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EDITORIAL

Investigation and assessment of airway and lung inflammation: we now have the tools, what are the questions?

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Rhinitis, asthma, acute and chronic bronchitis, bronchiolitis, fibrosing alveolitis and emphysema are examples of a wide spectrum of inflammatory conditions of the conducting airways and lung. Whilst all share an inflammatory component they differ markedly in their clinical expression, progressive nature, pathology and responsiveness to anti-inflammatory treatment. The reasons for this remain unclear but are probably multiple and include the type of stimulus, chronicity of the reaction, the absolute and relative concentration and phenotypes of inflammatory and structural cells involved, the balance of pro-inflammatory cytokines and, importantly, the anatomic site of the reaction (*e.g*., central conducting or peripheral respiratory zones). Genetic predisposition will also contribute significantly to individual susceptibility and clinical outcome. We now have more tools and validated methods to assess and monitor airway and lung inflammation than ever before and it is vital to understand not only the wide range of techniques that are available, but also how and when they may be applied usefully to aid diagnosis, treatment and our basic understanding of the underlying disease processes. Most of these methods are summarized in this month's supplement of the journal (*European Respiratory Journal*, Supplement 26): there is particular emphasis on their application in studies of asthma and chronic obstructive pulmonary disease (COPD), but they are equally well suited to study a range of inflammatory conditions of the airways and lung which I believe we should now begin to compare and contrast.

The airway lining mucosa forms the individual's first contact with inhaled irritants, infection and allergens and is the site at which immune responses are initiated by immuno-competent cells in association with resident antigen presenting cells. Inflammation is "the response of vascularized tissue to lung injury" and, normally its purpose is to repair, restore and, if necessary, remodel the injured tissue. The key signs of acute inflammation, recognized by Celsus (30 BC–20 AD) are redness, swelling, heat and pain and loss or altered function, the last described by Galen (130–200 AD). In addition, Lord Florey recognized that acute inflammation at moist mucosal surfaces, such as the gut and airways, include injury to and sloughing of surface epithelium and hypersecretion of mucus. Apart from the reddening and swelling which may be observed macroscopically the microscopic changes of inflammation include changes of vascular calibre and blood flow, tissue oedema (as a result of alterations of vascular permeability) and leucocyte emigration. If acute, there is oedema, the inflammatory cell infiltrate is predominantly of polymorphonuclear cells (mainly neutrophils) and the res-ponse to injury, infection or allergen is of short duration leading to resolution, healing and repair as often occurs, *e.g*., in bacterial pneumonitis and viral induced bronchio-litis in children. However, if the injury is repeated (low grade) or severe then there may be a switch to persistent or chronic inflammation which may be inappropriate (as in autoimmune conditions), too severe or persistent and in the wrong anatomical location and lead to an abnormal tissue remodelling (*e.g*., enlargement or destruction of tissue components). The result is altered function or failure to function normally as in, for example, bronchiolitis obliterans, pulmonary fibrosis (fibrosing alveolitis) or emphysema. The reasons and mechanism(s) involved in the switch to chronicity remain unclear and an understanding of this is critical to future effective prevention and treatment of several persistent inflammatory conditions of the conducting airways and lung.

To examine and characterize the structural and inflammatory changes at distinct anatomical sites: open lung, transbronchial, nasal or bronchial or brush biopsy, bronchoalveolar lavage, spontaneous or induced sputum analysis and examination of blood and exhaled air provide examples of direct and indirect ways of assessing and monitoring airway and lung inflammation *in vivo*. Bronchial biopsy may sample the site of inflammation directly and as such is a powerful technique to assess the nature and magnitude of the inflammation [1], but the sample size, depth of the airway wall which is sampled and permitted frequency of biopsy for longitudinal follow-up investigation are limited and the site of interest may be too peripheral and open lung biopsy may not be justified. In such cases other, less direct, techniques which provide distinct but complementary information need to be applied and these form the focus of the accompanying supplement [2]. As such the supplement to this months issue of the Journal represents the experience of 60 internationally recognized experts who have endeavoured to define, characterize and treat the inflammation of asthma and COPD. Apart from their potential for application in the clinic, these techniques may be used for investigative research into a number of inflammatory conditions, and to focus on challenging issues and questions, some of which are now highlighted below.

The upper and lower respiratory tracts (URT and LRT, respectively) share similar cellular and humoral defence mechanisms and it is common to find that diseases of the nose and paranasal sinuses (*e.g*., rhinosinusitis), pharynx,

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larynx and LRT occur in association. For example, there is some evidence that active allergic rhinitis may induce in an unexplained way a remodelling process (*i.e.,* thickening of the reticular basement membrane) in the lower airways in subjects who are otherwise nonasthmatic [3]. Conversely there are anecdotal reports that effective therapy aimed at the URT can also improve symptoms associated with the LRT (*e.g.,* asthma). How, then, are these associations of the URT and LRT mediated? Are they *via* neural pathways, migration and homing of lymphocytes belonging to the mucosal-associated lymphoid system or by other mechanisms?

The allergic manifestations and inflammation of allergic rhinitis and asthma are similar. Both are immunoglobulin (Ig)E-mediated conditions of hypersensitivity which involve increased vascular permeability, tissue oedema and production of mucus. During the "late" response to seasonal exposure to allergen, these changes are associated with the local tissue accumulation of activated (CD4+ T-helper (Th) lymphocytes, eosinophils (and perhaps basophils) regulated *via* the production of type 2 T-helper (Th2)-like pro-inflammatory cytokines, particularly interleukin (IL)-4 and IL-5 [4]. Whilst the pattern of allergic inflammation is similar in allergic rhinitis and atopic asthma, the thickening and hyaline appearance of the reticular basement membrane so characteristic of asthma is not as prominent in rhinitis and the increase in smooth muscle mass (a characteristic of fatal asthma) is restricted to the airways of the LRT. Thus, the resultant effects of the allergic inflammation are altered by the differing anatomy and histology of the upper and lower airways.

As with allergic rhinitis, atopic asthma is now recognized as an inflammatory condition of the airways in which there is an infiltration of the mucosa by inflammatory cells very early in the course of the condition: there is a tissue eosinophilia and a predominance of T-lymphocytes of the CD4 (Th) subset [5]. The activation of Th cells results in the release of cytokines, particularly IL-4, IL-5 and IL-10 which characterize the "allergic" profile of inflammation. Release of these pro-inflammatory cytokines, together with chemokines such as eotaxin specific for eosinophils [6], leads to the recruitment of eosinophils (not neutrophils) from bronchial vessels, their activation and the release of a range of highly charged molecules which are thought to damage mucosal tissue. It is interesting that recent reports demonstrate up-regulation of eotaxin gene expression and protein production by airway surface epithelial cells, bronchial and bronchiolar smooth muscle and bronchial capillary endothelium as well as by migratory cells such as the alveolar macrophage [6, 7].

In this respect, airway wall structural cells must also be considered as "inflammatory cells" which contribute significantly to the inflammatory response. What then are the relative roles of structural and migratory inflammatory cells in different inflammatory conditions of the airways and lung?

The recruited and activated eosinophils show extensive "piecemeal" degranulation of eosinophils but in addition there is "cytolyosis" with the release of clusters of free eosinophil granules (cfegs) [8]. This cytolytic process, which may make eosinophil identification difficult, was described as early as 1922 [9], but has only now received renewed interest [10]. Eosinophil apoptosis is considered to be a normal route for eosinophil clearance and its induction by corticosteroids is thought to occur in allergic inflammation. However, electron microscopic examination of bronchial biopsies in asthmatics treated successfully with steroids fails to show significant numbers of apoptotic eosinophils whereas apoptopic neutrophils and intraepithelial mast cells are relatively frequently found even in the mucosa of healthy subjects (A. Rogers, personal communication). It would seem more likely that migration of eosinophils (and neutrophils) through the surface epithelium and into the lumen is the normal route for their clearance. However, their exit may be retarded by the up-regulated expression of epithelial intercellular adhesion molecule-1 (ICAM-1), which would cause their retention and accumulation within the surface epithelium in conditions of eosinophilic and neutrophilic inflammation. What then is the functional relevance of eosinophil cytolysis and apoptosis and may their presence or absence explain the differing pathologies of distinct eosinophilic conditions? For example, why may a substantial number of patients with chronic cough and sputum (*i.e*. bronchitic) present with a marked sputum eosinophilia and yet have no history of asthma and have airways responsiveness within the normal range [11]? The kinetics of eosinophil and eotaxin production and clearance are complex and the timing is probably crucial to the levels of tissue and sputum eosinophilia [12]. We need to research the kinetics of several chemokines further. If the presence of inflammatory cells in the airway lumen is a reflection of their clearance from the lung, how, then, do we interpret changes in their number during treatment?

In fatal asthma there is a marked inflammatory cell infiltrate throughout the airway wall and also in the occluding plug where they are usually present as concentric lamellae indicative of several episodes of inflammation prior to the terminal event. Tissue lymphocytes are abundant and eosinophils are characteristic and neutrophils are often sparse or retained within vessels. Certainly small conducting airways are involved [13]. The inflammation may spread to alveolar septae which immediately surround the airway and may affect adjacent arteries, but, in the author's opinion and experience, asthma is a disease of the conducting airways and not of the respiratory portion of the lung. Is it really so? Recent results showing eosinophils present within alveolar walls have challenged this idea [14] and it will now be important to apply a number of methods of assessment of inflammation in order to understand whether these findings are relevant to asthma pathology. There is an association of tissue eosinophilia and the airways hyperresponsiveness in mild stable asthma: yet in fatal asthma the extent of tissue eosinophilia varies greatly with each case and, interestingly, with the duration of the terminal episode [15–17]. The longer the terminal episode the higher the concentration of eosinophils [15] and these are particularly abundant in the large (central) airways in fatal asthma [18]. In contrast, acute sudden death in asthma is reported to be associated with high numbers of neutrophils and plugging of the airways [17, 19]. We do not yet understand why this should be.

What distinguishes severe, life-threatening asthma from mild but asymptomatic asthma? It is the writer's opinion that it is bronchial smooth muscle and alterations to it that occur as the result of chronic severe inflammation. There is recent evidence that bronchial smooth muscle shows phenotype plasticity: in response to allergen challenge

there may be de-differentiation to form a "synthetic"/myofibroblast-like phenotype which may migrate towards the subepithelial zone to form new muscle blocks which appear and function abnormally [20]. Which are the key cytokines in this process? Are comparisons with the inflammatory changes already described in atheroma relevant and helpful to our understanding of the asthma process at the severe end of the spectrum? If so, we may have discovered novel targets for anti-asthma treatment.

Macrophages may also increase in number, particularly in the more severe intrinsic form of asthma [21] and in this regard there is some similarity with the inflammation of COPD. Does the increase of this cell type mark the tendency to a less reversible and more severe asthmatic condition? Another cell type, the mast cell, initiates the immediate response to allergen exposure by release of mediators such as histamine, but mast cells may also be an important source of IL-4 and other pro-inflammatory cytokines whose secretion may trigger the induction of subsequent persistent production of IL-4 and IL-5 by lymphocytes [22]. Does this shift mark the switch from acute to chronic inflammation? The development of persistence may depend upon the stimulation of inflammatory cell precursors already present or recently recruited to the bronchial mucosa which may then mature into the eosinophils and mast cells characteristic of allergic inflammation. This has been referred to as the "tissue directed" inflammatory response [23] and requires further study to identify inflammatory cell precursors within the tissues as well as blood borne progenitors that may increase in response to allergen [24].

Analysis of sputum in smokers with chronic bronchitis in its stable phase shows a pattern of inflammation in which macrophages predominate whilst eosinophils and metachromatic (mast) cells are scarce [25]. The increase in sputum production may be initiated by and is reported to be associated with the extent of tissue inflammation [26]. Electron microscopic and immunohistochemical techniques have only recently been applied to examine the nature of the inflammatory infiltrate in chronic bronchitis. There is certainly evidence of inflammation in bronchial biopsies of subjects with stable disease and in "exacerbations" of bronchitis [27–28]. Bronchial mononuclear cells appear to form a predominant cell type and in contrast to asthma there are relatively few eosinophils (in the absence of an exacerbation of infection). The mononuclear component comprises lymphocytes, plasma cells and macrophages, the last a consistently reported cell type [29, 30]. In bronchial biopsies significant increases are reported in the numbers of CD45 (total leucocytes), CD3 (T-lymphocytes), CD25 activated and very late activation antigen (VLA)-1 positive cells and of macrophages [29]. There may be a moderate increase in the number of tissue eosinophils compared to that found in normal healthy controls and their numbers are reported to increase markedly to within the range found in asthma during "exacerbations" of bronchitis [31]. It has been suggested that, in contrast to asthma, they do not degranulate: this requires investigation by electron microscopy. Many lung conditions other than asthma are, of course, associated with peripheral blood and tissue eosinophilia and, from a mechanistic point of view, it would be informative to understand why this may be so. These observations emphasize the fact that, although they are important, studies of inflammation alone are insufficient to explain the differing pathologies, symptoms and prognosis of the airway and lung conditions in which we are interested [32]. We also need to understand the interrelationships between inflammatory cells and tissue remodelling/destruction which eventually lead to irreversible damage.

In smoking-associated COPD, T-lymphocytes and neutrophils are present in increased numbers in the surface epithelium, as are T-lymphocytes and macrophages in the subepithelium. We have reported that it is the CD8+ lymphocyte subset in smokers that increases in number and proportion and have shown that the increase of CD8+ cells is significantly associated with loss of lung function as measured by forced expiratory volume in one second (FEV_1) [30]. Importantly, this contrasts with the predominance and activation of the CD4+ T-cell subset, which is characteristically increased in mild atopic asthma and highlights the contrasting roles of T-lymphocyte subsets in COPD and asthma. Increasing pigmentation of sputum-derived macrophages and increased numbers of neutrophils are also associated with poor lung function [33]. Bronchoalveolar lavage fluid (BALF) from subjects with chronic bronchitis demonstrates high numbers of neutrophils [27, 34]. These probably derive from the surface epithelium or respiratory portion of the lung. Increased glutathione (GSH) myeloperoxidase (MPO; a marker of neutrophils and eosinophils) and eosinophil cationic protein (ECP) are also associated with reduced FEV_1 [35]. Interestingly, the high numbers of neutrophils and MPO found in lavage fluid from subjects with COPD is not reflected in their numbers in the bronchial mucosa, at least in the subepithelial zone (often referred to as the lamina propria) of biopsies obtained from the same subjects [27, 30, 36]. This may represent the inability of bronchoscopy to sample distal portions of the lung to which neutrophils may be preferentially recruited in COPD, or it might be due to the relatively rapid migration of neutrophils across the airway wall of the more proximal airways, sampled by bronchoscopy. Alternatively, the location of the histological section in which the inflammatory cells are counted may not be that in which neutrophils accumulate [37]. The preference of neutrophils for surface epithelium and that comprising mucus-secreting glands has recently received attention [37, 38].

Pigmented alveolar macrophages were the main inflammatory cell of the respiratory bronchiolitis and alveolitis first described in young smokers. In more severe cases the peribronchiolar inflammation of small airways disease (chronic bronchiolitis) consists mainly of lymphocytes as-sociated with a mild mural fibrosis which may be respons-ible for the subtle abnormalities detected by sophisticated tests of small airway function. The T-cell functional phenotype and cytokine profile of the bronchiolar inflammation in smokers is yet to be characterized and it will be important in future studies to determine whether the pattern and character of the inflammation seen in central (large) airways reflects that seen in the walls of small airways and alveoli. There is already some evidence that it does [39].

Epidemiological studies have demonstrated a significant relationship between cigarette smoking and severity of emphysema but the mechanism(s) by which cigarette smoke causes such respiratory bronchiolar and alveolar wall destruction is still the subject of much research. An imbalance

between proteolytic enzymes and protease inhibitors in the lung, favouring an excess of enzyme and in particular elastases has and continues to be a favoured mechanism. In addition, the imbalance between oxidants and antioxidants probably contributes by allowing an excessive oxidant burden to degrade the normal protease inhibitor screen. When activated, alveolar macrophages may release a variety of oxidants. These proposed mechanisms involve interactions between cigarette smoke, alveolar macrophages, chemoattractants, neutrophils, elastases, endogenous and exogenous oxidants, protease inhibitors, antioxidants and lung connective tissue, primarily elastin, which undergoes repeated destruction, synthesis and degradation. In spite of this, experimental animal models of cigarette smoke-induced emphysema have proved difficult to develop. The destruction of the respiratory zone is also considered to be the result of an inflammatory reaction, much of which is centred on respiratory bronchioli and largely initiated by products of inhaled tobacco smoke. As with the more proximal airways, T-lymphocytes and macrophages also appear to play a role in the lung parenchyma [39, 40].

Are CD8+ (cytotoxic) cells directly involved in destruction of the respiratory acinus? It is now becoming clear that cytotoxic lymphocytes (which include "natural killer cells") can destroy their target cells by multiple mechanisms, which include release of granule-derived proteins such as perforin and granzymes [41]. These proteins may punch holes in cell membranes and trigger an endogenous pathway of apoptosis resulting in dissolution of their target cell nuclear membrane and deoxyribonucleic acid (DNA) fragmentation. These processes may not be restricted to major histocompatibility complex (MHC) and are likely to be new areas of research for those interested in how it is that inflammatory responses may damage the lung.

Recruitment of T-cells of the CD8 phenotype may relate to viral infection: the observations of latent adenoviral proteins in COPD indicates that the effects of viruses may persist [42]. How, then, might the effects of virus and cigarette smoke interact? Are the patterns and effects of inflammation on airway and lung tissue different in the case of virus or cigarette smoke alone or in combination? These are issues that can be addressed experimentally but they will also need to be answered in humans using the variety of investigative techniques outlined in this month's supplement.

Finally, cryptogenic fibrosing alveolitis (CFA; also referred to as idiopathic pulmonary fibrosis) and that associated with vascular disease, systemic sclerosis (FASSc), are also both inflammatory conditions of the lung which result in collagenous thickening of the alveolar wall rather than its emphysematous destruction. An understanding of the reasons for the very different outcomes of the inflammatory processes of emphysema and fibrosing alveolitis is required. We know that activated T-lymphocytes (*i.e.*, CD25+ and CD45RO+ cells) are also present in increased numbers in fibrosing alveolitis. Gene expression for IL-4 and IL-5, whilst characteristic, is not unique to asthma and this Th2 pattern also occurs in CFA [43]. By contrast, the inflammation of fibrosing alveolitis (associated with) systemic sclerosis (FASSc) is associated with gene expression for IL-4, IL-5 and interferon (IFN)-γ (*i.e.* a mixed Th2/Th1 phenotype). However, both fibrotic conditions are associated with increase of IL-8 gene expression [44].

In conclusion, the severity and nature of the inflammation and its consequences (be they obstructive or restrictive) are highly dependent on the type, dose and persistence of the insult and the predominant site in the lung at which the inflammatory reaction occurs. By comparing and understanding the subtleties of the inflammatory processes of these pathologically distinct conditions and the contributions made by different inflammatory, and also structural cells, we will be able to understand better their interaction with the genetic factors which predispose an individual to tissue damage rather than resolution and repair. Such a fundamental understanding will help in the design of novel, more effective and incisive treatment. It is hoped that the variety of methods presented in this month's Journal supplement will facilitate rapid progress in addressing the many questions that continue to challenge us as scientific and clinical researchers alike.

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