

immunoprofiler continued...

Molecular Mode of Action:

Extracellular cues are transmitted through the cell by a network of signal transduction molecules. A compound's mode of action can be further characterized by identifying pathways affected such as:

- Identify inhibitors or agonists of cell signaling pathways by determining the phosphorylation state of intracellular proteins such as Akt, CREB, ERK1/2, GSK-3 β , HSP27, I κ B α , JNK, p38 MAPK, p70S6K and ZAP-70. Concentrate on a specific pathway, such as T cell activation, or screen multiple pathways at once.
- Screen compounds for their ability to activate or inhibit a specific pathway using cell lines harboring a luciferase reporter gene under the control of NF κ B, STAT-1, STAT-3, AP-1, CREB or NFAT responsive elements.
- Determine second messenger levels, such as cAMP or calcium, in compound treated cells.
- Screen compounds for their ability to inhibit enzyme activity: kinase activity, cyclooxygenase activity (COX-1, COX-2), monoamine oxidase activity (MAO), aggrecanase activity, matrix metalloproteinase activity.

Gene Expression Analysis

In addition to examining protein production, we also offer gene expression analysis in many of our *in vitro* and *in vivo* models. Using branched DNA (bDNA) signal amplification in association with bead-based multiplex technology¹, we can analyze up to 30 genes in a single sample allowing for high-throughput screening of small volume samples. Sample types include whole blood, cultured cells, fresh or frozen tissues and formalin fixed or paraffin embedded tissue. Providing high sensitivity, a high dynamic detection range and a high level of reproducibility, our gene expression service allows the ability to compare effects of gene induction in different tissues as well as investigate effects of drug treatment on disease.

1. QuantiGene Plex 2.0 System, Panomics, Inc.

THE STUDY OF LUNG FUNCTION: WORTHWHILE OR MEANINGLESS IN PRE-CLINICAL ALLERGIC ASTHMA MODELS?

In recent years there has been much discussion as to whether methods of studying lung function in pre-clinical models of allergic lung inflammation are worthwhile. Some researchers maintain that the physiological differences in rodent lung function versus human lungs mean that lung function studies in rodents are meaningless.

Despite this, the most consistent diagnostic feature of asthma is airway hyperresponsiveness (AHR) in response to chemicals such as Methacholine or Adenosine. For this reason, many researchers feel that in order for an asthma therapeutic to be efficacious, it must be shown to affect AHR.

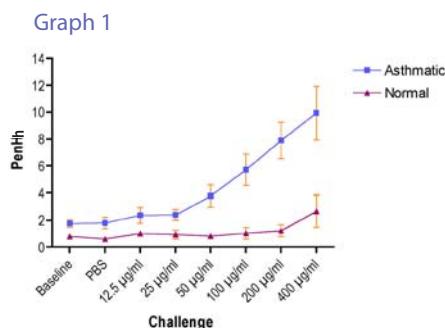
Although much research on this topic has been carried out, we still do not fully understand why the AHR response occurs. Airway inflammation involving cytokines such as IL-4, IL-5 and IL-13 and cells such as mast cells and eosinophils as well as neurogenic abnormalities are believed to contribute to AHR.

There are several different methods of assessing AHR in preclinical studies. *In vitro*, the contraction of smooth muscle samples after electrical stimulation can be assessed. *In vivo*, the measurement of lung resistance or compliance can be assessed following administration of Methacholine or other bronchoconstrictive chemicals. Such *in vivo* analysis can be carried out invasively on tracheotomized and ventilated animals. These are very labour intensive and time consuming methods, but they do result in determination of airways resistance and dynamic compliance. Another *in vivo* option is whole-body plethysmography using unrestrained conscious animals. This is used for more high-throughput screening and determines factors such as Penh (see below).

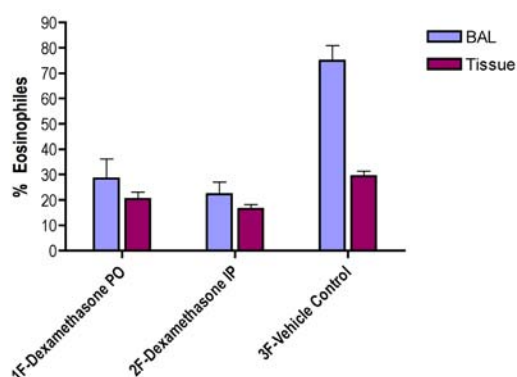
During whole-body plethysmography the animals are placed in small Perspex boxes and the pressure changes that occur as the animal breathes in and out whilst being challenged with a bronchoconstrictive agent are measured. This method has the advantage that the breathing pattern is natural. Various measurements can be applied. One common parameter measured is the enhanced pause (Penh). Penh is empirically derived from the pressure changes in the box and can be used as a measure of patterns of respiration. Inspiration and expiration are processed as a waveform of the box pressure-time signal and are recorded by computer. Changes to the early expiration that can occur due to bronchoconstriction will alter the waveform of the box pressure-time signal and can be quantified.

There are publications showing strong correlations between Penh and airway resistance and that Penh correlated to eosinophil numbers (1) as well as publications indicating that Penh is not a relevant readout for lung function plethysmography in mice (2).

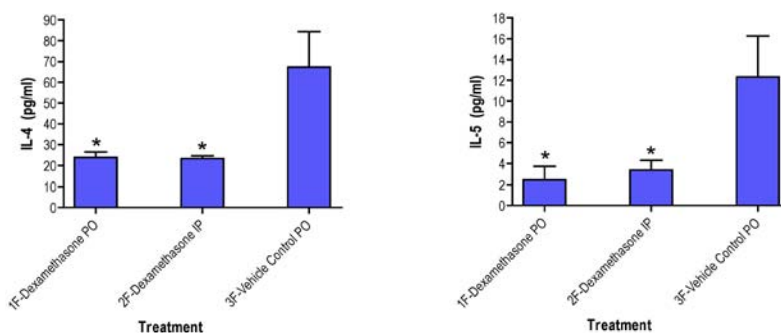
Generally several different concentrations of the bronchoconstrictive agent are used in order to generate a curve for Penh as can be seen below. In this graph the nonselective adenosine receptor agonist 5'-N-ethylcarboxamido adenosine (NECA) was nebulised and administered to the mice in



increasing concentrations from 12.5ug/ml to 400ug/ml. NECA was administered for 2 minutes and the lung response was measured for 5 minutes afterwards. Naïve healthy animals were studied along side mice that had been induced to have an allergic lung inflammation against ovalbumin. It is obvious that the lungs of asthma-induced mice respond to lower concentrations of NECA than healthy mice (graph 1). Typical results for inflammatory cell influx and cytokine responses in asthmatic mice are represented below.



Graph 2: Percentage of granulocytes that are eosinophils within BAL and lung tissue



Graphs 3, 4: Production of IL-4 and IL-5 within lung airways. *Asterik indicates a P-value <0.05 lower for Dexamethasone than for vehicle.

As the prevalence of asthma, along with asthma-associated morbidity and mortality, increases worldwide, asthma is an important therapeutic target for the biopharmaceutical industry. The development of new asthma therapeutics depends upon suitable pre-clinical models that reproduce the airway inflammation, mucus hypersecretion or airway hyper-responsiveness. MD Biosciences offers *in vitro* and *in vivo* models of asthma for determining efficacy of potential asthma compounds. Visit www.mdbiosciences.com to learn more.

References

1. Hamelmann, E., et al 1997. Am. J. Respir. Crit. Care Med. 156:766.
2. Lennart K., et al 2002. J. Appl. Physiol. 93:1198–1207.

PRE-CLINICAL ASTHMA MODELS

In Vivo Models

- Traditional 28-day OVA Allergic Asthma model
- Rapid 14-day OVA Allergic Asthma model

Readouts available:

- Histology of lung
- Flow cytometry and cytokine analysis of BAL
- Anti-OVA antigen-specific IgG/IgE
- Airway hyperresponsiveness
- Gene expression

In Vitro Models

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



MOUSE OVA-IgE ELISA:

Catalog #OVA-IGE96

Measure OVA-specific IgE in mouse serum and cell culture supernate samples.

- Ready to use delivering results in 2.25 hours
- Stringent quality control so you get accurate and reproducible results
- Measure levels down to 7.8 ng/mL with a sensitivity <3.8 ng/mL

Visit www.mdbiosciences.com to place an order.