

Smoking-Related Lung Injury

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Introduction

The idiopathic interstitial pneumonias (IIPs) are a group of largely unrelated non-neoplastic disorders that were originally described by Dr. Averill A. Leibow in the mid 70's. Patients usually present with prolonged symptoms (months) of dyspnea and the lung biopsy demonstrates varying patterns of interstitial fibrosis and inflammation. A substantial number of these patients are cigarette smokers and the following lung diseases have demonstrated a strong association with exposure to cigarette smoke. Histologic diagnosis is often challenging as the major task surrounds evaluating varying degrees of fibrosis and inflammation, which is somewhat subjective. The final diagnosis is most accurate when the histology is combined with knowledge of respiratory physiology and the distribution of disease as demonstrated on chest imaging.

Presentation Highlights

- Provide an approach to combined radiologic-pathologic diagnosis in patients with cigarette smoke-related lung injury based on the normal physiologic gradients in the lung for air, blood, and lymphatic flow
- Describe the histologic findings in patients with cigarette smoke-related lung injury
- Correlate the histologic findings with pulmonary pathophysiology and resulting radiologic abnormalities

Respiratory Bronchiolitis-Associated Interstitial Lung Disease (RB-ILD) and Desquamative Interstitial Pneumonia (DIP) are best thought of as a spectrum of smoking-related fibrotic and inflammatory reactions. Respiratory bronchiolitis is found in the lung biopsies of essentially all smokers although in-and-of-itself, it rarely causes symptoms. It is characterized by the presence of pigmented macrophages within and around respiratory bronchioles. Mild peribronchiolar fibrosis may occur. A small percentage of smokers with histologic respiratory bronchiolitis will present with significant dyspnea and hypoxemia. The patients are almost always heavy smokers in their fourth or fifth decade who improve after cessation of smoking. The chest radiograph demonstrates airway thickening in the majority of patients, but may be normal. The chest CT reveals centrilobular nodules and ground glass with an upper lobe predilection. Mild upper lobe emphysema is also common. Evidence of air trapping may be present on

expiratory phase CT scans. In this setting, the clinical-radiologic-pathologic diagnosis of RB-ILD may be applied. The differential diagnosis for this appearance includes hypersensitivity pneumonitis and pulmonary Langerhans' cell histiocytosis.

DIP is now considered to be a more extensive form of respiratory bronchiolitis in which the macrophages are found within the alveolar spaces across the secondary lobule in addition to those found in the peribronchiolar region. Rarely, the disease occurs in non-smokers. Patients present with dyspnea and low lung volumes. The chest radiograph is usually characterized by patchy lower lobe ground glass. However, the chest radiograph is normal in up to 25% of cases. The chest CT demonstrates ground glass opacity in a peripheral and lower lobe distribution in the majority of cases. Reticular opacities and irregular lines are usually found in the lung bases and honeycombing is found in less than one third.

Pulmonary Langerhans' Cell Histiocytosis (PLCH) is an isolated form of Langerhans' cell histiocytosis that primarily affects cigarette smokers. PLCH is characterized histologically by bronchiolocentric interstitial stellate nodules that are composed of a variable mixture of cells including plasma cells, lymphocytes, neutrophils, pigmented cells, and Langerhans'. The nodules frequently cavitate and form thick- and thin-walled cysts, which are thought to represent enlarged airway lumina. PLCH lesions display temporal microscopic heterogeneity, early lesions are cellular with little fibrosis, these progress to late lesions composed of dense collagen with minimal cellularity. In advanced cases, stellate fibrotic scars are surrounded by enlarged, distorted air spaces, so-called 'paracicatricial emphysema or air-space enlargement'. Affected patients are typically young adults who often present with cough and dyspnea. Characteristic radiographic features of PLCH are bilateral nodular and reticulonodular areas of opacity that predominantly involve the upper and middle lung zones with relative sparing of the lung bases. High-resolution chest CT shows nodules and cysts in the same distribution and allows a confident prospective diagnosis of PLCH in the appropriate clinical setting. In typical cases, a predominantly nodular pattern is seen on CT scans in early phases of the disease, whereas a cystic pattern predominates in later phases. The radiologic abnormalities may regress, resolve completely, become stable, or progress to advanced cystic changes. Treatment consists of smoking cessation, but corticosteroid therapy may be useful in selected patients. Chemotherapeutic agents and lung transplantation may be offered to patients with advanced disease. The prognosis of PLCH is variable with frequent regression, stabilization, or recurrence of disease that does not correlate with cessation or continuation of smoking.

Nonspecific Interstitial Pneumonia (NSIP) remains a controversial diagnosis and represents those patients who have interstitial lung diseases that do not fit cleanly into the above diagnostic categories. Those patients with the cellular form of NSIP have an excellent prognosis, while increasing fibrosis on lung biopsy is

associated with poor survival. NSIP may be the presenting manifestation of collagen vascular disease or hypersensitivity pneumonitis and should prompt an investigation for these diseases. The diagnosis of NSIP may also be made in those patients with inadequately sampled idiopathic pulmonary fibrosis (IPF); in this case, imaging is important and the finding of typical IPF on computed tomography carries a poor prognosis despite the histologic diagnosis NSIP. In some cases of NSIP, the fibrosis is associated with cigarette smoke alone.

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Smoking-related airway field carcinogenesis – the molecular basis of pre-neoplasia

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Tobacco smoke carcinogens

Tobacco smoking is considered to be the cause of around 90% of all lung cancers, possibly slightly more than this for males and rather less than this figure in females but data will vary by population and country. There are over 4000 chemicals identifiable in tobacco smoke; just over half of these derived from the combustion of the tobacco itself, the remainder the result of tobacco agriculture and processing in the manufacture of tobacco products. Over 50 of these chemicals are recognized as toxins or carcinogens by the US FDA. Among those substances regarded as carcinogenic are polycyclic aromatic hydrocarbons (PAHs) and N-nitrosamines; these are probably the most important, together with others which include aldehydes, aromatic amines, ethylene oxide and other organic compounds but also inorganic substances including arsenic and chromium-based compounds, radioactive elements such as radon, lead-210 and polonium-210, and oxidizing free radicals. Amongst these the most closely studied are the PAH benzo(a)pyrene and an N-nitrosamine known in abbreviated form as NNK. NNK is a tobacco and lung-specific carcinogen which has been particularly linked with the development of adenocarcinoma. Between 10-20% of smokers get lung cancer.

Lung carcinogenesis pathways

There are two relatively well characterized pathways of lung carcinogenesis; one involving the epithelium of mostly central bronchi, the other originating in the epithelium of the peripheral bronchioles and alveoli, the so-called terminal respiratory unit (TRU). Squamous dysplasia (SD) and carcinoma-in-situ (CIS) are the recognized pre-invasive precursor lesions in bronchial carcinogenesis, giving rise (probably) to most squamous cell carcinomas of the bronchus but possibly some small cell lung cancers (SCLC) and other tumours. In the TRU, the putative precursor lesions equivalent to SD & CIS in bronchial carcinogenesis are atypical adenomatous hyperplasia (AAH) and localized non-mucinous bronchioloalveolar carcinoma which is probably better classified as adenocarcinoma-in-situ (AdCIS). While the association between SD/CIS, and the tumours arising from it, and smoking is strong, there is a lesser link with smoking and

AAH/AdCIS. Nonetheless, the strength of association and frequency of these lesions in smokers with adenocarcinoma suggests a likely relationship.

The carcinogenic process

The exact chemical events which take place when carcinogenic compounds 'damage' cellular DNA is really beyond the scope of this discussion. Activated carcinogens form bulky DNA adducts which may lead to misreading during DNA replication in proliferating cells and thus genetic alterations (mutations) in daughter cells. Adducts may especially form at endogenously methylated CpG islands, explaining the specific G-T transversions common in smoking-related tumours. Those alterations which are non-lethal and pass down through generations of cells may or may not alter cellular functions, the critical steps in carcinogenesis involve the dysregulation of processes involved with the cell cycle, cell proliferation, receptors and down-stream effectors of intracellular signaling pathways, DNA repair mechanisms and cell death pathways (apoptosis). The malignant phenotype involves the diminution or loss of a variety of cellular homeostatic functions and controls; cellular dysregulation leading to more autonomous behaviour by the affected cell population. Those recognizable pre-invasive lesions mentioned above are the morphological expression of some but not all of the genetic changes which are required for the full expression of the invasive, malignant cell phenotype. Many molecular events are recognized as contributing to this process, the significance of them all is not understood, nor is there a comprehensive understanding of the process overall. It is clear however, that molecular events predate the onset of morphological changes.

Xenobiotic polymorphisms and other heritable factors

Tobacco smoke contains active carcinogens, and so-called pro-carcinogens which are paradoxically converted into active carcinogens by host xenobiotic metabolizing enzymes (XMEs). There are many types of XMEs including the cytochrome P450s (CYPs), Glutathione S Transferases (GSTs), N-Acetyl Transferases (NATs), enzymes such as NQO1, those involved in transport and excretion of metabolized carcinogens and many others. These enzymes are variable in their prevalence and efficacy and individual enzymes show person-to-person heritable variability (polymorphisms) determined by single nucleotide changes in genetic code leading to single amino acid structural changes in the enzyme protein (single nucleotide polymorphisms or SNPs). While so-called phase 1 XMEs such as the CYPs convert procarcinogens into active

carcinogenic compounds, phase 2 XMEs such as the GSTs, NATs and NQO1 convert potentially toxic compounds into less toxic soluble forms to facilitate their elimination by transport and excretion enzyme systems. These polymorphisms vary enzyme activity and efficacy, and thus influence the total effective exposure of lung epithelium to tobacco carcinogen, depending on the overall balance between carcinogen activation and detoxification/elimination conferred on any individual by their particular XME polymorphisms.

CYP1A1 and 1B1 (metabolizing PAHs), CYP2A6 and 2E1 (metabolizing nitrosamines), and GSTs M1 and T1 are associated with variable DNA damage in smokers depending on the polymorphisms present. Polymorphisms of CYP1A1 and GST M1 have been shown in several studies to be related to lung cancer risk; in some studies the inherited combination of enzyme polymorphisms appears important. It has been suggested that high risk CYP polymorphisms are more associated with increased squamous cell carcinoma risk while GST M1 together with P53 gene polymorphisms may be more deterministic of adenocarcinoma.

DNA repair enzymes may abrogate DNA damage caused by adduct formation. Nucleotide excision repair pathway enzymes ERCC1-3 and base excision repair pathway enzymes such as XRCC1 & 3, MGMT and OGG1 have all been studied in the context of smoking induced carcinogenesis. Again there is evidence that polymorphism of these genes may lead to inherited poor DNA repair efficiency and increase lung cancer risk. Certain ERCC1 polymorphisms have been linked to excess risk of small cell lung carcinoma.

The constitutive molecular environment in the pulmonary epithelium of the smoker accounts for individual variation in the risk from environmental carcinogens in tobacco smoke, through variable carcinogen activation, deactivation and elimination, and DNA repair. This genetic influence on lung cancer risk has been shown in other ways. Familial risk of lung cancer, regardless of smoke exposure, has been linked to heritable genes at the 6q23-25 locus. More recently, there has been considerable interest in polymorphisms of the nicotinic acetylcholine receptor gene cluster at 15q24-25.1 (CHRNA5-A3). As well as the A3, A5 and B4 subunits of this receptor complex in lung epithelial cells actually binding carcinogens, variants of this gene cluster seem to be associated with greater addiction to nicotine and there is evidence of consequential greater tobacco consumption and increased risk of lung cancer as well as other smoking-related diseases in these groups.

Lung carcinogenesis: a field effect

As many as 80 – 90% of smokers do not develop clinical lung cancer in their life time. This does not mean that their pulmonary epithelia were 'immune' to the effects of tobacco carcinogens. For the fully invasive malignant phenotype to be expressed in lung epithelial cells it has been suggested that between 3 and more likely closer to 12 critical genetic alterations are required. Furthermore the multi-hit theory of human carcinogenesis requires that these critical events occur in the correct order as there is some inter-dependency, and that the genetic events are non-lethal and heritable. Simple statistics support Sir Richard Doll's observation that getting cancer is rather a matter of bad luck. Many, probably most, smokers may have lung epithelial cells replicating the first few necessary 'genetic steps', fewer smokers will have fewer cells with more of the changes on board, only 10-20% of smokers achieve the full set in a single cell clone. We do not understand the relationship between the existence of fewer critical genetic changes and the emergence of morphologically recognizable pre-invasive precursor lesions. We do know that smokers have detectable genetic changes in morphologically normal bronchial epithelium, but that more changes are present in SD and CIS. These early genetic alterations must occur in the stem cell populations in either the bronchial or TRU epithelial compartments. Only by propagation in these immortal cell populations, can the genetic changes persist in the smoker long enough for further sequential critical changes to accumulate. The recognition of separate stem cell populations in these two epithelial compartments in the lung fits nicely with the recognition of corresponding precursor lesions and invasive cancers of differing biology arising from these respective locations. Early genetic alterations in stem cells, including possibly mutations of P53, lead to the development of clonal patches of altered cells. These patches acquire survival advantage and increased proliferation. There is evidence that in the head and neck and oesophageal epithelium these patches may exceed 7cm in diameter without being morphologically different from 'normal'. These patches then undergo clonal divergence, a process presumably synchronous with the emergence of morphologically recognisable pre-invasive precursors, then, perhaps, invasive disease. It has been suggested that such patches in bronchial epithelium contain somewhere between 40,000 and 360,000 cells, which would equate with an epithelial area of between 4 - 80 sq mm. Limited data from autofluorescence bronchoscopy studies and other work suggests that while SD lesions in bronchi are more often less than 1.5mm in diameter,

rather less than half measure between 1.6 and 4mm across. Bronchial CIS lesions have been measured at between 2 and 17mm in diameter with an average of 9mm.

Presumably these morphologically recognizable lesions represent further evolution of genetic change in the clonal patches. These small pre-invasive lesions may be numerically fewer than more prevalent but invisible clonal patches bearing fewer genetic changes.

Considerably less is known about peripheral TRU epithelium and its stem cells in this context. AAH lesions are generally less than 3mm in size and rarely over 10mm in diameter.

Molecular changes in Bronchial carcinogenesis

There is a substantial literature on the molecular changes which are identifiable in SD & CIS in bronchi. While most of this work is not specifically related to smoking, given that tobacco smoke is considered the most important aetiological factor, we may infer that most of the changes are a consequence of tobacco carcinogenesis. Some studies have made a distinction between smokers and non-smokers.

'Normal' bronchial epithelium

Molecular changes may be detected in morphologically normal bronchial epithelium in smokers. Chromosomal aneusomy is common in bronchial epithelial cells of long term smokers. Hypermethylation of certain genes may be found in morphologically normal bronchial mucosa in patients who have lung cancer but at levels much lower than in the tumours themselves; P16 and APC are especially noted in smokers but RASSF1A, MGMT, RARbeta, GSTP1, CDH13 and PAX-5 may also be methylated. Minor levels of human telomerase reverse transcriptase (hTERT) and chromosomal loss of heterozygosity (LOH) have been described even in normal mucosa in chronic bronchitics. LOH in particular has been detected at 3p and 9p, in the non-neoplastic mucosa of lungs resected for squamous cell carcinoma. Such losses are also found in pre-invasive lesions; in such cases the losses are greater and more widespread. Among the earliest losses are those in 3p14.2 (FHIT), 3p21 (RASSF1A, FUS-1), 3p22-24 (BAP-1), 3p25, 9p21 (P16INK4a – CDKN2) and sometimes at 17p13 (P53) (associated

Tumour Suppressor Genes possibly lost shown in parenthesis). Not all patients and not all samples show losses and when multiple foci of loss are found most appear to be clonally unrelated. In a comparative study of 3p losses, while 47% of smokers showed loss in 3p, none of the never smokers did. In those smokers who do not have cancer and who give up smoking, genetic abnormalities persist in their bronchial epithelium for many years as does their risk of lung cancer, which diminishes slowly but never reaches the lowest levels seen in never smokers.

While the genetic abnormalities found in the 'normal' epithelium of those with squamous carcinoma may be limited, it is characteristically very extensive in those with SCLC; almost as much alteration as may be seen in SCLC itself. This epithelium, lacking features of SD/CIS, has been described as 'genetically scrambled', showing high degrees of allelic losses and frequent mutations. It has been suggested, therefore, that SCLC arises directly from this genetically altered respiratory epithelium, without any recognizable pre-invasive lesions (the so-called parallel theory of evolution), as opposed to the sequential (theory) passage through stages of dysplasia prior to the onset of invasion.

A recent gene expression profile study of microdissected bronchial epithelium from smokers and non-smokers demonstrated a differentially expressed 23 gene set including 10 XME genes, 2 oncogenes (FGFR3 and LMO3) and one TSG (HLF).

There is also evidence that inflammatory mediators may also have a role in upregulating several pathways which are important in carcinogenesis, including simple proliferation and basal cell hyperplasia. Given that upregulation of cell cycle activity is an obligatory early event in carcinogenesis, this may well be an important mechanism, whether or not the inflammation is tobacco-induced. Nicotine may be able to stimulate proliferation in bronchial epithelial cells via the NACH receptor.

All of this is evidence that there is a 'field' change at a genetic level, presumably caused by tobacco carcinogens, which precedes and is required for the evolution of recognizable pre-invasive bronchial lesions.

Squamous Dysplasia and Carcinoma-in-situ

There is very extensive literature describing genetic and molecular alterations in SD/CIS lesions. Molecules and pathways involved in cell proliferation, cell cycle regulation, neovascularisation, cell death pathways including p53, bcl2 and related proteins, putative TSGs such as FHIT and RARbeta, the HER tyrosine kinase driven pathways

including downstream molecules such as AKT and K-RAS, and the important p16INK4a-cyclinD1-CDK4-RB pathway.

Some literature reviewing these data is listed below. In general there is evidence that as the SD/CIS sequence progresses, so does the frequency and extent of abnormality in many of these pathways. In conjunction with these specific changes, the LOH found in morphologically normal epithelium in smokers is found more frequently and more extensively in SD and CIS; losses at 18p21-23, 13q14 (Rb) and 5q (APC-MCC) emerge. There has been considerable interest in trying to identify which genetic changes are the key to the onset of invasion and which might predict such an event. While there is little evidence and less consensus on this topic, high expression of p53 or Cyclin D1 and loss of p16 may be associated with greater risk of invasion, as may a high bcl2:bax expression ratio. The presence of 3p LOH is also associated with lesion progression. It is also worth noting that while there is some evidence that higher grades of lesion are more likely to progress to the 'next stage', there is ample evidence that ANY grade of lesion, including CIS may regress; progression of disease is by no means a given. Evidence varies as to whether smoking increases the chances of progression, or that cessation improves the chances of regression. All of these studies are hampered by difficulty in longitudinal observation of the same lesion in the clinical setting; biopsy may remove completely or alter the natural history of the lesion, any apparent 'skipping' of a stage in progression could reflect rapid progression between biopsy intervals. The P16-CyclinD1-CDK4-Rb pathway appears to be frequently dysregulated in bronchial carcinogenesis: this is possibly an obligatory step. Phosphorylation of Rb protein leads to its dissociation from bound E2F transcription factor protein and this initiates progression from G1 into S-phase of the cell cycle. The CyclinD1-CDK4 complex promotes this process; p16 inhibits it by inhibiting CDK4. Thus, cell cycle progression may be promoted by loss of p16 or Rb function, or up-regulation of CyclinD1. There is evidence, mainly from animal models, that the manner in which this pathway is disrupted is associated with, and may even be deterministic of, the type of tumour which arises from the bronchial epithelium. Loss of p16 function, probably by a combination of LOH at 9p21 and hypermethylation of the remaining allele, is associated with squamous cell carcinoma while RB loss is associated with SCLC development.

Molecular changes in peripheral airway (TRU) carcinogenesis

Compared with data available on smoking-related molecular events in bronchial carcinogenesis, much less is known about this in peripheral adenocarcinogenesis. There are very few such studies of morphologically normal peripheral lung epithelium and while there is an expanding literature on the genetics of AAH and AdCIS, little of this can be related to smoking effects. This literature is dominated by work from eastern Asia, especially Japan. Smoking seems to account for fewer lung cancers in this region, adenocarcinomas are particularly common, as are patients with AAH and AdCIS. How these factors inter-relate is unclear. In clinicopathological studies of AAH in Japan, there is no clear association between smoking and AAH prevalence, in Scotland most patients with AAH are smokers but this finding could be confounded by a lack of non-smoking lung cancer resections. AAH may be caused by tobacco-carcinogens, but also by other factors; it seems likely that AAH lesions may progress under the influence of such toxins.

'Normal' peripheral epithelium

In one study peripheral lung tissue in adenocarcinoma patients failed to show evidence of 3p, 9p or 17p LOH in smokers or non-smokers. In another, using HG-U133A gene expression array analysis, twenty seven genes were differentially expressed in the peripheral lung between smokers and non-smokers. Among the 7 upregulated genes were CyclinE1 and the XME GPX-2; down-regulated genes included XME FMO3. Another HG-U133A-based study identified a smoking gene signature in non-neoplastic peripheral lung, which seemed to persist for years after smoking cessation. Amongst the genes showing differential expression in smokers were up-regulation of CYP1B1 and several genes related to cell cycle promotion (NEK2, CENPF, TTK). Immune response genes were down-regulated, most notably CX3CR1, which is located at 3p21.3, a locus frequently deleted in smokers.

AAH and AdCIS

Genetic data related to smoking are lacking in these lesion. As with SD/CIS, there are studies with AAH and AdCIS looking at a variety of pathways related to carcinogenesis. Cell proliferation is certainly increased in AAH and AdCIS compared to the surrounding lung tissue. CYP1A1&2, CYP2B1&2 and CYP2E1 have been demonstrated in excess amounts in AAH. CyclinD1 was shown to be upregulated but neither p16 nor Rb loss was found in AAH. On the other hand there have been reports of 9p and 13q LOH in limited numbers of AAH lesions. Hypermethylation of p16 in AAH has also been found.

Loss of FHIT does not, however, seem to be a feature of AAH or AdCIS development. There is evidence of limited over-expression of p53 protein in AAH, more so in higher grade lesions and in AdCIS. Evidence of P53 mutation is scanty, as is 17p LOH, although both these features are more common in higher grade lesions. LOH studies in AAH and AdCIS in general find less change than in SD/CIS, although a low level incidence of various 3p, 9p and even 13p losses are reported. In AdCIS rather more losses are described, including additional loss at 5q, 11q, 18q and 22q. Of interest is the finding of 9q losses on AAH, including putative TSGs TSC-1 & -2. Most of these data are derived from Japanese studies where many of the patients with AAH will be non-smokers. The variability of findings, especially for p53 could be explained by smoking differences within and between study groups.

Adenocarcinomas

The genetics of invasive adenocarcinomas may provide clues to those events which are important before invasion occurs. A range of studies on established adenocarcinomas has looked for differences between smokers and non-smokers. Smokers adenocarcinomas, even at a small size, tend to be more solid-pattern, necrotic and invasive, implying more rapid progression of disease. They are more frequent in the upper lobes.

An LOH study showed rather more losses at 3p (FHIT) and 17p (P53) in smokers adenocarcinomas compared to those in non-smokers. Those gene expression array studies mentioned above also demonstrate significant differences in the up and down regulation of various genes, related to XMEs, cell cycle progression, cell-cell adhesion and cell migration.

Key pathways

There is now substantial evidence that the EGFR and related pathways downstream of the EGFR membrane bound receptor are of key importance in carcinogenesis within the TRU. This pathway complex may be driven by EGFR mutation or KRAS mutation; such mutations appear to be mutually exclusive, and there is good evidence that the former mutation is associated with adenocarcinomas in never smokers while the latter is more often found in smokers' tumours. It has also been shown that KRAS mutant smokers' adenocarcinomas are more likely to have p16 methylation.

Two further Japanese studies, without smoking data, provide further points of interest. Firstly, that while maintaining exclusivity within AAH and AdCIS, KRAS mutation was more frequent than EGFR mutation in AAH but that progression of disease seems to be associated with a rapid loss of KRAS mutation but more frequent EGFR mutation. This implies that EGFR mutation is more important in progression of AAH, at least in this population. Reported data on the frequency of EGFR mutation in AAH are however, widely variable. KRAS mutation is more frequent in the more advanced stages in European and North American studies, perhaps due to a greater prevalence of smoking. Secondly, certain KRAS polymorphisms are associated with adenocarcinomas and multiple AAH lesions.

It is tempting to believe that adenocarcinogenesis in the smoker is promoted through KRAS mutation and RAS signaling rather than EGFR mutation and PI3K/AKT mediated pathways.

While most consideration is given to AAH as a precursor of peripheral TRU type adenocarcinomas, atypical proliferations of bronchiolar or small bronchial epithelium have been suggested as possible adenocarcinoma precursors. Chromosomal gains and losses, p16 loss, p53 overexpression, KRAS and EGFR mutations have all been described in this context but any the relationship with smoking is unknown.

Conclusion

There is good evidence that there are fields of cellular change within the lung in patients who smoke. A bronchial epithelial field change is more associated with squamous carcinoma and possible small cell lung cancer. A field change in the peripheral lung epithelium is associated with adenocarcinogenesis.

Inherited polymorphisms of various genes including those encoding for the nicotinic acetylcholine receptor, various xenobiotic metabolizing enzymes and DNA repair enzymes determines how an individual 'interacts' with tobacco carcinogens and confers variable risk of developing lung cancer. Particular enzymes coupled with changes in smoking pattern and carcinogen constituents of smoke may explain why one or other of these fields of cancerization develops in any particular individual.

A range of genetic changes can be identified, including in morphologically normal airway epithelium, which appear to be important in lung carcinogenesis and which are related to tobacco smoke exposure. The frequency and range of these changes increases from

normal epithelium through atypical/dysplastic lesions and carcinoma-in-situ to invasive disease.

These molecular changes have the potential to be targets for early detection of lung cancer and chemoprevention of the disease. Smoking cessation, however, still appears to be the 'best bet' for reducing lung cancer mortality.

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Smoking-Related Small Airway Disease
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Background:

Small airways are defined as airways with an internal diameter of less than 2mm. The small airways are devoid of cartilage and consist of the membranous, terminal and respiratory bronchioles. The small airways consist of simple columnar cells (ciliated and non-ciliated) and rare neuroendocrine cells overlying a thin layer of connective tissue and smooth muscle. Small airways normally contribute little to airway resistance but abnormalities may have a disproportionate effect on lung function.

Overview of small airway disease:

Small airways may be the primary site of pathology or may be affected in association with a wide array of lung diseases including bronchiectasis, asthma, chronic obstructive pulmonary disease (COPD), respiratory bronchiolitis-interstitial lung disease/desquamative interstitial pneumonia (RB-ILD/DIP), hypersensitivity pneumonitis (HSP), cryptogenic organizing pneumonia (COP), Langerhans cell histiocytosis (LCH) and the more recently described airway centered interstitial fibrosis/bronchiolocentric interstitial pneumonia, among others.

Overall, the small airways have not been as extensively studied as the interstitial lung diseases and thus there is no consensus classification for small airway pathology. Further, the histologic findings are largely not specific for a particular etiology and as such, a multi-disciplinary approach is warranted in the evaluation of small airway pathology.

General histologic patterns of small airway disease:

While the focus of this program is on small airway disease related to cigarette smoking, the following is a brief overview of the spectrum of small airway disease in order to provide an appropriate context of COPD in the larger arena of small airway pathology. Smoking related small airway disease may exhibit chronic peribronchiolar inflammation or fibrosis in either a pattern of constrictive bronchiolitis or peribronchiolar metaplasia; patterns which are non-specific by themselves.

Non-specific patterns of small airway disease generally fall into two broad categories-cellular/inflammatory vs fibrotic. The findings may be subtle. The adjacent lung may have foamy macrophage accumulation and/or dilatation of distal bronchioles with mucostasis, which should prompt evaluation of the small airways for pathologic findings. The following represents an overview of histologic patterns of small airway disease and the most commonly associated etiologic agents.

Cellular bronchiolitis may consist of acute (neutrophilic inflammation), acute on chronic (chronic inflammation within the bronchiolar wall and neutrophils within the lumen) or chronic inflammation. The patterns by themselves are not specific and there is much overlap among the potential etiologies for each. In all situations it is important to look for sources of infection, particularly viral inclusions or aspirated material. In all cases it is important to know the clinical distribution of disease, as localized disease may imply association with aspiration or bronchiectasis whereas diffuse disease has differing implications. Acute bronchiolitis typically occurs in association with infection, fume

exposure, aspiration or occasionally Wegener's. Acute or chronic typically occurs in association with infection, inflammatory bowel disease, collagen vascular disease or aspiration. Chronic bronchiolitis may occur distal to bronchiectasis or in association with collagen vascular disease, inflammatory bowel disease or aspiration, among other etiologies.

Fibrotic small airway disease consists of three patterns: Constrictive bronchiolitis, intraluminal fibrosis (bronchiolitis obliterans) and peribronchiolar metaplasia with fibrosis (Lambertosis). An associated inflammatory component may or may not be present. Constrictive bronchiolitis consists of subepithelial collagen deposition with airway narrowing and constriction, and is generally considered to be secondary to chronic airway damage with abnormal healing response. Constrictive bronchiolitis is associated with mosaic air trapping on CT and may be extremely subtle histologically, requiring evaluation of multiple levels or the use of trichrome and elastic stains. Constrictive bronchiolitis may occur secondary to a number of disorders including transplant rejection, fume/toxin exposure, infection, collagen vascular disease, drug reaction, secondary to cigarette smoke or distal to bronchiectasis. Some cases are idiopathic.

The intraluminal pattern (bronchiolitis obliterans) consists of organization of luminal inflammatory exudates evidenced by polypoid plugs of granulation tissue. This pattern rarely occurs as an isolated finding and is more often seen in association with airspace organizing pneumonia as seen in COP or HSP. Isolated bronchiolitis obliterans does occasionally occur and has been reported in association with fume inhalation among other etiologies.

Peribronchiolar metaplasia and fibrosis is a non-specific finding probably representing the end result of a wide range of airway injuries and is frequently seen as incidental findings in resections for lung cancer where it is felt to be likely secondary to chronic smoking. Of note, three groups have described a pattern of interstitial lung disease consisting only of this finding using differing names-bronchiolocentric interstitial pneumonia, peribronchiolar metaplasia-interstitial lung disease and airway centered interstitial fibrosis. Two of the studies report a poor prognosis. The significance of this pattern and its relationship to other lung disease requires further study.

Some patterns of bronchiolitis, while not entirely specific, might point to certain etiologies. These include follicular bronchiolitis, eosinophilic bronchiolitis and granulomatous bronchiolitis. Follicular bronchiolitis consists of lymphoid hyperplasia +/- airway obstruction and may be seen in association with collagen vascular disease, particularly rheumatoid arthritis or secondary to bronchiectasis. Eosinophilic inflammation more typically involves the large airways but may be seen as an extension of asthma, allergic bronchopulmonary aspergillosis or drug reaction. Granulomatous bronchiolitis should suggest infection, sarcoid, hypersensitivity pneumonitis, aspiration, Crohn's disease or drug reaction. Two other rare entities, diffuse panbronchiolitis and diffuse idiopathic neuroendocrine cell hyperplasia (DIPNECH) are also specific patterns of disease centered on the small airways.

Small airway disease in the context of smoking-related lung disease:

Described smoking related lung diseases include RB-ILD/DIP, LCH and COPD. More recently, RB-ILD with fibrosis and airspace enlargement with fibrosis have been described, and further study of the significance of these patterns is warranted.

COPD is typically considered to encompass chronic bronchitis and emphysema but in actuality consists of four anatomic lesions, including emphysema and chronic bronchitis as well as small airway remodeling and vascular remodeling/hypertension. While the lesions frequently occur together, the mechanisms for the alterations in each anatomic lesion are thought to differ.

From a clinical standpoint, evaluation of the severity of COPD is based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria which are summarized below:

GOLD Staging System for COPD Severity

Stage	Description	Findings (based on postbronchodilator FEV1)
0	At risk	Risk factors and chronic symptoms but normal spirometry
I	Mild	FEV1/FVC ratio less than 70 percent FEV1 at least 80 percent of predicted value May have symptoms
II	Moderate	FEV1/FVC ratio less than 70 percent FEV1 50 percent to less than 80 percent of predicted value May have chronic symptoms
III	Severe	FEV1/FVC ratio less than 70 percent FEV1 30 percent to less than 50 percent of predicted value May have chronic symptoms
IV	Very severe	FEV1/FVC ratio less than 70 percent FEV1 less than 30 percent of predicted value or FEV1 less than 50 percent of predicted value plus severe chronic symptoms

Direct measurements of pressure and flows within the lung indicate that the small airways are the major site of airway obstruction in COPD. Reduced expiratory flow results from reduction of the lumen by peribronchiolar fibrosis, thickening of small airway walls and occlusion by mucus. Remodeling of small airway wall tissue has been found to correlate more with decreased FEV1 than inflammation

As early as 1957, Leopold and Gould made the observation of inflammation and connective tissue deposition in small airways in association with emphysema, which led to the postulation that extension of the chronic inflammatory process from the terminal into the respiratory bronchioles initiated centrilobular emphysematous destruction. Since that time, most research has focused on emphysema for a variety of reasons, and small airways have only more recently returned as an intense focus of study. Emphysema and small airway disease are typically found in association with one another, and thus a brief review of the mechanisms of emphysema follows for background and comparison. Interestingly, small airway disease appears to be independent of the presence of chronic bronchitis/large airway disease.

The classic theory of emphysema is based on the protease/anti-protease theory, extending from the knowledge that patients with alpha-1-antitrypsin (A1AT) deficiency

develop emphysema. The premise of this theory is that smoke incites an inflammatory reaction which, in turn, results in the release of proteases which overcome the anti-proteolytic defenses and ultimately lead to matrix destruction and emphysema. A variety of proteases have been implicated including serine proteases, metalloproteases and cysteine proteases, although which inflammatory cells and proteases are most critical is complex and controversial. It is generally accepted that smoke invokes an inflammatory response which induces pro-inflammatory cytokines and that smoke induces an increase in expression of chemoattractant and pro-inflammatory mediators, but the precise mechanisms are the subject of much debate. The following represents an overview of some of the more widely studied mediators and is by no means comprehensive. The interested reader is referred to the many excellent reviews on this subject, some of which are referenced below.

Neutrophil elastase is a serine protease that was originally postulated in the development of emphysema. Most studies have supported a role for neutrophils and neutrophil elastase in the development of emphysema. The hypothesis is further supported by lavage studies demonstrating increased neutrophils correlating with increased levels of desmosine and hydroxyproline, which are markers of elastin and collagen breakdown, respectively. Most studies have also demonstrated that tumor necrosis factor-alpha is presumed to be a driver of inflammatory cell influx.

Metalloproteases (MMP) have also been widely studied in regard to the development of emphysema. In mouse models, cigarette smoke increases levels of MMP's 2, 9, 12, 13 and 14. MMP-9 and MMP-12 are of particular interest as they degrade elastin. A link between the original neutrophil elastase theory and metalloproteases exists in that neutrophil elastase activates MMP-12 and decreases tissue inhibitor of metalloprotease-1 (TIMP-1). MMP-12 had also been found to degrade A1AT. Thus, MMP-12 likely has both a signaling role and a direct destructive role. Additionally, MMP inhibition/deletion eliminates development of emphysema in mouse models.

Failure of repair is a key difference between the development of emphysema and small airway remodeling, which generally occurs in association with increased fibrosis. In emphysema, alveolar walls fail to regenerate new matrix. Smoke decreases lysyl oxidase which is critical in the formation of collagen. Smoke also interferes with cell proliferation, chemotaxis, production of matrix components by fibroblasts and increases apoptosis. Why the alveoli and small airways respond differently to the same stimuli has continued to be a puzzle and subject of debate.

Small airway disease has not been as extensively studied as emphysema, but has been assumed to follow similar pathways as emphysema. Hogg, et al (Hogg, *NEJM* 2004), evaluated small airways from 159 surgical specimens and correlated the pathologic findings with the clinical GOLD stage. In this study, progression of COPD correlated with an increase in volume of tissue in the wall, accumulation of mucus exudates, and the percentage of airways containing neutrophils, macrophages, CD4 cells, CD8 cells, the total volume of B-cells and the presence of lymphoid aggregates. Such findings appear to support the role of inflammatory cells in the development of small airway disease. However, Churg, et al (Churg *AJRCCM* 2006), in an elegant study using laser capture microdissection, demonstrated that smoke upregulated gene expression of type 1 pro-collagen and profibrotic cytokines, particularly those related to TGF-beta signaling. Of interest, the elevations were seen at 2 hours following exposure and then decreased, in contrast to BAL inflammatory cells which

increased slowly over 24 hours. Such findings suggest that upregulation of the fibrotic response may be independent of inflammation.

Other studies have demonstrated that upregulation of TGF-beta effects the Smad signaling pathway which results in increased collagen production. A possible role of VEGF and increased microvessel density in small airway remodeling has also been demonstrated but these findings are inconsistent. The role of neutrophils remains uncertain, although some studies suggest that oxidant release from neutrophils and macrophages may potentiate TGF-beta release.

Genetic predisposition

Only 10-20% of heavy cigarette smokers will develop COPD and COPD cases have been observed in familial clusters. Thus, it would appear that COPD develops in genetically susceptible individuals following sufficient exposure to cigarette smoke. Genetic polymorphisms related to levels of antiproteases, metalloproteases, pro-inflammatory and pro-fibrotic cytokines have been reported; however, molecular studies of genetic risk factors predisposing to the development of COPD are still in the infancy stage and will be the subject of much further study.

Future issues and directions:

COPD continues to be a major source of morbidity and mortality. The factors governing matrix destruction in emphysema in contrast to matrix production in small airway remodeling continues to be investigated. Evaluation of molecular targets will hopefully lead to the identification of potential therapeutic targets, although translation of findings in animal models to those in humans has been imperfect. For example, TGF-alpha inhibitors diminish the development of emphysema in mouse models but have not been demonstrated to be clearly efficacious in humans at this time. Genetic factors contributing to the risk of developing COPD will also be an area of further study. The recent description of fibrotic lung disease associated with cigarette smoking in conjunction with emphysema and airspace enlargement also requires further elucidation.

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