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Research paper

In vitro and *ex vivo* models of human asthma

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ABSTRACT

Asthma is an inflammatory disorder of the conducting airways which undergo distinct structural and functional changes leading to non-specific bronchial hyperresponsiveness (BHR) and airflow obstruction that fluctuate over time. It is a complex disease involving multiple genetic and environmental influences whose multifactorial interactions can result in a range of asthma phenotypes. Since our understanding of these gene–gene and gene–environment interactions is very poor, this poses a major challenge to the logical development of 'models of asthma'. However, use of cells and tissues from asthmatic donors allows genetic and epigenetic influences to be evaluated and can go some way to reflect the complex interplay between genetic and environmental stimuli that occur *in vivo*.

Current alternative approaches to *in vivo* animal models involve use of a plethora of systems ranging from very simple models using human cells (e.g. bronchial epithelial cells and fibroblasts) in mono- or co-culture, whole tissue explants (biopsies, muscle strips, bronchial rings) through to *in vivo* studies in human volunteers. Asthma research has been greatly facilitated by the introduction of fibreoptic bronchoscopy which is now a commonly used technique in the field of respiratory disease research, allowing collection of biopsy specimens, bronchial brushing samples, and bronchoalveolar lavage fluid enabling use of disease-derived cells and tissues in some of these models. Here, we will consider the merits and limitations of current models and discuss the potential of tissue engineering approaches through which we aim to advance our understanding of asthma and its treatment.

1. Introduction

Epidemiological studies have shown that the prevalence and severity of asthma has increased remarkably over the last decades both in children and adults. It is estimated that 300 million people suffer from the condition worldwide, with up to 18% of the population being affected in some countries [1]. Furthermore, the asthma related costs, which include direct costs for healthcare as well as indirect costs due to working days lost, have been rising dramatically and, in Europe, are estimated to be around €17.7 billion per annum [2]. A recent assessment of asthma across Europe (Brussels Declaration) has identified a substantial unmet clinical need with those 10% of patients with severest disease accounting for ~50% of the health costs [3]. Asthma prevention has not been achieved; once established there is no cure and there are currently no medications that can alter its natural history. Management is primarily directed towards suppressing inflammation with corticosteroids and relieving bronchoconstriction with bronchodilators.

Asthma is a chronic inflammatory condition defined primarily by clinical characteristics; it is often considered to be a syndrome rather than a disease, as patients exhibit a range of different phenotypes including differences in the type of inflammation or response to therapy [4–6]. Although many studies have been undertaken to investigate the complex mechanisms of asthma, our understanding of the disease is still limited. This makes asthma one of the biggest challenges for researchers and clinicians. The complexity of asthma and the likelihood that the underlying disease mechanisms are multifactorial probably explain why there remains an unmet clinical need for new therapeutic strategies. In order to fully understand the underlying mechanisms of asthma, there is on the one hand a need for detailed characterisation of the subtypes of asthma and on the other hand the need for good experimental models that reflect these different phenotypes. Together, these approaches may result in new therapeutic strategies and more efficient patient-tailored treatments. This review will give an overview of the complexity of asthma and discuss the human tissue/cell based models used to study asthma mechanisms *in vitro* and *ex vivo*.

2. Definition of asthma

The hallmarks of asthma are airway inflammation, airway hyper-responsiveness (AHR), reversible airway obstruction and

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symptoms of cough, wheeze and breathlessness. Asthma is associated with episodic exacerbations which can be triggered by a multitude of factors; the most common are viral infections, allergen exposure, exercise and cold air [7]. Considerable effort has been invested into the development of national and international guidelines for the management of asthma in order to improve diagnosis, patient care and treatment [1]. Diagnosis of asthma is mainly based on medical history of symptoms and measurement of lung function. Commonly used methods to evaluate lung function are by spirometry: the measurement of Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC) and Peak Expiratory Flow (PEF). While lung function tests can enhance the confidence of a symptom-based diagnosis and provide complementary information about asthma control, they are not strongly correlated to the symptoms. In patients with normal lung function but asthma-like symptoms, a diagnosis can also be based on measurement of airway responsiveness to challenges such as inhaled methacholine, histamine, mannitol or exercise. Asthmatic patients normally show a 20% fall in FEV_1 in response to lower agonist concentrations than non-asthmatic subjects, but despite the high sensitivity, these tests lack specificity [8]. Further methods for the diagnosis of asthma can include the measurement of exhaled NO concentration and the number of granulocytes in induced sputum as a marker of airway inflammation [9,10]. In certain cases, a more invasive fiberoptic bronchoscopy can be performed in specialist centres in order to obtain biopsies for histological analysis and bronchoalveolar lavage (BAL) fluid for differential cell counts and analysis of proinflammatory mediators [11].

3. Phenotypes and treatment of asthma

Phenotypes of asthma are very heterogeneous, ranging from mild to severe or from intermittent to persistent airway obstruction. Strictly speaking, there are currently no validated and specific biomarkers based on underlying disease mechanisms which may allow classification of asthma into subtypes and development of more efficient patient-tailored therapies [12,13]. However, the measurement of exhaled NO and the count of eosinophils in sputum have been shown to be of value for prediction of corticosteroid responsiveness [14], and more recently, serum periostin levels have been used to define 'Th2 high' asthmatic subjects who may show treatment benefit with anti-IL-13 [15]. On the basis of clinical characteristics, cluster analysis of asthma patients in primary and secondary care has revealed five clusters divided by symptoms and eosinophilic inflammation: early onset atopic asthma, benign asthma, obese non-eosinophilic asthma, early symptom predominant and inflammation predominant asthma [4]. Other attempts to classify asthma phenotypes are based on symptoms and the responsiveness to drug treatments. For example, asthma phenotypes can be divided into allergic or intrinsic non-atopic asthma; or according to granulocyte cell counts in induced sputum into eosinophilic, neutrophilic, mixed granulocytic, or paucigranulocytic phenotypes. Responsiveness to drugs suggests a corticosteroid insensitive and an aspirin-intolerant subgroup of asthma. For more details about asthma subgroups, the reader is referred to other recent reviews [5,6].

The Global Initiative for Asthma (GINA) has published recommendations and guidelines for the management of asthma. The report 'Global Strategy for Asthma Management and Prevention' is updated yearly in December. Since 2006, the recommendations for asthma management are based on clinical control rather than asthma severity. Asthma medications can be grouped into asthma 'controllers', which are taken on a regular basis to suppress airway inflammation, and 'relievers', which are bronchodilators and are taken only when needed. To date, the most effective controllers of persistent asthmatic airway inflammation are inhaled corticosteroids. Reliever therapies are inhaled short-acting β_2-agonists and are predominately used in acute asthma exacerbations in order to relieve bronchospasm. Leukotriene modifiers, such as cysteinyl-leukotriene 1 (CysLT1) receptor antagonists (montelukast, pranlukast, zafirlukast) and 5-lipoxygenase inhibitor (zileuton) are also controllers, but since their effect is less than low doses of inhaled corticosteroids, they are mostly used in combination with corticosteroids. Another class of controllers used in combination with corticosteroids is the long-acting inhaled β_2-agonist. Combination therapy has a higher efficacy and allows lower doses of inhaled corticosteroids compared to corticosteroids alone. Theophylline in sustained-release formulation can also be used in combination with inhaled corticosteroids but is less effective than long-acting β_2-agonists. Due to its bronchodilator effect, short-acting theophylline is also used as a reliever, but there is controversy about the benefit in the treatment of asthma exacerbations [1]. For some patients with allergic asthma that remains uncontrolled despite use of high doses of corticosteroids, anti-IgE therapy (omalizumab) can be an option. However, there is still an unmet clinical need for new treatments, especially for patients with severe, uncontrolled asthma. Therefore, there is a need for detailed characterisation of the underlying disease mechanisms that might help in identifying new therapeutic targets.

4. Pathogenetic mechanisms of asthma

Asthma is characterised by inflammation and structural remodelling of the conducting airways, although with increasing chronicity the small airways can also become affected. The structural changes of the remodelled airways are characterised by goblet cell metaplasia and hyperplasia, subepithelial fibrosis and increased collagen deposition throughout the airway wall, thickening of airway smooth muscle and increased angiogenesis [16]. Asthma is a complex disease that involves both genetic and environmental factors. It was originally thought that asthma is mainly mediated by allergic airway inflammation triggered by exposure to environmental allergens. However, epidemiological studies have shown that up to 40% of the population in industrialised countries are atopic, but only 7% of this group are affected by asthma [17]. These findings imply that there are other underlying mechanisms involved in the development of asthma beyond atopy. New hypotheses are pointing to the importance of the formed elements in the airways, especially the airway epithelium in the development of asthma [18]. This concept is supported by recent studies which have identified a number of asthma susceptibility genes including *PCHD1*, *IL-33* and *ORMDL3* [19] that are expressed in epithelial cells, as well as other genes such as *ADAM33* [20] which are found in mesenchymal cells [21,22].

The airway epithelium forms the first line of defence against inhaled environmental agents and a breakdown in innate defence mechanisms is thought to play a central role in asthma pathogenesis. The barrier properties of the epithelium can be divided into three broad functions: (i) the physical barrier formed by close cell–cell contacts, for example, tight junctions, (ii) the chemical barrier which is formed by the secretions of epithelial cells, for example, mucus, anti-oxidants, etc., and (iii) the immunological barrier which is involved in innate immunity and which interacts with cells of the innate and adaptive immune system through expression of adhesion molecules and release of mediators including cytokines and chemokines [23]. There is evidence that these barrier functions of the epithelium are abnormal in asthma [24], either due to genetic or environmental influences. Although not well explored, environmental factors like pollution or cigarette smoke may cause epigenetic changes which result in altered gene