



Asthma: Measuring the Breadth of New Medicines

With an array of preclinical models available, choosing the right one may seem overwhelming.

Dr Alison Kerr and Dr Gordon Meiklejohn at MD Biosciences offer a guide

Dr Alison Kerr graduated with a PhD in Infection and Immunity at the University of Glasgow. With nine years' experience in respiratory inflammation, Alison joined MD Biosciences in March 2005. Currently Director for Asthma and Respiratory Pathway Inflammations, she is responsible for directing the development, optimisation and implementation of *in vivo* models.

Dr Gordon Meiklejohn gained his degree in Parasitology from the University of Glasgow and went on to conduct his doctoral research there with Professor Paul Garside. Gordon's studies specialised in the field of the immunomodulation of the adaptive immune response and he has continued this focus at MD Biosciences, where he is the Study Director for the company's mode-of-action platform.

A range of preclinical models is available for the researcher attempting to establish the efficacy of a novel therapy for human asthma. The situation is further complicated by the fact that each of these models can have a variety of readouts. The importance of choosing a suitable model is highlighted by the fact that currently licensed therapies can have variable efficacies depending upon the model and endpoints utilised (1). This article discusses the models that are currently available and highlights new advances in the field (techniques such as magnetic resonance imaging (MRI) or adoptive transfer of trackable immune cells) that can help researchers gain information on the mode-of-action of their therapy.

ASTHMA AS A THERAPEUTIC TARGET

The prevalence of asthma, along with asthma associated morbidity and mortality, continues to increase worldwide. An estimated 5-12 per cent of the world's population now suffers from the disease. Asthma is therefore an important target for the biopharmaceutical industry. Development of new therapeutics depends upon suitable preclinical models. The goal of a preclinical asthma model is to reproduce the airway inflammation, mucus hypersecretion or airway hyperresponsiveness (AHR) seen in human asthma.

CURRENTLY AVAILABLE PRECLINICAL MODELS

The majority of these utilise rodents. These are sensitised to a model antigen such as ovalbumin (OVA), house dust mite antigen or cockroach allergen in combination with an adjuvant such as alum. Most models utilise multiple sensitisation steps, followed by one or more local challenges with the antigen into the lungs. The whole process typically takes around one month. Some of these models have the added advantage of being suitable for use to study effects of test items in other pulmonary disorders such as rhinitis.

TYPICAL ENDPOINTS MEASURED

Lung Function

A range of endpoints can be studied in preclinical asthma models. It is possible to measure the functionality of the lungs, although the small size of rodent lungs has proven to be problematic.

The most common measurement of lung function is to study AHR using non-invasive whole-body plethysmography. This assesses pressure fluctuations within an airtight box giving a readout termed enhanced pause (penH). PenH is not a physical measurement, but is rather empirically derived from the size and timing of pressure changes in the box.

A lot of recent literature on mouse lung function testing indicates that penH is not relevant to human (2). This is due to the fact the mouse lung appears to close complete areas of the lung during AHR testing (3). In addition, it appears that AHR in mice has no involvement of smooth muscle shortening, which is thought to be a major factor in humans. Another issue preventing lung function tests from being correlated between mice and humans is that airways in human lungs have far more branches than do mouse lungs.

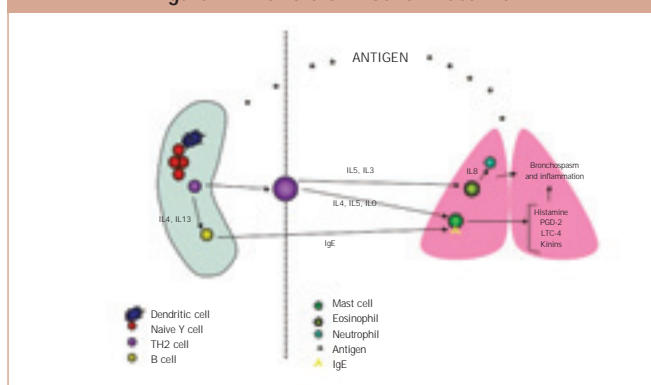
In a recent article, Lomask used a mathematical model to qualify the use of penH whereby changes in humidity and temperature of the air inside the box of the whole body plethysmograph are minimal and the calculations are based upon at least 20 breaths in order to reduce the impact of animal movement upon the readout (4). PenH is now generally used as a relatively high-throughput screen.

More specific and sensitive but invasive methods of measuring AHR have been developed recently. Mice that are anaesthetised and intubated via the orotracheal route breathe spontaneously and can be used to assess lung resistance and dynamic compliance (5). This method has the additional advantage that repeated measurements could be taken from the same animal; in effect that animal can act as its own control.

Asthmatic Immune Response

In contrast to lung function measurement, the immune response during asthma is well preserved between mice and humans. In human asthma, eosinophils and lymphocytes are found to infiltrate the bronchial mucosa. Increased mucus secretion and production of Th2 associated cytokines such as IL-4, IL-5 and IL-13 are also found. IL-4 induces differentiation of CD4 T cells into Th2 cells, induces the proliferation of activated B cells and is the major cytokine involved in B cell class switching to IgE (the antibody isotype most associated with human asthma). IL-5 is

Figure 1: The role of T cells in asthma

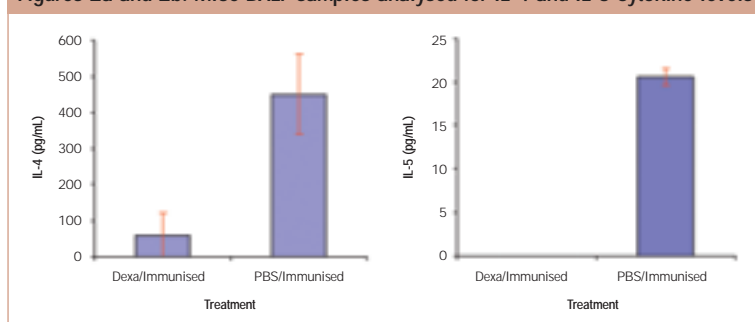


involved in eosinophil activation and also facilitates B cell growth and antibody production. The activities of IL-13 and IL-4 show a high level of overlap, although it is thought that IL-4 acts primarily in the initial sensitisation, with IL-13 more important during secondary exposure to the allergen. In addition to inducing IgE production, IL-13 can induce AHR, goblet cell metaplasia and airway glycoprotein hypersecretion, which all contribute to airway obstruction.

All of these parameters can easily be studied in preclinical asthma models. Cells in the bronchoalveolar lavage fluid (BALF) and lung tissue can be phenotyped by eye on cytospins preparations, in lung histology sections or using fluorescent antibodies raised against various cell markers in combination with a fluorescence activated cell sorter (FACS) machine. The results of these assays provide information on the type of cells that are present within the lung airways and tissues. In a BALF sample from a normal lung, macrophages make up greater than 90 per cent of the cells present. Lymphocytes constitute less than 10 per cent, eosinophils and neutrophils around 1 per cent each and mast cells less than 1 per cent. In a sample from a preclinical asthma model, eosinophils can make up 50-60 per cent of the cells with lymphocyte and neutrophils levels also increased greatly and macrophage levels correspondingly decreased.

Mast cells are central to the development of asthma due to their ability to release an array of preformed and newly synthesised inflammatory mediators such as cytokines, leukotrienes and prostaglandins (see Figure 1). Mast cells are also thought to be involved in the tissue remodelling that occurs later in asthma. It can therefore be of interest to study their location and degranulation in preclinical asthma models. This can be done easily by staining lung sections with toluidine blue or by assessing the level of mast cell protease in serum.

Figures 2a and 2b: Mice BALF samples analysed for IL-4 and IL-5 cytokine levels



Staining lung sections with alcian blue/periodic acid-Schiff stain normally assesses variations in mucus secretions. This identifies mucin glycoconjugates expressed by goblet cells. These cells are extremely rare in healthy lungs but their numbers increase markedly in asthmatic lungs and can easily be counted in lung sections.

Cytokine levels within the BALF can be measured in a variety of ways. Individual cytokines can be quantified using reverse transcription PCR or ELISAs. Combinations of multiple cytokines can be measured in the same sample using Luminex® technology. Luminex technology can also be used for other cellular products that may be of interest in asthma such as cell signalling pathways and matrix metalloproteinases. Typical results from analysing the immune response in a preclinical asthma model are shown in Figures 2a and 2b.

RECENT DEVELOPMENTS IN ENDPOINTS

The above readouts are well established and have been utilised for many years without significant alteration. In recent years, models that make use of methods mainly used in academic research have been made available to the researcher seeking to outsource to a contract research organisation.

MAGNETIC RESONANCE IMAGING

Larger pharmaceutical companies have been using MRI in the drug research and development fields for several years (6). This imaging technique utilises powerful magnetic fields to align the nuclei within the atoms of the subject. Radio wave pulses are then applied and the nuclei release some of this radio frequency energy, which is then detected by the MRI equipment. The images basically represent a distribution of protons (hydrogen nuclei) within the water and fat in tissues.

The main advantage of MRI is that it is non-invasive so repeated measurements are possible on the same subject, so once again an animal can act as its own control. In addition, it is possible to gather very high resolution images.

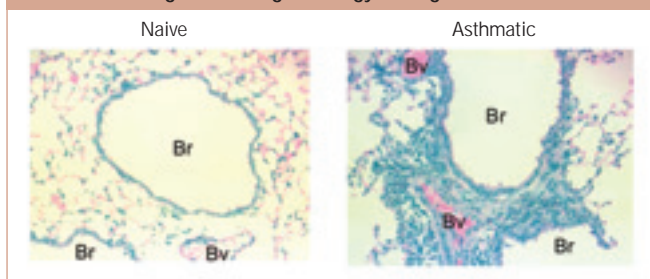
The lungs have proven to be one of the most problematic organs to image via MRI due to their air content, as well as the movement of the heart and lungs associated with breathing. Such problems can be addressed by using hyperpolarised noble gases such as ³He to enhance the contrast within the lungs or by restricting ventilation of the subject until after the image acquisition period. Another possible solution is to allow the subject to breathe normally and simply average a higher number of signals. This method has been

applied to study the development of oedematous regions following sensitisation and subsequent challenge with OVA (7). Detection of the oedema signal via MRI correlated well with the oedema seen in histological sections as well as the eosinophil influx into the BALF.

ADOPTIVE TRANSFER

Another recent advance in preclinical asthma models is the ability to gather information on the mode-of-action upon the immune response of a test item.

Figure 3: Lung histology during asthma



By utilising transgenic T cells that are specific for OVA, it is possible to track these cells in the lungs and the peripheral and draining lymph nodes as asthma is induced. This model makes use of the fact that T cells are central to the immune response in asthma. As shown in Figure 2, T cells are involved from very early on in the immune response, as soon as a dendritic cell presents antigen. The naïve T cells then differentiate into Th2 cells and release cytokines, which induce B cells to class switch to IgE. Binding of IgE to the high affinity IgE Fc receptor on the surface of mast cells leads to crosslinking of IgE, which in turn activates mast cells, causing them to degranulate and release a range of mediators such as histamine, prostaglandins and leukotrienes, which leads to bronchoconstriction. The activated T cells in the lungs also release cytokines such as IL-3 and IL-5, which act to recruit and activate eosinophils, mast cells, more lymphocytes and neutrophils both within the lymph nodes and lung. Thus T cells are involved at many stages in the development of asthma. By tracking T cells and any effect exerted upon them by a test item, we can determine at which stage and where a test item is affecting the antigen-specific immune response during asthma.

An additional benefit of adoptive transfer models is that they provide information on the immune response as it occurs, whereas traditional models only provide information on the final immune response (antibody levels, for example). Indeed, using adoptive transfer, it is possible to gather *in vivo* information from extremely early on in the immune response (8). This technology has been well described in the literature, and adoptive transfer of cells is now a well established immunological technique (9) although the application of this technology to asthma models is a more recent development (10,11,12).

Using this technology, it is possible to determine whether a test item is able to affect a range of events during an immune response, such as the activation of T cells, the clonal expansion of T cells, the Th1/Th2 bias of an immune response or the activation of T cells (13). Of note are the facts that adoptive transfer technology can also be applied to other diseases such as rheumatoid arthritis and that this technology can be utilised as a powerful first step in identifying the mode-of-action of a test item in the absence of a disease setting.

This technology can also provide information on what type of disease a test item is likely to be efficacious in. For example if it is discovered that the test item modulates the cytokine balance towards a Th2 response, then it would not be sensible to test the item in an asthma model but rather in a disease with a Th1 mediated pathology such as the collagen induced arthritis model of rheumatoid arthritis.

CONCLUSIONS

Preclinical asthma models remain an important tool for the industry. Most models available offer well-established readouts such as pulmonary cell influx and antibody levels, which have good correlation with human disease. Recent developments in models now offer researchers the ability to not only discover whether or not the test item is effective against asthma, but can also inform on timing site and mode-of-action. ♦

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