

Asthma as a therapeutic target: choosing a pre-clinical model

The prevalence of asthma, along with asthma-associated morbidity and mortality, continues to increase worldwide. It is estimated that between 5-12% of the world's population now suffer from the disease. Asthma is therefore an important target for the biopharmaceutical industry. Development of new therapeutics depends upon suitable pre-clinical models. The goal of a pre-clinical asthma model is to reproduce the airway inflammation, mucus hypersecretion or airway hyper-responsiveness seen in human asthma.

Currently available pre-clinical models

The majority of pre-clinical models of asthma utilize rodents. Rodents are sensitized 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 utilize multiple sensitization steps, followed by one or more local challenges with the antigen into the lungs. The entire process typically takes approximately one month. Some of these models have the added advantage of being suitable for use in studying effects of test items in other pulmonary disorders such as rhinitis.

Typical endpoints measured

Lung function

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 airway

hyper-responsiveness (AHR).

Asthmatic immune response

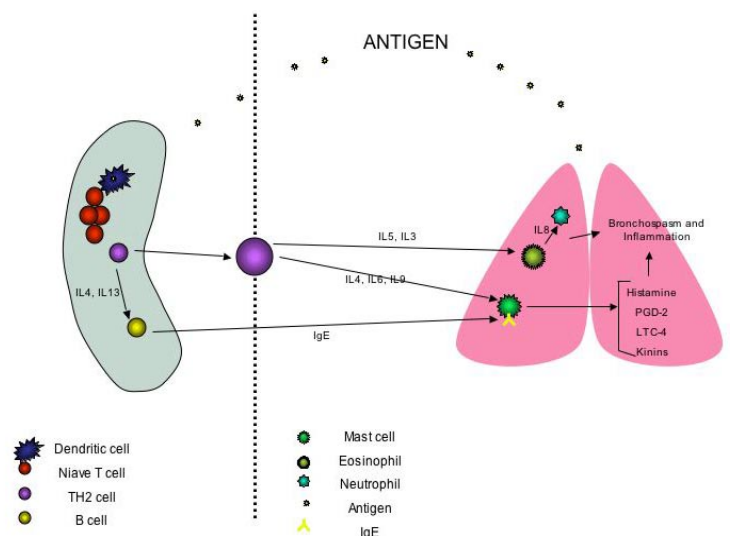
In contrast to lung function measurement, the immune response during asthma is well preserved between mice and humans. In human asthma, eosinophils and lymphocytes infiltrate the bronchial mucosa. Increased mucus secretion and production of Th2-associated cytokines such as IL-4, IL-5 and IL-13 are also 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 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 sensitization while IL-13 is 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 pre-clinical asthma models.

Mast cells are central to the development of asthma due to their ability to release an array of pre-formed and newly synthesized inflammatory mediators such as cytokines, leukotrienes and prostaglandins (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 pre-clinical asthma models.

Recent developments in endpoints

The above readouts are well established and have been utilized for many years without significant alteration. In recent years models that make use of methods such as MRI or adoptive transfer have led to several advances in the end measurements.

Figure 1. The roll of T cells in asthma



Pre-clinical Asthma Models

Traditional 28 day OVA Allergic Asthma:

- Histology of Lung
- Flow cytometry and cytokine analysis of BAL
- Total & antigen specific IgG/E

Rapid 14 day OVA Allergic Asthma:

- Histology of Lung
- Flow cytometry and cytokine analysis of BAL
- Total & antigen specific IgG/E

OVA combined with adoptive transfer:

- Traditional assessments plus...
- Flow cytometry of BAL for proportion of Tg T cells
- Flow cytometry of draining & peripheral lymph nodes for proportion of Tg T cells
- IHC of lungs and lymph nodes for Tg T cells by light microscopy or LSC

In vitro asthma models:

- Cytokine stimulated human lung epithelial model
- Cytokine stimulated human bronchial smooth muscle cell

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Adoptive transfer

The use of adoptive transfer techniques in pre-clinical asthma models provides the ability to gather information on the mode of action upon the immune response of a test item. By utilizing 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 1, T cells are involved from very early 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 cross-linking of IgE, which in turn activates mast cells causing them to degranulate and release a range of mediators such as histamine, prostaglandins and leukotrienes leading 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 exerted effect 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 (e.g. antibody levels). 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 (1). 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 utilized 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. 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

Pre-clinical asthma models remain an important tool for the pharmaceutical industry. MD Biosciences offers several models that offer well-established readouts such as pulmonary cell influx and antibody levels, which have good correlation with human disease. Additionally, we are able to 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 by utilizing the adoptive transfer asthma model.

References

1. Marshall FA, et al., (2005) J Immunol, 175:5817.

Inflammatory Bowel Disease

An early event thought to participate in the pathogenesis of inflammatory bowel disease (IBD) is the disruption of the gastrointestinal epithelial barrier. This disruption leads to the mixing of microbial pathogens from the lumen with antigen presenting cells in the lamina propria producing an inflammatory response. The resulting pro-inflammatory cytokines and chemokines recruit and activate leukocytes, regulate the integrity of the epithelial barrier and stimulate the production of chemokines from epithelial cells. Together, these events lead to chronic inflammation in the intestines.

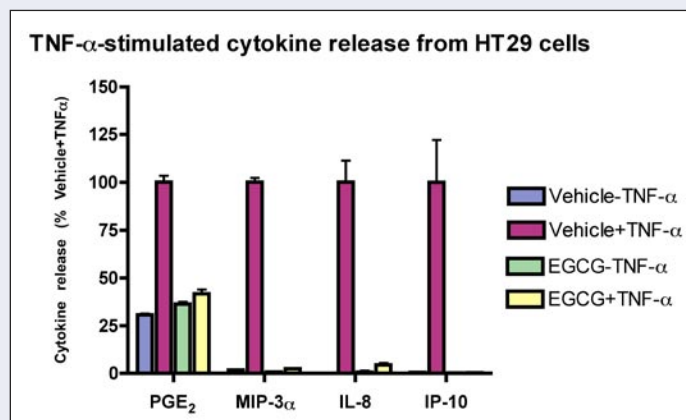
MD Biosciences offers two IBD in vitro assays designed to evaluate the effect of a compound on two of these events:

Macrophage-induced intestinal epithelial cell damage model.

A monolayer of human colon adenocarcinoma cells is cultured on a semipermeable support membrane above LPS-stimulated human macrophages in the presence and absence of test compound. Macrophage-induced epithelial monolayer damage is determined by measuring the transepithelial electrical resistance (TEER).

Cytokine-stimulated human colon adenocarcinoma cell model.

The human colon adenocarcinoma cell line HT29 is stimulated with TNF- α to mimic chronic inflammation in the presence and absence of test compound. Cell culture media is removed at 6 and 24 hours after stimulation and assayed for the following inflammatory mediators: PGE₂, IL-8, IP-10, and MIP-3 α .



In addition to the in vitro models, MD Biosciences performs the TNBS model for the study of IBD. TNBS is administered intrarectally to induce an intestinal pathology that is driven by IL-12. This model is a 7 day model using sulfasalazine as a control.