responses induced by cigarette smoke. Reflexes initiated by nervous receptors in the airways normally augment mucus secretion in response to airway insult. Absorbed nicotine, however, bypasses its usual reflex circuits by directly stimulating ganglion cells.[64] In guinea pigs acute inhalation of low doses of cigarette smoke stimulated airway goblet cell secretion by activating cholinergic nerves (via parasympathetic ganglion stimulation) whereas exposure to high doses of the vapour phase of cigarette smoke stimulated capsaicin-sensitive sensory nerve endings.[65] In rats both control and previously exposed "bronchitic" animals showed exhibited tran-

sient increases in fucose (a mucus marker) secretion when cigarette smoke was blown through their laryngotracheal segments in situ.[66] In the cat in situ studies have indicated the complexity of reflex responses involved; cigarette smoke passed directly into the feline trachea segment stimulated mucin secretion, while smoke passed directly into the lower airways stimulated secretion in the lung segment. If cigarette smoke was first passed through the larynx, however, no augmentation occurred. It seems necessary for nicotine to be absorbed either directly into the tracheal segment or into the blood (via the alveolar spaces) for the autonomic ganglia innervating the airway submucosal glands to be stimulated. This reflex pathway predominates over that of airway irritant reflexes.[67]

Nicotine was a powerful mucus secretagogue when applied to ferret tracheal segments in vitro.[68] Exposure of organ cultures of rat trachea to cigarette smoke condensate induced loss of secretory activity and ciliary function,[35] and exposure of rat tracheal explants to varying amounts of cigarette smoke for 10 minutes induced dose-related blebbing of the apical membrane and loss of cilia.[69] The cigarette smoke component acrolein reduced ciliary beat frequency in cultured bovine bronchial epithelial cells,[70] while acetaldehyde – another major component of tobacco smoke – impaired ciliary function and beat frequency by inhibiting ciliary dynein ATPase activity and binding to ciliary proteins critical in the functioning of dynein and tubulin.[71]

Thus, cigarette smoke and/or many of its components can have direct effects on the secretory and transport functions of airway epithelial cells. Obviously such alterations can play an important part in the pathogenesis of chronic airway disease – for example, decreased mucociliary clearance can result in airway obstruction and increased susceptibility to microbial infection.

Influences of cigarette smoke on airway epithelial regulatory functions
AIRWAY NEUROPEPTIDE REFLEX RESPONSES AND BRONCHOCONSTRICTION

Airway reflex responses to irritants such as cigarette smoke are important in mediating changes in mucus secretion and these pathways often involve release of tachykinins, a family of neuropeptides including substance P, neurokinin A, and neurokinin B. When released from sensory nerve endings in the airways tachykinins act on various receptors eliciting a typical pattern of response. These collective effects – often referred to as "neurogenic inflammation" – include mucus hypersecretion, smooth muscle contraction, increased airway mucosal plasma extravasation (associated with increased vascular permeability), increased neutrophil adhesion, and cough.[72] Tachykinins are normally rapidly hydrolysed by the membrane-bound metalloenzyme, neutral endopeptidase (NEP), which is widely distributed in the airways. Excessive release of tachykinins, however, decreases the capacity of NEP enzymatic degradation, or a combination of these effects

may result in exaggerated neurogenic responses.

Cigarette smoke exposure stimulated primary afferent sensory nerves in rats, eliciting tachykinin release and resultant mucosal oedema of tracheal tissue.[73] Chemical irritants in the vapour phase of cigarette smoke are primary elicitors of the permeability response.[74] Inhalation of cigarette smoke in guinea pigs also augments airways responsiveness to inhaled substance P and capsaicin,[74] effects associated with decreased airway NEP activity.[75] Supernatants from bovine bronchial epithelial cells exposed to tachykinins exhibited increased neutrophil chemotactic activity,[76] and direct pretreatment of bovine bronchial epithelial cells with substance P significantly increased adherence of cocultured neutrophils.[77]

Pulmonary neuroendocrine cells – for example, neuroepithelial bodies and small granule cells – are a poorly understood group of epithelial cells in the airways, and cigarette smoking has been associated with this hyperplasia and variable increases in respiratory tract concentrations of the bombesin-related peptides.[78] Bombesin-related peptides are a family of neuropeptides similar to amphibian bombesin. They are present in several human body systems including the respiratory tract where they have a role(s) in immunological lung functions.[79] They exert chemoattractant activity for monocytes, mitogenic activity for bronchial epithelial cells, and bronchoconstrictive activity. These peptides are involved in cigarette smoke-associated lung inflammation, fibrosis, and/or airway obstruction,[79] and function as essential autocrine factors for many small cell lung cancers. Since cigarette smoke inactivates NEP, and NEP normally hydrolyses bombesin-related peptides, it has been proposed that decreased airway NEP activity may also be causally related to the development of these carcinomas.[80]

Nitric oxide exhibits potent relaxing activity in both canine and guinea pig airways.[81] Unstimulated bovine bronchial epithelial cells in culture are capable of converting L-arginine to L-citrulline, one of the biosynthetic pathways of nitric oxide.[82] Moreover, exposure of the epithelial cells to cigarette smoke extract prevented this conversion, as did treatment of the cells with competitive inhibitors of nitric oxide synthase.[82] Competitive inhibitors of nitric oxide synthase also decrease ciliary beat frequency in cultured bronchial epithelial cells,[83] and the free radical reactant product of nitric oxide and low molecular weight thiols can form *S*-nitrosothiol adducts which have potent relaxation effects on airway smooth muscle.[84]

Finally, another potential cause of smoke-induced airway obstruction could be the development of airway inflammation and associated increases in release of cytokines. For example, guinea pig tracheas cultured with inflammatory cytokines such as IL-8 and TNFα demonstrated increased synthesis of endothelin-1, a potent bronchoconstrictor.[85]

AIRWAY INFLAMMATION

Perturbations of the airway[73] epithelial cell barrier induced by cigarette smoke may lead to adverse changes resulting in airway inflammation (table 2). Specific mechanisms involved in this inflammation include: (a) increased synthesis and/or release of inflammatory mediators (for example, arachidonic acid metabolites, chemotactic agents) or, alternatively, decreased synthesis of protective mediators (for example, chemotactic factor inactivators); (b) synthesis of proinflammatory cytokines; (c) modulation of cell adhesion molecules; and (d) modulation of immunoregulatory processes.

Cigarette smoke induces an acute inflammatory reaction in the airways and lung parenchyma. Peripheral leucocytosis[86] and increases in bronchoalveolar lavage neutrophil and total inflammatory cell counts have also been demonstrated in cigarette smokers.[87] In one study both current and ex-smokers had increased total inflammatory cells and neutrophils in the walls of their membranous bronchioles compared with non-smokers,[32] and they also had similar numbers and types of airway inflammatory cells present regardless of concurrent emphysematous changes.[32 88]

Airway epithelial cells are an important source of inflammatory mediators[9] since responses to various stimuli may involve epithelial release of chemotactic activity for leucocytes.[89] Cigarette smoke has proved to be a "reliable" stimulus for inducing release of such chemotactic activity, although the specific mediators released vary among species in both in vivo and in vitro studies. Exposure of young non-smoking humans to environmental tobacco smoke for three hours promoted systemic priming of neutrophils and increased circulating neutrophil counts, neutrophil chemotaxis, and neutrophil release of oxidants upon stimulation.[90] Similarly, human bronchial epithelial cell cultures exposed to smoke extract release significantly greater neutrophil chemotactic activity than controls.[82] Nitric oxide appeared to play a part in this response as pretreatment of the cultures with a nitric oxide synthase inhibitor (L-NMMA) abrogated this increase, while addition of L-NMMA to supernatants failed to inhibit neutrophil chemotactic activity.[82]

At concentrations comparable to levels observed in the plasma of smokers nicotine appeared to enhance the neutrophilic response to other chemotactic peptides.[91] At higher concentrations, however, nicotine inhibited the neutrophil chemotactic response and sponta-

Table 2 Potential mechanisms of epithelial cells serving as effector cells

Neutral endopeptidase enzymatic degradation of tachykinins
Production of bombesin-related peptides
Production of nitric oxide
Production of endothelin-1
Major histocompatibility complex class II antigen expression
Production of arachidonic acid metabolites (for example, prostaglandins, thromboxanes, leukotrienes, hydroxyeicosotetraenoic acids)
Production of chemotactic agents
Production of chemotactic factor inactivators
Cytokine production (for example, GM-CSF, G-CSF, IL-6, IL-8)
Cell adhesion molecule changes (for example, ICAM-1)
Production of fibronectin and other extracellular matrix molecules

neous migration.[92] Nicotine pretreatment of mice appeared to enhance neutrophil influx towards the "source of inflammation" when the chemotaxin, zymosan-activated serum, was injected into the pleural space.[92]

Smoke-induced recruitment of neutrophils was complement-dependent in mice: C5-sufficient mice, but not C5-depleted mice, exposed to cigarette smoke had increased neutrophil chemotactic activity in their bronchoalveolar lavage fluids.[93] Others suggest that the mechanism of neutrophil influx into the lungs relates to smoke-induced loss of functional activity of chemotactic factor inactivator (CFI). CFI normally inhibits C5a-directed neutrophil chemotaxis by binding to GcGlobulin, an essential cochemotaxin of C5a.[94] While specific smoke-induced effects on other airways or alveoler protease inhibitors remain largely unknown, cigarette smoke inactivation of α-protease inhibitor is a contributing factor in the development of smoking-related emphysema.[95]

Airway epithelial cells contain both linoleic acid and arachidonic acid, which are available for metabolism and release via oxidative pathways and are capable of metabolising arachidonic acid to prostaglandins, thromboxanes, leukotrienes, and hydroxyeicosotetraenoic acids (HETEs), although the exact profile of eicosanoids is dependent on the species of origin and the nature of the stimulus – for example, ozone, nitrogen dioxide.[96][97] Since these eicosanoids are associated with effects ranging from enhanced mucus secretion to smooth muscle contraction and inflammatory cell chemotaxis their release following exposure to cigarette smoke has also been evaluated. In humans cigarette smoking causes increased urinary excretion of the 2,3-dinor metabolites of thromboxane A_2, but not prostacyclin (PGI),[98] and reduces salivary concentrations of PGE_2, $PGF_{2\alpha}$, leukotrienes, and 12-HETE.[99] Bronchoalveolar lavage fluid obtained from Wistar rats exposed to cigarette smoke contained increased concentrations of 15-HETE but not thromboxane B_2.[100] Conversely, bronchoalveolar lavage fluid from guinea pigs exposed to acrolein (a low molecular weight aldehyde found in cigarette smoke) had increased concentrations of both $PGF_{2\alpha}$ and thromboxane B_2.[101] In vitro exposure of bovine tracheal epithelial cells to non-cytotoxic concentrations of acrolein caused increased release of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, 12-HETE, and 15-HETE. Exposure of isolated rat tracheal segments to cigarette smoke extract elicited a biphasic effect on muscarinic-stimulated eicosanoid synthesis: low concentrations potentiated production while higher concentrations inhibited this response.[102] Exposure of canine mast cells to cigarette smoke solution inhibited PGD_2 production but induced increased release of the preformed mediators, histamine and tryptase.[103]

Research over the last decade has expanded our knowledge of cytokines and their networks and their role in inflammatory responses of the respiratory tract. Airway epithelial cells themselves are now known to be capable of synthesising, releasing, and/or upregulating production of these locally acting mediators. Analysis of conditioned medium from human bronchial epithelial cells in primary culture demonstrated the presence of GM-CSF, G-CSF, and IL-8.[104] The presence of GM-CSF and G-CSF in the conditioned media caused increased in vitro survival of cultured peripheral blood neutrophils.[104] IL-8 is the major cytokine of the human nasal epithelium,[105] although conditioned media from human nasal epithelial cell cultures also contained GM-CSF, G-CSF, and IL-6.[106] There are too few data available on the influence of cigarette smoke on cytokine production by airway epithelial cells, but exposure of human *alveolar* epithelial cells to a phenol-rich glycoprotein present in cigarette smoke condensate was associated with increased synthesis of IL-1 and IL-6. Exposure also elevated steady state levels of mRNAs for IL-1, IL-6, and platelet derived growth factor (PDGF-A and PDGF-B) as detected by in situ nucleic acid hybridisation.[107] As assessed by bioassays, cigarette exposure of cultured human and guinea pig alveolar macrophages decreased IL-7 and TNF activity.[108] Additionally, lipopolysaccharide-stimulated alveolar macrophages from smokers caused less IL-1 and IL-6 production than alveolar macrophages from non-smokers.[109] This may be important for the increased susceptibility of smoking patients to respiratory infections, and to the decreased incidence of immune-mediated pulmonary diseases such as sarcoidosis in smokers.[110]

Human bronchial epithelial cells have recently been shown to express the α_{2-6} integrins, molecules which interact with many extracellular matrix components. Cultured human respiratory cells also express intercellular adhesion molecule 1 (ICAM-1) on their surfaces. Adherence of neutrophils to airway epithelial cells via ICAM-1 may be an important factor in inflammatory airway diseases.[111] The processes by which neutrophils migrate to sites of airway inflammation are complex and beyond the scope of this report. Briefly, however, as reviewed recently,[112] initial steps of neutrophil recruitment involve a process known as "rolling" whereby neutrophils begin to decelerate and attach to the endothelial surface of postcapillary venules at or near sites of tissue injury or inflammation. Adhesion molecules of the selectin family (P-, E-, and L-selectin) are essential components in this process. Neutrophils subsequently arrest and spread over the endothelial cell surface via interactions between their β_2-integrins (CD11a/18 and CD11b/18) and endothelial cell ICAM-1. Once firmly attached, leucocytes migrate through vessel walls, extracellular matrix components, and between normally adherent epithelial cells following chemotactic and/or haptotactic gradients. Migration requires careful regulation of proteolytic enzyme secretion and transformation of the cell into a deformable, motile state. Once near or at the site of airway injury or inflammation, leucocyte retention involves critical concentrations of specific cytokine(s) – for example, GM-CSF – and continued interaction with epithelial cell adhesion molecules. Interestingly enough, proinflammatory cytokines – for example, IL-1, TNFα – increase cell

surface expression of ICAM-1 and neutrophil adhesion to tracheal epithelial cells in vitro.[111] Selective induction of human tracheal ICAM-1 expression in human tracheal epithelial cells has also been shown in response to interferon γ treatment.

Cigarette smoke elicits a number of neutrophil migratory and cell adhesion changes. Neutrophil transit time through the human pulmonary vasculature is prolonged by inhalation of cigarette smoke.[112] Studies in rabbits have shown that smoking-induced neutrophil retention in pulmonary vasculature occurs without corresponding changes in pulmonary haemodynamics.[113] In hamsters acute exposure to mainstream smoke for five minutes provoked neutrophil rolling and subsequent adhesion to the endothelium of both postcapillary venules and arterioles. Adhesion steps were mediated via 5-lipoxygenase metabolites of arachidonic acid (leukotrienes).[114] In vitro exposure of neutrophils to both the whole particulate and vapour phase of cigarette smoke reduced the deformability (hence migratory capability) of neutrophils via effects on the actin component of their cytoskeleton.[115] Also, plasma myeloperoxidase concentrations are elevated immediately after exposure to cigarette smoke which suggests that cigarette-induced lung damage is related to activation of leucocytes still within the pulmonary capillary bed.[113] Further evidence is provided by intravascular neutrophils in certain lung regions of rabbits exposed to smoke which showed increased expression of CD11/CD18 coincident with decreased L-selectin expression.[116] After subacute (two week) exposure rats did not show altered endothelial neutrophil adhesion compared with sham-exposed animals; however, after treatment with capsaicin they had more vascular adherent neutrophils, suggesting that cigarette smoke potentiates neutrophil adhesion in association with neurogenic airway inflammation.[117] Exposure of monolayers of bovine bronchial epithelial cells to cigarette smoke extract significantly increased adherence of both cocultured neutrophils and mononuclear cells.[118] Peripheral blood neutrophils exposed to cigarette smoke in vitro and then cocultured with alveolar epithelial cells demonstrated decreased adherence which did not increase after stimulation with f-Met-Leu-Phe.[119]

As mentioned previously, cigarette smoking is associated with a decrease in certain immune mediated pulmonary diseases[110] while smoking and/or exposure to environmental tobacco smoke has been associated with increased respiratory infections including sinusitis, otitis media, bronchitis, and pneumonia,[5] conditions presumably related to alterations in immune function.[120] Immunological effects induced by cigarette smoke include reduction of bronchus associated lymphoid tissue (BALT), decreased immunoglobulin concentrations due to suppression of B cell antibody production (except for IgE, which is increased), decreased helper T cell numbers, decreased natural killer cell activity, and increased circulating soluble IL-2 receptors.[120 121] Infections asociated with cigarette smoke may be related to effects such as decreased mucociliary clearance of inhaled infectious agents. Other smoking-related immunological effects on airway epithelial function could involve alterations in the expression of major histocompatibility complex class II antigen (MHC class II) or alterations in the function of airway dendritic cells. Expression of MHC class II antigens was increased in bovine bronchial epithelial cells exposed to supernatants from activated lymphocytes.[122] Antigen presenting functions of pulmonary dendritic cells (which typically express ICAM-1) are downregulated via interactions with resident alveolar macrophages.

Repair of injured areas of airway epithelium (resulting from either direct cigarette smoke-induced injury or as a consequence of airway inflammatory processes) may be impaired by ongoing cigarette smoke exposure. Cigarette smoke degrades hyaluronic acid,[123] decreases fibronectin release from cultured bovine bronchial epithelial cells,[124] and delays migration of bronchial epithelial cells to fibronectin.[125]

The airway epithelial cell therefore has the potential to act as an inflammatory cell, responding to smoke/and or many of its components by releasing a number of humoral and inflammatory mediators that may enhance the inflammatory reaction in the airways and contribute to the pathogenesis of chronic disease. The role of airway epithelial cells as potential effector cells in the response to smoke or many other insulting or injurious agents is a research area that has received much recent attention, and it is possible that these cells can produce a number of other as yet unidentified compounds that can contribute to airway inflammation.

Cigarette smoke combined with other pulmonary insults

Synergistic effects of cigarette smoke with other respiratory insults have been reported – for example, a synergistic effect of asbestos exposure and cigarette smoke in bronchogenic carcinoma.[126] A mechanism of this synergy suggests that both deleterious agents provoke generation of reactive oxygen species in the airways.[127] In guinea pigs exposure to cigarette smoke combined with intratracheal instillation of amosite asbestos fibres resulted in increased penetration of the asbestos fibres into the airway walls.[128] Cigarette smoke exposure also impairs clearance of amosite asbestos fibres (particularly the shorter fibres) from both alveolar macrophages (30-fold increase in fibre number) and pulmonary tissue (eightfold increase).[129] Excised rat tracheal segments exposed to cigarette smoke followed by amosite asbestos have increased epithelial uptake of the fibres, and uptake was still enhanced when smoke exposure preceded asbestos treatment by up to 48 hours.[130] Again, reactive oxygen species played a part in smoke-mediated fibre transport into the tracheal epithelium.[131] Cigarette smoke exposure of tracheal epithelial explants also directly enhanced uptake of inert dust – that is, non-fibrous titanium dioxide, talc, and fibrous silicon carbide – while treatment with scavengers of reactive oxygen species only blocked uptake of some of these particles.[132] Finally, exposure to cigar-

ette smoke has also been associated with potentiation of pulmonary injury following instillation of bleomycin,[133] irradiation-induced pneumonitis,[134] and ozone inhalation.[135]

Conclusions

Cigarette smoke and many of its individual components, both gaseous and particulate, can generate various lesions in epithelial cells of the airways. Interactions between these stimuli and airway epithelial cells are just beginning to be elucidated. Although studies of cigarette smoke-induced effects on individual cells of the airway epithelium are exceedingly useful for eliciting underlying mechanisms of respiratory injury and disease, the overall respiratory system response depends ultimately upon the integrated action of all effector cell populations with their corresponding target cells. To fully understand how defence mechanisms within the respiratory system attempt to intervene against cigarette smoke while attempting to repair previous smoke-induced damage, these interactions of airway epithelial cells with other host defence/repair mechanisms must be more completely understood.

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