Drugs for the Treatment of Respiratory Diseases

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CAMBRIDGE
UNIVERSITY PRESS

PUBLISHED BY THE PRESS SYNDICATE OF THE UNIVERSITY OF CAMBRIDGE
The Pitt Building, Trumpington Street, Cambridge, United Kingdom

CAMBRIDGE UNIVERSITY PRESS
The Edinburgh Building, Cambridge CB2 2RU, UK
40 West 20th Street, New York, NY 10011–4211, USA
477 Williamstown Road, Port Melbourne, VIC 3207, Australia
Ruiz de Alarcón 13, 28014 Madrid, Spain
Dock House, The Waterfront, Cape Town 8001, South Africa

http://www.cambridge.org

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First published 2003

Printed in the United Kingdom at the University Press, Cambridge

Typeface Utopia 8½/12 pt. *System* QuarkXPress® [SE]

A catalogue record for this book is available from the British Library

Library of Congress Cataloguing in Publication data

Drugs for the treatment of respiratory diseases / edited by Domenico Spina . . . [et. al.].
p. ; cm.
Includes bibliographical references and index.
ISBN 0 521 77321 0 (hardback)
1. Lungs – Diseases – Chemotherapy. 2. Respiratory agents. I. Spina, Domenico.
[DNLM: 1. Respiratory Tract Diseases – drug therapy. 2. Anti-Allergic Agents – therapeutic use. 3. Respiratory System Agents – therapeutic use. WF 145 D7947 2002]
RC756 .D784 2002
616.2′4061–dc21 2002023390

ISBN 0 521 77321 0 hardback

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1

Pathology of asthma and COPD : inflammation and structure

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Introduction

It is widely recognized that neither asthma nor COPD are disease entities but rather each is a complex of inflammatory conditions that have in common airflow limitation (syn. obstruction) whose reversibility varies (Fig. 1.1). The characteristics and distinctions between mild stable asthma and COPD have been reviewed[1,2]. However, these differences become less clear when the conditions become severe or there are exacerbations due to infection or other cause. An understanding of whether or not there are fundamental differences of inflammation and airway/lung structure between these two conditions is relevant to clinical decisions regarding both initiation and long-term treatment and to patient management during exacerbations. In the longer term it is of value to the design of specific therapy for asthma and COPD and to their prevention. Whilst the definitions of asthma and COPD highlight the differing degrees of airflow variability and reversibility[3,4], there is a prevailing clinical impression that, with age, there is often overlap and a progression from the reversible airflow obstruction of the young asthmatic to the more irreversible or 'fixed' obstruction of the older patient with COPD. The Dutch hypothesis encompasses the idea that both conditions are extreme ends of a single condition[5]. In the author's opinion it may, in the future, be less relevant to be concerned with the clinical labels of 'asthma' or 'COPD' and more important to ascertain and target treatment to the predominant pattern of inflammation and structural change that prevails in each patient.

Asthma may be divided into extrinsic (also called allergic or atopic), intrinsic (late onset or non-atopic) and occupational forms. At this time the pathologist cannot distinguish between these distinct clinical forms of asthma: there are alterations that appear to be common to all forms. COPD is associated, usually, with the smoking habit as the relationship between cigarette smoking and COPD is a strong one statistically. Three conditions can contribute, the degree varying in each patient, to the clinical expression of COPD: chronic bronchitis (syn. mucus hypersecretion), chronic bronchiolitis (syn. small airways disease) and emphysema, inflammatory conditions broadly affecting bronchi (airways with cartilage in their wall), bronchioli (membranous or non-cartilaginous airways) and lung parenchyma respectively. In both asthma and COPD, the persistence of distinct inflammatory cells initiated by allergen or cigarette smoke, respectively, is probably responsible for most of the structural change and usually referred to as 'remodelling': interactions with the effects of acute and chronic infection and genetic predisposition are clearly important also.

The chapter focuses on the patterns of infiltrating inflammatory cells in asthma and COPD and the associated remodelling of the airway wall. First, airway wall thickening is considered, particularly in asthma, remodelling is defined and the relationship between inflammation and remodelling discussed briefly. Lumenal secretions obtained as sputum or lavage and asphyxic plugging of the airways with mixtures of mucus and inflammatory exudate are discussed briefly. The chapter then divides into two

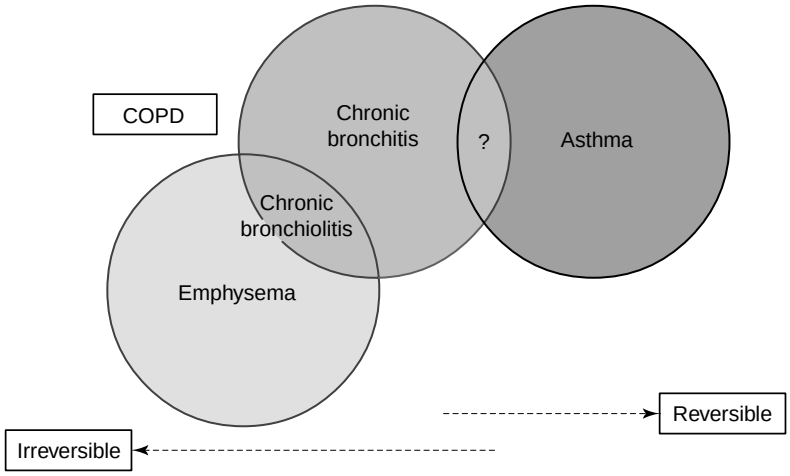


Fig. 1.1 Venn diagram illustrating the overlap between asthma and COPD.

major sections considering first inflammation and then remodelling in asthma and COPD. The results of examination of the conducting airways by flexible fibre-optic bronchoscopy are included as this technique has provided the means by which the early inflammatory and structural alterations of asthma and COPD have been compared, free from the complications of end stage disease[6].

Airway wall thickening

The airway walls in asthma are thickened by the remodelling process by between 50 and 300% of normal and there is lumenal narrowing, which is further compromised by excessive mucus admixed with an inflammatory exudate (Fig. 1.2, see colour plate section). In cases of fatal asthma, the longer the duration of asthma, the thicker becomes the airway wall[7]. However, it has been suggested that airway wall thickness *per se* is not a requirement for asphyxic fatality as a group of relatively young asthmatics (i.e. with a relatively short history of asthma) had an airway wall thickness not significantly different from that of non-asthma controls. Lumenal secretions and plugging are likely the greater contribution to asthmatic death in these young cases of fatal asthma[7]. All tissue structural components, as well as inflammatory cell infiltration and edema, can contribute to the observed thickening; however, in the last mentioned study it is thickening of the (outer) adventitial layers that was most pronounced in the older group with the longest duration of disease. The airway walls are also thickened in COPD. One systematic study has described changes in large airway dimensions in relation to the lung function of patients with COPD and found wall area internal to the muscle to be significantly thickened over the entire range of cartilaginous airways measured[8]. The relative contributions of the airway wall components contributing to the thickening, however, vary with airway generation.

Inflammation and remodelling

Acute inflammation is the response of vascularized tissue to injury: the inflammatory reaction is designed to protect the host and to restore tissue and its function to normal. One generally accepted proposal is that the accelerated decline in forced expiratory flow over time in COPD, and that which occurs also in an important subset of asthmatics, is the direct result of a switch from acute, episodic, to chronic inflammation and to consequent airway and parenchymal remodelling[1]. The proposal is

attractive but, as yet, there is no convincing evidence that the remodelling process is dependent upon the prior development of chronic inflammation. It is equally plausible that the processes responsible for the development of chronic inflammation are distinct to those responsible for remodelling. The last consideration has important implications for the design of disease modifying therapy: thus those agents that are effective antiinflamatory compounds will not necessarily prevent or attenuate the process of remodelling for which new classes of drugs will be required.

Definition

The concept of 'remodelling' implies that a process of 'modelling' must have preceded it. The lung, in utero, undergoes extensive modelling and remodelling yet these processes are entirely appropriate to the normal process of lung development. Many of the cytokines and growth factors thought to be pro-inflammatory in asthma and in COPD are also expressed normally without detriment to the developing lung; these include: members of the fibroblast growth factor family, the transforming growth factor family, epithelial-derived growth factor, granulocyte–macrophage colony stimulating factor, platelet-derived growth factor, vascular endothelial growth factor and hepatocyte growth factor[1,9]. Accordingly the working definition of remodelling proposed herein recognizes that the process of remodelling *per se* is not of necessity abnormal. It is: an alteration in size, mass or number of tissue structural components that occurs during growth or in response to injury and/or inflammation. It may be appropriate, as in normal lung development or that which occurs during acute reaction to injury, or 'inappropriate' when it is chronic and associated with abnormally altered tissue structure and function as, for example, in asthma or COPD.

In wound healing (in the skin) the components of an appropriate response include: clot formation, swelling/edema, rapid restitution of the denuded areas by epithelial dedifferentiation, proliferation and migration from the margins of the wound. This is normally associated with an inflammatory reaction, i.e. early infiltration of the injured tissue by neutrophils and later by lymphocytes and macrophages. Reticulin is deposited within days and this may mature to form interstitial collagen, a scar, within 2–3 weeks. In addition, healing may involve contraction of the surrounding tissue (in the case of an open wound), by myofibroblasts that may proliferate transiently in relatively large numbers[10]. Vasodilatation, congestion and mucosal oedema are also cardinal signs of acute inflammation and the angiogenesis of the granulation tissue is an integral part of the reparative response. Thus, normal tissue architecture and function is restored consequent to an entirely appropriate inflammatory reaction with which there has been an associated remodelling process. Each of these stages in normal wound healing and many of the inflammatory cell types and cytokines involved appear also in asthma and in COPD, but in these last two conditions both the inflammation and remodelling persist and result in exaggerated remodelling inappropriate to the maintenance of normal (airway) function. The reasons for the persistence of the inflammation are unknown but may be the result of repeated inhalation of allergen or exposure to high concentrations of allergen, irritation (e.g. by tobacco smoke) or persistent infection or a genetically influenced abnormal host inflammatory response or a defective repair process.

Lumenal secretions

Sputum and bronchoalveolar lavage

The examination of spontaneously produced or saline-induced sputum has become a much used and relatively non-invasive method for determining the extent of inflammation in the asthmatic airway[11,12] (Fig. 1.3(*a*), 1.2(*b*), see colour plate section). Corkscrew-shaped twists of condensed mucus (Curshmann's spirals), clusters of surface airway epithelial cells (referred to as Creola bodies and named after the first patient in whom they were described)[13], and the presence of Charcot–Leyden

crystals, composed of eosinophil cell and granule membrane lysophospholipase (Fig. 1.3(*a*), (*b*), see colour plate section[14]), together with eosinophils and metachromatic cells, are characteristic features of sputa obtained from asthmatic, but not bronchitic patients[15]. Sputum eosinophilia has, however, also been reported in non-asthmatics in the absence of the airways hyper-responsiveness (AHR) characteristic of asthma[16]. In contrast, sputa from bronchitic patients may be mucoid or, during infective exacerbations, purulent when neutrophils may be present in large numbers. BAL in mild (allergic) asthma demonstrates the presence of sloughed epithelial cells, the numbers of which show an association with AHR,[17] and of eosinophils and their highly charged secreted products (such as eosinophil cationic protein (ECP) and major basic protein (MBP))[18]. In contrast, in smoker's bronchitis, macrophages are the most usually reported cell type and neutrophils are numerous as are their secreted products.

Airway plugging

Examination, postmortem, of cases of fatal asthma has shown that the lungs are hyperinflated and remain so on opening the pleural cavities due to the widespread presence of markedly tenacious airway 'plugs' in both large (segmental) and small bronchi (Fig. 1.4(*a*)), see colour plate section). Even intrabronchial inflation with fixative to a 1.5-metre head of pressure hardly moves these sticky lumenal plugs[19]. Histologically the airway plugs in asthma are composed predominantly of inflammatory exudate together with mucus in which lie: eosinophils, lymphocytes and desquamated surface epithelial cells. The arrangement of the eosinophilic elements of the plug is often as concentric, multiple lamellae suggesting that several episodes of inflammation have led to their formation rather than a single terminal event (Fig. 1.4(*b*), see colour section). The non-mucinous, proteinaceous contribution is the result of increased vascular permeability and includes a fibrin. Electrostatic interaction of positively charged (cationic) eosinophil products and serum constituents and negatively charged (due to carboxyl and sulfate groups) mucin likely contributes to the particular stickiness of the airway plug. There are, however, reports of sudden death in asthmatics in which intraluminal plugs are absent[20] but these are rare. In the absence of a history of smoking, emphysema in fatal asthma and right ventricular hypertrophy is uncommon. However, areas of atelectasis and petechial hemorrhages may be present in asthma due to bronchial obstruction, reabsorption collapse and repeated forced inspiratory efforts. The asthmatic who has smoked will likely have features which overlap between asthma and COPD and, in these cases, there may be focal evidence of centriacinar (i.e.bronchocentric) alveolar destruction (see Fig. 1.4(*a*), colour plate section).

Inflammation

To the physiologist, inflammation is characterized by cardinal signs: redness, heat, swelling, pain and loss of normal function. To the pathologist, inflammation is recognized in tissue sections as congestion of vessels together with the recruitment (i.e. margination within and emigration from vessels) of a variety of morphologically and immuno-phenotypically distinct inflammatory cells. It is now recognized that both asthma[21,22] and COPD are inflammatory conditions albeit the relative magnitude and site of the inflammatory infiltrate and the predominant inflammatory cell phenotype differs.

Asthma

Studies of biopsies obtained by fibreoptic bronchoscopy or at open lung biopsy in asthma demonstrate the presence of an inflammatory cell infiltrate even in patients with newly diagnosed asthma[23]. The infiltrate comprises CD3 immuno-positive (T) lymphocytes of the CD4 (i.e. T-helper) subset and eosinophils[17,24–26]. An increase in leukocytes, including lymphocytes and eosinophils, occurs in relatively mild atopic, occupational and intrinsic asthma and it is associated with an increase of 'activation' markers for both lymphocytes (CD25 + cells)

and eosinophils (EG2 + cells)[21,24,26–28]. In symptomatic atopic asthmatics, in electron microscopic studies, irregularly shaped lymphomononuclear cells appear and these may represent ultrastructural forms of the CD25 + (activated) lymphocyte. EG2 is a marker for the cleaved ('secreted') form of eosinophil cationic protein that can be found both within eosinophils and diffusely in the wall, often in association with the epithelial reticular basement membrane. Eosinophil-derived products such as major basic protein[29] together with toxic oxygen radicals and proteases probably all contribute to the epithelial fragility described in asthma (see below). Eosinophil cytolysis or disintegration and release of granules[30,31] and of cytokines may also stimulate nearby fibroblasts to produce additional reticulin and so induce thickening of the reticular basement membrane.

In fatal asthma there is a marked infiltrate throughout the airway wall, in sputum and also in the occluding plug. Compare Fig. 1.5(*a*) and (*b*), see colour plate section, see Figs. 1.3(*a*) and 1.4(*b*), see colour plate section: lymphocytes are abundant[22,32,33] and (EG2+)eosinophils are characteristic (Fig. 1.6, see colour plate section)[22,34,35]. Neutrophils are sparse in mild asthma[21] albeit they are present in relatively large numbers in sputa during infective exacerbations[36], in biopsies of severe asthmatics refractory to high dose treatment with corticosteroids[37] and in status asthmaticus when death is sudden (i.e. within 24 hours of the attack)[38]. It has been suggested based on examination of biopsy tissue that two forms of asthma may be usefully distinguished: those with a relatively high eosinophil count and those with predominant neutrophilia[39]. The inflammation of the airway wall may involve the adjacent pulmonary artery[33] and, in small (distal) airways, may spread to surrounding alveolar septae[40]. Alveolar walls may thus show evidence of eosinophilic infiltration[40] and alveolar spaces may contain a fibrillar-rich component, most likely fibrin (author's unpublished observations). However, destruction of the parenchyma (i.e. emphysema) is not a feature of asthma. Thus, both small and large airways may be inflamed in asthma: transbronchial biopsy studies of relatively severe asthma and studies of resection tissue in asthmatics have demonstrated infiltration of bronchioli by eosinophils and lymphocytes[40,41]. There are also recent data in severe asthma that demonstrate the inner wall to be infiltrated by neutrophils in numbers considerably greater than in larger airways[42]. Thus the pattern of inflammation in severe asthma appears to be different from that in mild and, in order to be effective, treatment needs to be tailored accordingly. The association of tissue eosinophilia and asthma is a strong one. However, the extent of tissue eosinophilia varies greatly with each case and with the duration of the terminal episode[22,38,43]. The variation may be due, in part, to eosinophil degranulation, which makes cell identification difficult. In comparison with mild asthma, fatal asthma is reported to be associated with a higher concentration of eosinophils in the large airways and a reduction of lymphocytes in the peripheral (smaller) airways[35].

The role of the activated T-helper (Th) lymphocyte in controlling and perpetuating the chronic inflammatory reaction in asthma has received much attention[24,44]. The T-lymphocyte is thought to control allergic inflammation via the selective release of the proinflammatory cytokines (interleukins) IL-4 and IL-5, which characterize the T-helper (type 2) phenotype[45]. IL-5 gene expression has been shown to be increased in bronchial biopsies from symptomatic atopic asthmatic subjects[46] (Fig. 1.10), and this is supported by studies of cells obtained at bronchoalveolar lavage[47,48] and peripheral blood[49]. IL5 appears to be a key cytokine required to induce terminal differentiation of eosinophils and, together with IL4, enhances their vascular retention and longevity in tissues. It is also a key cytokine in the late phase reaction to allergen challenge[48]. IL4 is also increased in atopic asthma[50,51] and may be important in both the initiation and persistence of allergic inflammation. IL4 encourages the selective recruitment of eosinophils by up-regulating adhesion molecules (V-CAM) on bronchial endothelium whose ligand on the eosinophil cell surface is VLA-4. The last is absent from the surface of neutrophils

and helps to explain the eosinophil predominance in mild asthma. There is currently debate as to the involvement of IL4 in asthma of the intrinsic (i.e. non-atopic) form[52]. IL4 and IL5 are not, however, unique to asthma and may occur in other inflammatory conditions such as fibrosing alveolitis[53]. Whilst IL5 may be important in promoting eosinophil terminal differentiation, and the release of eosinophils into the blood from bone marrow, other molecules such as eotaxin and RANTES (regulated on activation normal T-cell expressed and secreted) are involved as selective chemokines inducing eosinophil emigration from blood vessels and their migration through the mucosa to the airway lumen from whence they are cleared[54–56]. The same or distinct molecules may be involved in eosinophil activation, a process about which little is as yet known. Symptomatic asthma is associated with the production of additional cytokines including TNFα, GM–CSF, IL1β, IL2 and IL6[45,57]. GM–CSF has also been reported to increase during the late phase reaction to allergen[58]. In addition to their production of toxins and lipid-derived mediators, eosinophils themselves may also produce proinflammatory cytokines and growth factors as evidenced by their gene expression for TNFα, IL6 and GM-CSFβ[45,59,60]. Macrophages have been reported to increase in number in more severe asthma of the intrinsic form[28].

Mast cells have long been thought to play a key role in the immediate (type I sensitivity) reaction in asthma through their release of a variety of mediators including those which bronchoconstrict i.e. histamine, prostaglandin D_2 and leukotriene D4. Mast cells are now thought to act as an important source of IL4 and other proinflammatory cytokines whose secretion may act as a trigger to the induction of subsequent persistent production of IL4 and IL5 by lymphocytes[61,62]. There are reports of decreases, increases and no change of mast cell numbers. Early biopsy studies demonstrated an apparent reduction in bronchial mast cell numbers in asthma due to their degranulation[63]. Studies of bronchoalveolar lavage report increased intralumenal mast cell numbers together with increased numbers of T-helper cells and eosinophils and evidence of histamine release and of eosinophil degranulation[18,64,65].

Although considered to be important in allergic conditions, little is known of the role of basophils in these conditions albeit there is evidence for increased recruitment of basophils and their precursors to sites of allergic reaction in atopic patients[66]. Asthma is also characterized by infiltration of the bronchial surface epithelium by dendritic cells (i.e. Langerhans' cell equivalent)[67]. These non-phagocytic histiocytes are rich in surface receptors and their functions are thought to include the presentation of antigenic information to T lymphocytes; very few Langerhans' cells are found in the normal lung although there is a rich network of their probable precursor dendritic cells[68]. Thus lymphocytes of the T-helper (CD4+) subset appear to be key to the controller cell and eosinophils the prime effector cell in mild asthma. However, with increasing severity of asthma and in infective exacerbations there is an increasing involvement of neutrophils and perhaps also of macrophages and these changes appear to be more refractory to conventional treatment with inhaled or even oral corticosteroids. Alternative approaches would seem to be required to treat more severe than mild asthma and the reasons for this may in part be explained by the altered pattern of inflammation.

COPD

T-lymphocytes appear also to be key controller cells in COPD but in contrast to asthma it is the CD8+ cells that are the predominant cells in COPD[69]. It is currently presumed that the majority of these CD8+ cells are T-lymphocytes of the cytotoxic/suppressor subset, but this is as yet unproven and these may also include natural killer cells and even a dendritic cell sub-type. The altered CD8:CD4+cell ratio appears, however, to be a fundamental distinction between the CD4+T-cell, allergen-driven process of allergic asthma in non-smokers and the CD8+T-cell, cigarette smoked-induced inflammation of COPD[70].

Smoking tobacco *per se* induces an inflammatory

response. Smoking shortens the transit time of neutrophils through the bone marrow, causes a leukocytosis and alters the immunoregulatory balance of T-cell subsets found in blood, bronchoalveolar lavage (BAL), and tissues of the conducting airways and lung[71–73]. Smoking initiates a peripheral blood leukocytosis and a reversible decrease in the normally high CD4 to CD8 cell ratio in blood of heavy smokers (i.e. > 50 pack–years). There is also a significant reduction of the CD4:CD8 + cell ratio in BAL fluid but not blood of a group of milder smokers (i.e. on average who have smoked 14 pack–years). The increase in the number of BAL and tissue CD8 + T-cells is positively associated with pack–years smoked[72,74,75].

Chronic bronchitis

Histological examination of airway tissues (taken at resection for tumour) from smokers demonstrates that inflammatory cells are present in and around the area of mucus-secreting submucosal glands and that scores of inflammation show a better association with the subjects who have symptoms of mucus hypersecretion than does gland size *per se*[76]. In bronchial biopsies of subjects with mild stable chronic bronchitis and COPD there is infiltration of the mucosa by inflammatory cells[75,77–79] (Fig. 1.7, see colour plate section): this is associated with up-regulation of cell surface adhesion molecules of relevance to the inflammatory process[80]. In the surface epithelium where, in contrast to the subepithelium, CD8 + cells normally predominate, Fournier and colleagues have demonstrated by comparison with non-smokers, an increase in all inflammatory cell types in smokers with chronic bronchitis and mild COPD[81]. In a subepithelial zone (also referred to as the lamina propria), bronchial lymphomononuclear cells appear to form the predominant cell type with scanty neutrophils (in the absence of an exacerbation): the lymphomononuclear component is composed of lymphocytes, plasma cells and macrophages. Significant increases are reported in the numbers of CD45 (total leukocytes), CD3 (T-lymphocytes), CD25 (i.e.activated) and VLA-1 (late activation) positive cells, presumed to be T-lymphocytes and of macrophages. The endobronchial biopsy studies of O'Shaughnessy and co-workers have demonstrated that by comparison with normal non-smokers, T-lymphocytes and neutrophils increase in the surface epithelium whilst T-lymphocytes and macrophages increase in the subepithelium of smokers with COPD[79,82]. In contrast to asthma, in COPD it is the CD8 + cell and not the CD4 + T-cell subset, which increases in number and proportion to become the predominant T-cell subset. Furthermore, the increase of CD8 + cells shows a negative association with the forced expiratory volume in one second (FEV1 expressed as a percentage of predicted). This novel distinction between the relative proportions in T-cell subsets of smokers with mild stable COPD and non-smoking mild asthmatics has received the support of subsequent studies of both resected tissues and bronchial biopsies[74,83,84]. The increase of the CD8 + phe‴ notype and of the CD8/CD4 ratio seen in the mucosa also occurs deeper in the airway wall in association with submucosal mucus-secreting glands in bronchitic smokers[83]. In addition neutrophils increase in the surface epithelium and glands especially when the disease increases in severity (Fig. 1.8, see colour plate section).

Similarity between COPD and asthma

COPD and asthma would seem to differ at the tissue level in a number of respects; for example the marked tissue eosinophilia and thickening of the reticular basement membrane of asthma (see below) is not usually a feature of COPD[85]. However, compared to normal healthy control tissue, there are a number of studies that report a small but significant increase in the number of tissue eosinophils in subjects with chronic bronchitis or COPD[76,79,86]. Sputum eosinophilia is also reported in cases of 'eosinophilic bronchitis', i.e. patients without a history of asthma and without bronchial hyperresponsiveness[12,87]. Furthermore, in mild COPD, the numbers of tissue eosinophils are markedly and significantly increased when there is an exacerbation of bronchitis (defined as a need by the patient to seek

medical attention due to a sudden worsening of dyspnoea or an increase in sputum volume or purulence)[88,89]. In such mild cases of COPD the exacerbation is associated with an increase in eosinophil chemoattractants, especially RANTES[90]. The bronchial mucus-secreting glands of smokers may also show gene expression for both IL4 and IL5 and the numbers of these cells are significantly higher in smokers with chronic hypersecretion as compared with their asymptomatic controls[91]. Thus, IL4, IL5 and eosinophil chemoattractant gene expression is not restricted to asthma and, like the recent reports of fibrosing lung disease[92], these regulatory cytokines can be expressed also in chronic bronchitic smokers.

Chronic bronchiolitis

Histologically, the earliest observed effects of cigarette smoke in small airways and surrounding alveoli is a marked increase in the number of macrophages and neutrophils, both in human and experimentally in animal studies. The increase is seen within both the tissue and lumena and can be detected in bronchoalveolar lavage fluid (BAL)[93]. Examination of small airway tissue in lungs resected from smokers also shows that the same profile of CD8- predominant inflammation reported in bronchial biopsies of the larger airways occurs deeper in the lung in both the 'small' airways[74,84] and also the lung parenchyma[94,95]. As with the findings in the large conducting airways there are significant negative associations of the numbers of CD8+cells and FEV1% of predicted in both the small (peripheral) conducting airways and lung parenchyma. However, at these sites the negative correlations are stronger than in the large airways. Thus the patterns of inflammation are similar at both proximal and distal sites. However, in contrast to the larger airways, the CD8+T-cell predominance in the small airways and lung parenchyma is more closely associated with decreased lung function in these subjects with COPD.

The infiltration of the airway wall by lymphocytes is associated with loss of alveolar attachments to the outer wall of small airways, a characteristic of centriacinar emphysema. The accompanying loss of radial traction and lung elastic recoil leads to early airway closure during expiration (Fig. 1.9(*a*), (*b*), see colour plate section). The loss of alveolar–bronchiolar attachments is thought to be due to the circumferential spread of small airway wall inflammation.

Emphysema

In the normal, the macrophage is the resident phagocyte of the alveolus: neutrophils are rarely present[96]. Neutrophils are recruited to the lung in smokers, albeit the extent of tissue neutrophilia is highly variable. On exposure to cigarette smoke, there is recruitment of macrophages and phagocytosis of cigarette smoke components. A macrophage alveolitis and respiratory bronchiolitis are the early changes in young cigarette smokers[97,98]. As in the large and small conducting airways in COPD, CD8+ cells also become the predominant inflammatory cell phenotype in the parenchyma and their numbers show a strong inverse correlation with FEV1% of predicted[95].

Inflammation and the pathogenesis of COPD

The neutrophil, the macrophage and the CD8+cell may each be involved in the destruction of the lung parenchyma by distinct mechanisms.

The neutrophil

The alveolar microcirculation is composed of a network of short interconnecting tubules of average diameter 5 μm. The average diameter of circulating neutrophils is 7.0 μm, which necessitates their deformation as they squeeze through capillary segments. Neutrophil traffic through the capillaries of the lung is normally slower (i.e. there is a higher transit time) than that of red blood cells as they are 700 times less deformable than RBCs[99]. Studies with radioactively labelled neutrophils have demonstrated that the normal delay in neutrophil transit is

further exaggerated, transiently, even in healthy subjects during smoking. Exposure of neutrophils to cigarette smoke in vitro and in vivo results in decreased deformability associated with polymerization of actin microfilaments[99,100]. This is the likely mechanism of the observed cigarette smoke-induced increase in transit time. Factors chemotactic for neutrophils, and which will induce their emigration from the microcirculation are released by the alveolar macrophages of smokers and the alveolar neutrophil population may increase from 1% to 5% of inflammatory cells. Cigarette smoke itself may contain substances chemoattractant for neutrophils, a possibility that is supported by the associated peripheral blood leukocytosis widely reported[101]. Cigarette smoke or factors released from cigarette smoke-exposed macrophages encourage the release of neutrophil elastase which may degrade lung elastin even in the presence of antiprotease[102–104]. Such neutrophil-derived serine proteases have been implicated in the pathogenesis of COPD since the appreciation of the emphysematous change that results from alpha-1 antitrypsin (AAT) deficiency in man. AAT protects against the proteolytic effects of neutrophil elastase, cathepsin G and proteinase 3, each of which has been shown to induce emphysematous change in experimental animal models of emphysema. These and earlier results led to the protease/antiprotease hypothesis in which emphysema develops if there is an imbalance favouring proteolytic digestion of the elastic framework of the lung (Fig. 1.10). Recent studies have shown that, when released from the cell, the concentrations of neutrophil elastase far outweigh the antiprotease in the immediate vicinity of the neutrophil cell surface. In the absence or reduction of the antiprotease this pericellular zone of proteolysis is greatly increased[105].

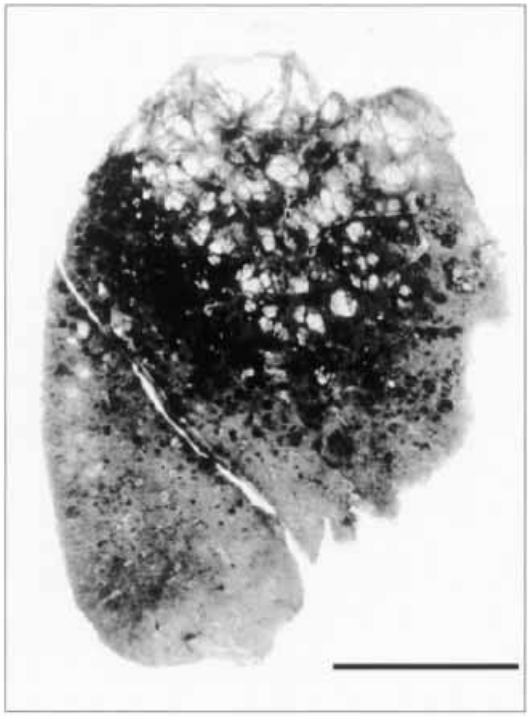


Fig. 1.10 Gross appearance of the cut surface of a lung in which the distribution of centracinar emphysema caused by proteolytic digestion is characteristically restricted to the upper aspects of each lobe. Scale = 10 cm. (By courtesy of Professor B. Heard.)

The macrophage

The role of the macrophage in the pathogenesis of COPD has been controversial. However, the data of recent studies support the hypothesis that the macrophage may play a critical role in regulating the inflammatory response and also directly in the tissue destruction associated with COPD[106,107]. Macrophages are able to synthesize significant amounts of matrix metalloproteinases (MMPs) including macrophage elastase (MMP12), collagenase 1 (MMP1), gelatinase B (MMP9) and others[106,108]. The hypothesis is that dysregulated expression of macrophage MMPs, induced either directly or indirectly by cigarette smoke, leads to the lung destruction characteristic of human emphysema. There is support for the hypothesis from experimental animal work using gene manipulated strains in conjunction with exposure to passive cigarette smoke[106]. MMP1 over expression in mice is associated with enlargement of airspaces suggesting

that collagen degradation may also be important in the generation of emphysema. Moreover, when macrophage MMP12-deficient (-/-)mice are exposed chronically to cigarette smoke, they fail to develop emphysema and fail to recruit macrophages to the lung: in contrast the smoke-exposed MMP12 intact (+/+) mice developed it[109]. Also in humans there are data from cultured macrophages of patients with COPD that show that there is increased expression of MMP1, MMP9 and MMP12[110]. MMP12 can be detected by immunohistochemistry and in situ hybridization in the alveolar macrophages of patients with emphysema but not in normal lung[106]. It appears that macrophage elastase is required for both macrophage accumulation and the emphysema that results from inhalation of cigarette smoke. The current working hypothesis is that cigarette smoke induces constitutive macrophages to produce MMPs that cleave lung elastin, generating fragments chemotactic for monocytes. This positive feed-back loop would perpetuate the accumulation of macrophages and lung tissue destruction. Zinc-containing metalloproteases released from the cigarette smoke-stimulated macrophages are not inhibited by the normal antiprotease of the alveolus and may thus also degrade alpha$_1$-antitrypsin *per se*[111].

The CD8 + cell

There are several differences between the CD4 + and CD8 + subsets of T-lymphocytes[112]. CD4 + T-cells have been well studied in the context of asthma and the Th2-type allergic response. However, until recently relatively little has been known about the CD8 + cell and smokers with COPD. CD8 + T-cells are generally associated with Th1-type immunity and play a role in generating protective immunity to *Mycobacterium* sp.[113]. LPS from gram negative bacteria cause selective activation of the CD8 + T-cell subset. Currently there is more speculation than knowledge about the role of this predominant cell in COPD. A recent study has shown they may interact with virus infected epithelial cells in a way that generates a chemotactic factor (MCP-1) for macrophages, an accumulation of which may then destroy host tissue[107]. $CD8^+$T-lymphocytes produce interferon gamma a cytokine that when overexpressed has been shown to induce emphysema experimentally in mice[114]. In addition, the CD8 + T-cell can produce perforin and granzymes, which may contribute to the apoptosis and cell destruction reported in emphysema[115]. Consequently the parenchymal damage associated with COPD could also be CD8 + cell driven.

One hypothesis is that individuals with a genetically determined low CD4 + /CD8 + T-cell ratio and those who smoke would be more likely to have an exaggerated CD8 + T-cytotoxic response to viral infection. Increased frequency of virally-associated exacerbations in this group would likely lead to lung tissue destruction and the development of COPD. In this respect the prevailing balance of CD4 + and CD8 + cells in the tissues is likely to be critical[70,116]. Finally, there are recent interesting data on the role of retinoic acid in influencing alveolar number and the repair of established emphysematous lung suggesting that nutrition may also act as an important additive factor in the emphysematous process[117]. Thus the patterns of inflammation in mild COPD in smokers and mild asthma in non-smokers differ but the distinctions become less clear when there is an exacerbation in mild COPD when eosinophilia develops in association with up-regulation of RANTES, an eosinophil chemoattractant usually thought of as characteristic of asthma. These alterations in COPD may explain why exacerbations in COPD may be responsive to corticosteroid treatment whereas the inflammation of ongoing mild to moderate COPD is not[118,119].

Vascular inflammation

There are few studies that examine the inflammatory process in pulmonary arteries of subjects with COPD. There is inflammation of these vessels due

probably to the close approximation of airways and pulmonary arteries and the spread of the inflammatory process from the bronchiolar wall to the adjacent pulmonary artery. An inflammatory process similar to that present in the conducting airways and in the lung parenchyma, consisting predominantly of CD8 + T-lymphocytes, has been reported in the adventitia of pulmonary arteries in smokers with COPD[95,120]. The vascular infiltration of CD8 + T-lymphocytes correlates with the degree of airflow limitation in these subjects[95], supporting a role for vascular inflammation in the progression of the disease. The vascular inflammatory process is also associated with impairment of endothelium-dependent vascular relaxation. The endothelium plays a crucial role in the regulation of vascular cell growth and tone through the release of endothelium-derived relaxing factors. Endothelial dysfunction, which results in an impaired release of these factors, has been shown in patients with end-stage COPD undergoing lung transplantation[121]. In this study, the degree of endothelial dysfunction was correlated with both the severity of pulmonary vascular remodelling and the arterial oxygen tension, suggesting that in end-stage obstructive lung disease, hypoxemia is the principal factor determining the endothelial dysfunction that leads to vasoconstriction. However, endothelial dysfunction and intimal thickening may be present also in smokers with mild COPD[122] who are not hypoxemic, indicating that factors other than hypoxemia might be capable of producing the vascular changes in smokers. It is possible that endothelial damage by cigarette smoking is the first vascular alteration occurring in COPD. This early alteration may predispose smokers to develop further vascular damage due to additional factors such as hypoxemia and inflammation, ultimately leading to the development of pulmonary hypertension[120].

Further studies of the distinctive patterns inflammation, cytokine gene expression and protein secretion in the airways and vessel walls of asthma and COPD should prove to be of scientific, clinical and therapeutic interest.

Remodelling

Surface epithelium

Histologically, damage and shedding of the airway surface epithelium are reported in asthma postmortem (compare Fig. 1.5(a), (b)) (Fig. 1.11(*a*), (*b*), see colour plate section) but this change is highly variable with some airways having completely intact surface epithelium in the presence of marked inflammation and other structural changes[123]. Subepithelial edema has been suggested to be one mechanism responsible for lifting of the overlying surface epithelium where this occurs[32]. Repeated loss of the epithelium induces a healing process as evidenced by squamous cell metaplasia and/or goblet cell hyperplasia. Histologically, damage and shedding of airway surface epithelium appears to be an early feature of asthma, particularly of the allergic form[124]: it has been reported in biopsy specimens of patients with stable mild disease and is not a usual feature of smokers with bronchitis or COPD (see Fig. 1.11(*a*), (*b*))[17,24,25,125]. Loss of superficial epithelium is accompanied by mitotic activity in the remaining epithelial cells in normal healthy individuals[126]. There is repeated epithelial regeneration in the form of simple and then stratified cells prior to restoration of the normal ciliated and goblet cell phenotypes, the entire process taking approximately 2 weeks. However, there are reports that such mitotic activity is deficient in asthma and this has led to the suggestion that there may be defective repair of epithelium in asthma with the consequent release of a range of factors that would promote a remodelling response[126,127]. These factors include epithelial-derived growth factor and granulocyte–macrophage colony stimulating factor and would induce alterations to the epithelial reticular basement membrane, via activation of adjacent fibroblasts/myofibroblasts, and deeper structures including bronchial smooth muscle, mucus-secreting glands and wall vessels. The release of these and other molecules including IL8, eotaxin and RANTES would also provide a chemoattractant gradient to

both inflammatory and phenotypically altered structural cells. Aggregations of platelets together with fibrillary material, thought to be fibrin have been observed in association with the damaged surface[24]. Such fibrin deposits are also seen during the late phase response following allergen challenge (author's unpublished results). The greater the loss of surface epithelium in biopsy specimens the greater appears to be the degree of airways hyperresponsiveness[24].

It is recognized that there is inevitably artefactual loss of surface epithelium during the taking and preparation of such small biopsy pieces, even normally, which makes interpretation of the epithelial loss seen in bronchial biopsies controversial. In the author's opinion, the observed loss reflects the fragility of the epithelium in vivo that facilitates sloughing during the bronchoscopy procedure. The fragility of the epithelium in vivo in asthma is supported by the frequent appearance of clusters of sloughed epithelial cells in sputa (see Fig. 1.3(*b*), see colour plate[124]) and the increased presence of bronchial epithelial cells in bronchoalveolar lavage of asthmatics with mild disease[17]. Other researchers have found no significant loss of epithelium in biopsies of mild asthmatics[128,129]: this may be due to differing methods of measurement of such loss or to differences in the severity of the patients sampled.

The fragility of the surface may be associated with disruption of epithelial cell tight junctions[130,131] and this may be facilitated by allergens *per se*, several of which have been shown to have proteolytic activity[132]. Tight junctions normally act as a selective barrier to the passage of ions, molecules and water between cells: their disruption may lower the threshold for stimulation of intraepithelial nerves leading to axonal reflexes, stimulation of mucus-secreting submucosal glands, vasodilatation and oedema through the release of sensory neuropeptides (i.e. referred to as neurogenic inflammation)[133,134]. Experimentally there is also evidence that the sensitivity of bronchial smooth muscle to substances placed in the airway lumen correlates strongly with the integrity of the surface epithelium[135]. Loss or damage of surface epithelium in asthma would thus lead to a reduction in the concentration of factors normally relaxant to bronchial smooth muscle with resultant increased sensitivity and 'reactivity' of bronchial smooth muscle.

Apart from their role as stem cells, the basal cells of normal pseudostratified surface epithelium have been suggested to act as a bridge, enhancing the adhesion of 'superficial' cells to epithelial basement membrane[136]. When superficial cells are lost in asthma the preferential plane of cleavage appears to be between superficial and basal cells[137], leaving basal cells still attached to their basement membrane. Epithelial cells also act as effector cells by their synthesis and release of cytokines such as IL-6, IL-8, GM-CSF and chemokines such as RANTES[138] and eotaxin. Disruption of the epithelium and attempts at repair may increase production of these proinflammatory cytokines by those cells that remain.

In contrast, epithelial loss is a less often reported feature of bronchial biopsies taken from smokers with bronchitis or COPD when goblet cell hyperplasia and/or squamous metaplasia are often seen (Fig. 1.11(*b*), see colour plate section)[139,140].

Reticular basement membrane

Thickening of the reticular basement membrane (i.e. the lamina reticularis) which lies external to (or below) the epithelium has long been recognized as a consistent change in allergic, non-allergic and occupational forms of asthma[24,32,141–143]: this may occur in response to repeated loss and healing of the surface epithelium (see Fig. 1.12(*a*)(*b*) and 1.5 (*a*)(*b*), see colour plate). Whilst there may be focal and variable thickening of the reticular layer in COPD and other inflammatory chronic diseases of the lung such as bronchiectasis and tuberculosis[143], the lesion, when homogenous and hyaline in appearance, is highly characteristic of asthma and is not usually found in COPD. The reticular layer appears to be absent in the fetus (at least up to 18 weeks of gestation)[144] but develops in normal, healthy individuals, presumably during early childhood: its thickening in asthma begins early[145], even before asthma is diag-

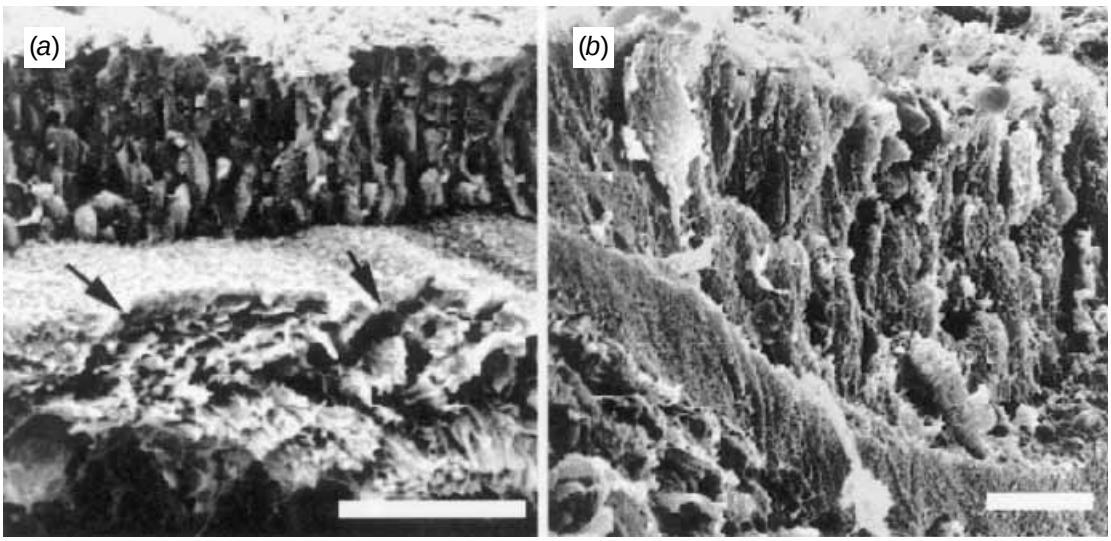
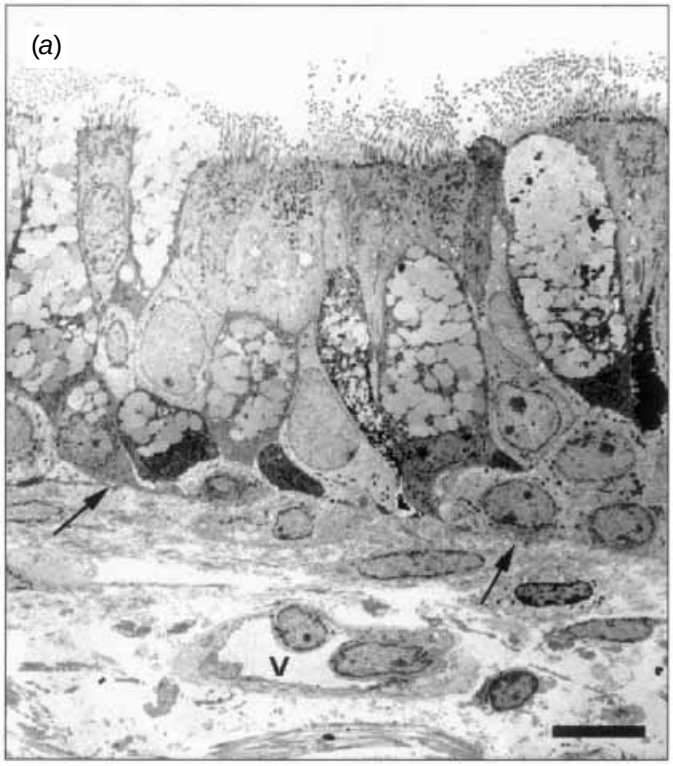


Fig. 1.12 Scanning electron micrographs demonstrating the airway mucosa in (*a*) the non-asthmatic with epithelium attached to a reticular basement membrane (RBM) of normal thickness (arrows) beneath which there is interstitial collagen. Scale = 50 μm. (*b*) A subject with a 25-year history of asthma, but who died of non-respiratory cause demonstrating the thickened RBM and damaged epithelium. Scale = 10 μm.

nosed[146]. The thickening remains even when asthma is mild and well controlled by antiasthma treatment[147] and is present in patients with a long history of asthma but who have not died of their asthma[142]. The extent of thickening is maximal early on in the development of asthma and does not appear to increase significantly with age, duration or severity of disease[7,145].

It should be noted that the basal lamina (i.e. the so-called 'true' epithelial basement membrane), which consists mainly of type IV collagen, glycosaminoglycans and laminin, is not thickened in either asthma or COPD. The thickening of the lamina reticularis (i.e. reticular basement membrane) (Fig. 1.13(*a*)(*b*)) which is immuno-positive for collagen types III and V together with tenascin[148] and fibronectin but not laminin has been referred to as 'subepithelial fibrosis'[141]. In the author's opinion this is an unfortunate use of the term as the thickened layer of reticulin is ultrastructurally different from the banded collagen that lies deeper in the airway wall or that which is characteristic of scarring. The reticular layer is composed of thinner fibres of reticulin linked to a tenascin-rich matrix in which there are sugars together with entrapped molecules such as heparin sulphate and serum-derived components such as fibronectin: these molecules may modulate the state of differentiation and function of overlying epithelium. In the author's opinion, swelling of this subepithelial reticular layer may also contribute to its thickening in asthma. Interestingly the thickened layer does not behave as a barrier to the transmigration of inflammatory cells, which by the release of enzymes (such as matrix metalloproteinases) or by the presence of pre-existing pores[149] can pass through it with apparent ease (see Fig. 1.13(*b*)). An association between the numbers of 'myofibroblasts' underlying the reticular layer and the thickness of the reticular layer has been demonstrated in asthma indicating these cells may secrete additional material contributing to its thickening[150].

In contrast to asthma, a study of bronchial biopsies, in carefully characterized patients with COPD, reports that the reticular layer is not thickened[140]. A recent report confirms this and demonstrates that the reticular layer in smokers with irreversible



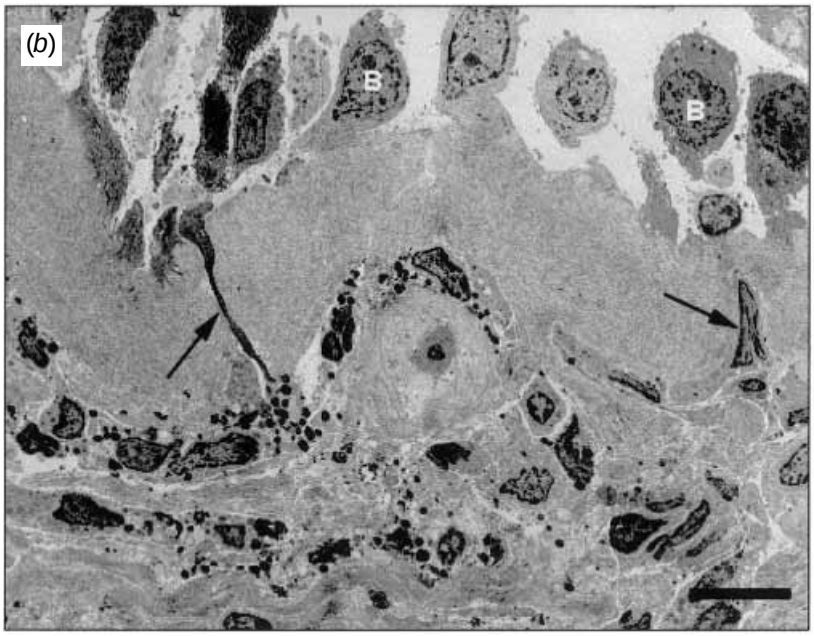


Fig. 1.13 Transmission electron micrographs of the epithelium and basement membrane: (*a*) normal epithelium of ciliated and goblet cells resting on the basal lamina (arrows) with relatively thin RBM and bronchial blood vessel (V) beneath. Scale = 10 μm. (*b*) Mild atopic asthmatic showing sloughing of basal epithelial cells (B) and thickened RBM and eosinophils with electron-dense granules beneath. Two mononuclear inflammatory cells (probably lymphocytes) are traversing the RBM (arrows). Scale = 10 μm.

disease is similar to that in normal healthy non-smokers and is significantly thinner than that of asthmatics who had been treated with inhaled corticosteroids[147]. There are, however, subpopulations of non-asthmatic smokers with COPD, defined by their smoking history and irreversibility to inhaled beta-2 agonist, who show significant airways reversibility (within the asthma range) to a 14-day course of oral prednisolone. These 'responders' have a thicker reticular basement membrane than normal and evidence of BAL eosinophilia: neither are present in the 'non-responder' group[151]. This interesting COPD group with a significant degree of reversibility demonstrates further the potential overlap that may exist between asthma and COPD at the tissue level.

Connective tissue

There is no consensus as to whether there is increased interstitial collagen in asthma or whether it increases with disease severity or duration. A recent study of bronchial biopsies obtained from asthmatics of varying severity reports increasing scores for collagen[152], whereas another reports no difference in collagen content[153]. Electron microscopic quantitative assessment of interstitial collagen in biopsies of mild asthmatics found no difference in the area of the mucosa occupied by collagen fibres[26]. There is similar controversy over loss of elastic tissue in asthma, one study demonstrating there is not[26] and others indicating that there is either elastolysis or altered ultrastructure of elastic tissue[154,155]. In contrast, airway wall fibrosis is generally, but not always, considered a feature of the airways in smokers who develop COPD, albeit these studies have focused on small rather than large airways[156–158].

Bronchial smooth muscle

The percentage of bronchial wall occupied by bronchial smooth muscle often increased substantially in fatal asthma[19] (Fig. 1.14(*a*), (*b*)). The absolute increase in muscle mass is reported to be particularly striking in large intrapulmonary bronchi of lungs obtained following a fatal attack as compared with that in asthmatic subjects dying of other causes[123]: it is an important contributor to the thickening of the airway wall and hence to the marked increase in resistance to airflow which may become life threatening[159–162]. Using a morphometric technique Dunnill showed that approximately 12% of the wall in segmental bronchi obtained from cases of fatal asthma was composed of muscle compared with about 5% in normals. Other studies have confirmed this trend in airways larger than 2 mm diameter and demonstrated a three- to fourfold increase over normal in the area of the wall occupied by bronchial smooth muscle[7,163,164]. The increase in muscle mass in small airways is not as great in absolute terms as in the large airways although as a percentage of the airway wall airway smooth muscle occupies a relatively larger percentage in the smaller than in the larger airway. Thus small increases of muscle in small airways may have a more significant effect functionally than similar increases in more proximal bronchi. In the absence of wheeze, values for muscle mass in segmental bronchi in chronic bronchitis and emphysema fall largely within the normal range but intermediate levels are present in so-called wheezy bronchitis[165,166].

Few studies in COPD have focused attention on the larger (cartilaginous) airways. One systematic study has described changes in large airway dimensions in relation to the lung function of patients with COPD[8]. These authors found that the wall area internal to the muscle was significantly thickened over the entire range of cartilaginous airways measured and that this was associated with a reduction in FEV/FVC. However, alterations in large airway smooth muscle mass were not observed and there was no correlation between muscle mass and airflow limitation. There was a positive association between peripheral airway inflammation and large airway inner wall area and the authors argued that their findings and those of others favour inflammation as the cause of the increasing inner airway wall thickness that occurs in both large and small airways in COPD. Airway smooth muscle increases significantly in the small airways in COPD[84,98,158,167]. In a

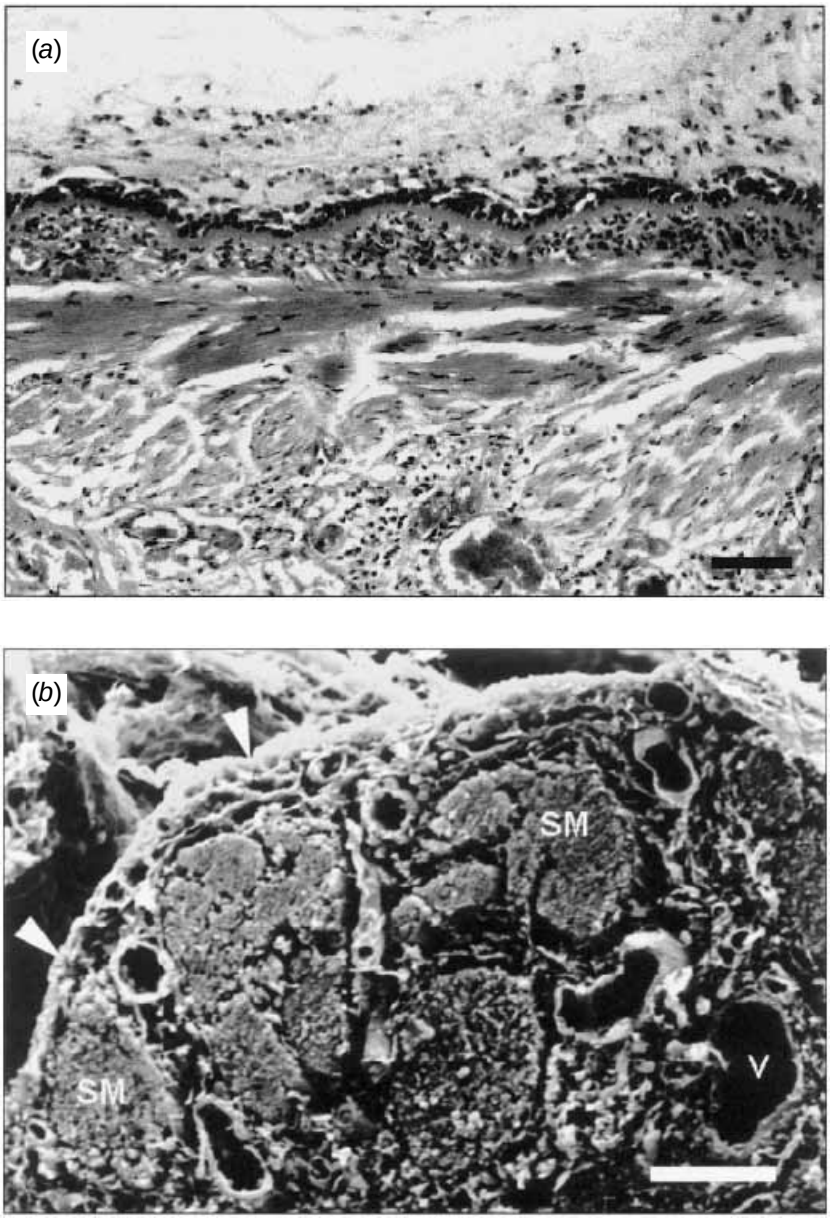


Fig. 1.14 Increased bronchial smooth muscle: (*a*) a histological section of the airway wall of a case of fatal asthma stained with H&E to show enlarged smooth muscle blocks lying relatively close to the surface epithelium. Scale = 80 μm. (*b*) Scanning electron microscopy of part of the mucosa in fatal asthma demonstrating the three-dimensional appearance of the enlarged blocks of bronchial smooth muscle (SM) and dilatation of bronchial vessels (V) which both contribute to thickening of the airway wall. The arrowheads show the position of the RBM from which the epithelium has been lost. Scale = 70 μm.

study of small (membranous) airways of 15 patients with COPD compared to the lungs of non-obstructed subjects and a group of asthmatic patients, it was only the airway smooth muscle area that was significantly increased in COPD[168]. In asthma, the increase in the wall area occupied by muscle, in absolute terms, is not as striking in small airways as in the large[123]. It is considered that the increased muscle mass that occurs at all generations of airway is likely to be the most important abnormality responsible for the increased airflow resistance observed in response to bronchoconstricting stimuli in both asthma and COPD[169]. Further studies and a greater understanding of the changes occurring in small airways is required as is a means of effective delivery of anti-inflammatory and antiremodelling therapy to this distal anatomic site. Whether the increase in muscle mass in asthma is due to muscle fibre proliferation (i.e. hyperplasia)[170] or hypertrophy is at present unclear. Two patterns of distribution of increased muscle mass have been described in asthma: one in which the increase is restricted to the largest airways and another in which the increase occurs throughout the airways: it is suggested that in the former hyperplasia of muscle fibres predominates whereas hypertrophy predominates when there is increased muscle occurring throughout the bronchial tree[171].

A newly proposed mechanism involves dedifferentiation of existing smooth muscle bundles. Cells that have ultrastructural features of both a contractile and secretory phenotype have been found in substantial numbers in the late phase response to allergen challenge. It has been suggested that, with repeated exposure to allergen, these may contribute to the increased mass of bronchial smooth muscle by a process of differentiation of existing smooth muscle and its migration to a subepithelial site where new muscle is formed[172]. The mechanisms involved in this response are likely to be similar to those occurring in atherosclerosis where there is vascular smooth muscle dedifferentiation and migration to form a neo-intima of increased vascular smooth muscle[173].

Mucus-secreting elements

The sources of the lumenal mucus that contributes to the airway mucus in both asthma and COPD are the submucosal glands and epithelial goblet cells. There is submucosal gland enlargement in both fatal asthma and COPD[19] and excessive production of mucus. The eosinophilic inflammatory exudate of asthma is probably responsible for the particularly sticky tenacious plugs that plug the airways and are associated with asphyxic death[174]. In asthma, there is dilatation of submucosal gland ducts, referred to as bronchial gland ectasia[175]. Whilst the characteristic condensed twists of mucus in asthma referred to as Curshman spirals (see Fig. 1.3(*b*) see colour plate section) are often said to represent the casts of small airways, their size is more in keeping with that of gland ducts which is their more likely origin. The normal proportion of serous and mucous secretory acini is retained in asthma whereas in COPD there is a shift towards a greater than normal predominance of mucous acini[176]. Goblet cell hyperplasia is a feature of both asthma[177] and bronchitis[178]. The mucous metaplasia that results in newly differentiated goblet cells in small bronchi and bronchioli of less than 2 mm diameter, where they are normally absent or sparse, is a feature of small airways disease in COPD[167]: whether mucous metaplasia also occurs in asthma is debated. It is considered by some that the mucus present at this distal site in asthma may have been aspirated from larger airways. In cases of fatal asthma where mucous metaplasia has occurred, the lumenal mucus secreted from surface goblet cells appears to remain adherent maintaining continuity between the cell's secretions and the plug, suggesting the secretory process or the mucin itself is altered in fatal asthma[177,179].

Airway vessels

Dilatation of bronchial mucosal blood vessels, congestion and wall edema are consistently reported features of fatal asthma and these can account for considerable swelling and stiffening of the airway wall

(Fig. 1.14(*b*), 1.15, see colour plate section[168,169,180]). There are indications that the increased proportion of the wall occupied by vessel may be due in part to a proliferation of bronchial vessels (angiogenesis)[181]. Whilst angiogenesis has been reported in mild asthma[182] it is particularly marked in severe corticosteroid-dependent asthma[183]. Whether these changes are the consequence of chronic allergic inflammation or due to the response to chronic (or latent) viral, mycoplasm or bacterial infection is not known.

Whilst proliferation of the bronchial vasculature is a feature of bronchiectasis and occurs in response to infection, changes to the bronchial vasculature have not been reported as a particular feature of COPD[168]. However, patients with moderate to severe COPD do have elevated pulmonary vascular pressures during exercise and there are structural changes in the pulmonary arteries consistent with endothelial dysfunction and pulmonary hypertension when compared with patients with minimal or no disease. Small (<500 μm) pulmonary vessels in airway obstructed smokers show intimal thickening as compared with those of non-obstructed non-smokers: in severely obstructed smokers, there is medial hypertrophy also[122,184,185]. Such structural changes likely contribute to the narrower lumens and vascular obstruction of these vessels. There is infiltration of the pulmonary arterial wall by T-lymphocytes. The CD8(+) T-cell phenotype is increased in both non-obstructed smokers and smokers with COPD compared with non-smokers and the intensity of the inflammatory infiltrate has been shown to correlate with both endothelium-dependent relaxation and intimal thickness[120].

Emphysema

Destruction of the lung parenchyma can be detected microscopically in the alveolar walls of smokers even when there is no evidence of airspace enlargement on gross examination (see Fig. 1.16). The microscopic measurement of such parenchymal destruction can, therefore, allow early identification of the disease, at a time when emphysema is not detectable macroscopically. The functional significance of such early destruction is demonstrated by its correlation with indices of airflow limitation and loss of elastic recoil of the lung[186].

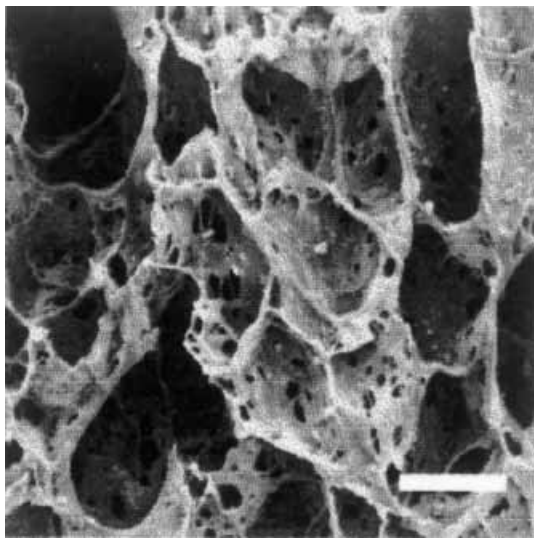


Fig. 1.16 SEM of human lung parenchyma illustrating microscopic emphysema in a smoker. Alveolar walls are peppered by fenestrae too small to be seen by the naked eye. Such early lesions probably result in loss of lung elastic recoil. Scale = 150 μm.

The two major forms of emphysema, centriacinar and panacinar, have distinct mechanical properties and distinct peripheral airway involvement[187]. In particular, lung compliance is greater in panacinar than in centriacinar emphysema, whereas the extent of peripheral airway inflammation is greater in the centriacinar than in the panacinar form. It is possible that, in centriacinar emphysema, airflow limitation is primarily a function of peripheral airway inflammation, as supported by the correlation between reduced expiratory flow and increased airway inflammation observed in this form of emphysema. By contrast, in panacinar emphysema, airflow limitation seems to be primarily a function of loss of elastic recoil, as supported by the correlation between reduced expiratory flow and increased compliance observed in this form of emphysema[187].

The current emphasis in smoking-related disease is on emphysema associated with loss of alveolar-

bronchiolar attachments. Bronchioles are supported within the lung by attachment of the adjacent alveolar walls. Loss of these attached alveolar walls and an increase in the interalveolar attachment distance (IAAD) appear to be associated with functional abnormalities, including a decrease in forced expiratory volume in 1 second (FEV_1) and abnormalities of tests of small airway function[188–191]. There is likely to be a role for airway wall inflammation in the selective loss of alveolar–bronchiolar attachments. It is possible that inflammatory cell activity may weaken the alveolar tissue and facilitate its rupture, particularly at the point where alveolar walls and airway adventitia meet and where the mechanical stress is likely to be greatest. This mechanism might provide an explanation for the relationship of airway inflammation and abnormalities of pulmonary function reported in smokers. Surprisingly, the majority of studies examining the pathology of COPD have been performed in subjects with mild to moderate disease, while pathological studies on subjects with severe COPD are few. The largest study, performed by Nagai and colleagues[192], showed that in subjects who had had severe disease both emphysema and peripheral airway abnormalities were present. Although the relative role of each of these pathologic lesions in the development of airflow limitation was difficult to establish, the authors concluded that emphysema was the more important. However, the findings of Nagai and colleagues should be interpreted with caution[156]. Their data indicate that, when emphysema is severe, loss of elastic recoil assumes overwhelming importance as the mechanism of airflow limitation, thus masking the effects of peripheral airway abnormalities. By contrast, when emphysema is mild, peripheral airways abnormalities do appear to play a role in causing airflow limitation.

While earlier suggestions that distinct clinical patterns of disease, referred to as 'pink puffers' and 'blue bloaters', represented morphologically different patterns of pathology detected postmortem, more recent studies have shown no correlation between the amount of macroscopic emphysema and chronic hypoxemia.

Studies of the relationships of macroscopically assessed emphysema and gas transfer or radiological changes have shown only moderate correlations. With microscopically assessed emphysema, however, carbon monoxide transfer coefficient (K_{CO}) shows a strong linear correlation ($r = 0.86$) in a group of patients, of whom only half showed macroscopic emphysema. When the microscopic assessment of emphysema is expressed in terms of an estimate of the density of alveolar tissue per unit volume of lung (AWUV), there is good correlation with assessment of emphysema using computed tomography (CT)[189,193]. Such studies, based on microscopic assessment of emphysema, represent a significant advance in the ability to identify early emphysema in life, and to follow its progression[194]. By application of combinations of quantitative histology and CT-determined lung volume data, Coxson and colleagues have been able to provide quantitative estimates of the extent of lung destruction in patients that may be followed longitudinally in the future: this will allow pathogenesis to be better understood and the effects of treatment to be determined[195,196]. Recent application of these methods has allowed the number of inflammatory cells present per unit surface area of lung parenchyma to be investigated in COPD. The data from these investigations indicate that emphysematous lung destruction is associated with a marked amplification of the inflammatory response in patients with emphysema compared to the lungs of smokers without emphysema but with equivalent smoking histories[195].

Emphysema in COPD, is also the likely consequence of a chronic CD8+cell inflammatory process. The current definition of emphysema excludes the presence of obvious fibrosis, yet it is now known that fibrosis may also occur even in the presence of alveolar wall loss[197,198]. The enlargement of alveolar spaces, distal to the terminal bronchiolus, in COPD may thus represent the consequence of lung injury and a failure to repair rather than of destruction *per se*. The focal fibrosis that may be identified in some cases of emphysema may represent the remainder of a repair component. Further

studies of the mechanisms that balance the production and degradation of collagen that occurs during the reparative and remodelling response to lung injury may yield important findings applicable to the treatment or prevention of the parenchymal lesions so important to COPD.

Airway wall nerves

The topic of airway wall innervation and its relation to asthma is a large one[133,134]. There are data suggesting that in fatal asthma there is an absence of (relaxant) vasoactive intestinal polypeptide (VIP)-containing nerve fibres and an increase in the numbers of substance P-containing fibres (stimulatory to bronchial smooth muscle) contrasting markedly with the innervation of the control lungs taken at resection from chronic smokers[199,200]. The reduction has not, however, been confirmed in examination of bronchial biopsies in mild asthma[201]. Whilst Sharma and colleagues have described a reduction of airway VIP and β-adrenoreceptors in cystic fibrosis, the densities of both VIP receptors and β-adrenoreceptors are reported to be similar in asthma to those of grossly normal tissue of the lungs of smokers resected for carcinoma[202,203].

Conclusions

The key points of comparison between asthma and COPD are summarized in Tables 1.1 and 1.2. There is evidence of airways inflammation in both asthma and COPD but there are marked differences in terms of the predominant anatomic site involved, the predominant pattern of inflammatory cells and the structural consequences of such inflammation. It will be of interest to learn whether further studies confirm or refute the hypothesis that chronic asthma and COPD are two distinct conditions that require equally distinct approaches to their management. This notion has received support from the recent findings of long-term trials of mild to moderate disease in which inhaled corticosteroids have been shown to be effective in the treatment of mild/moderate asthma but not so in COPD. However, the author predicts that the responses to any one treatment will vary from patient to patient depending not only on the diagnosis of 'asthma' of 'COPD' *per se* but rather on the particular prevailing patterns of inflammatory cells, cytokines and

Table 1.1. Asthma summary

The airway walls in asthma are thickened by inflammation and 'remodelling' and there is lumenal narrowing.
The association of tissue eosinophilia and asthma is a strong one and activated T-helper (CD4+) lymphocytes perpetuate the chronic eosinophilia.
Neutrophils are sparse in mild asthma but they are present in relatively large numbers in severe asthma
Mast cells play a role in the immediate (type I sensitivity) reaction in asthma: little is known of the role of basophils albeit they are considered important.
Epithelial fragility and loss are often but not always reported in asthma: healing or abnormal repair may be driving subsequent remodelling.
Thickening of the reticular basement membrane (i.e. the lamina reticularis but not the lamina densa) is a consistent change in allergic, non-allergic and occupational forms of asthma.
There is no consensus as to increased interstitial collagen in asthma.
The percentage of bronchial wall occupied by bronchial smooth muscle increases substantially in fatal asthma: there are several mechanisms that could explain the increase and there may be parallels with the changes in vessel walls in atheroma.
There is submucosal gland enlargement in fatal asthma and excessive production of mucus that, together with the inflammatory exudate, forms the sticky tenacious plugs that block airway lumena.
Dilatation of bronchial mucosal blood vessels, congestion and wall oedema are consistently reported features of fatal asthma: these can account for considerable swelling of the airway wall.
While corticosteroids are effective in treating the eosinophilic inflammation of mild asthma new treatments need to be found to treat the altered inflammation and the remodelling of severe asthma.
Inflammation and remodelling may respond to distinct classes of drug.