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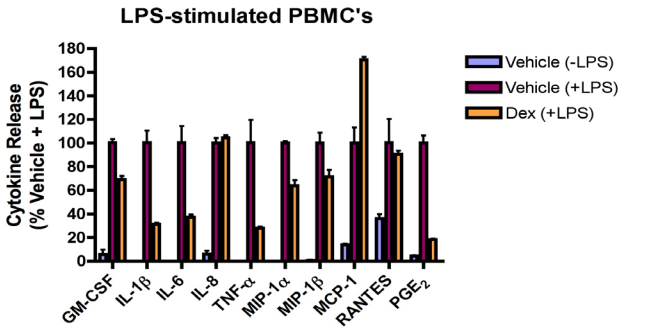
Contents

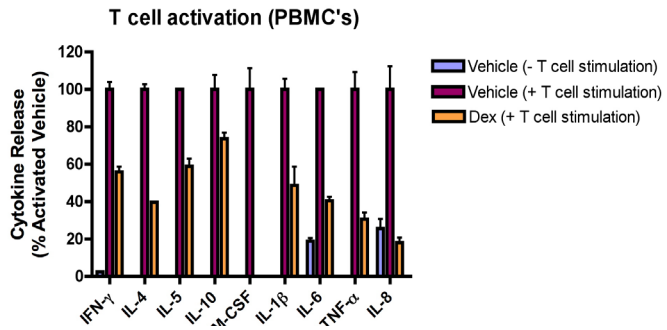
ImmuneProfiler™: *In Vitro* Drug Discovery Services

Cytokine and cell-based assays provide the ability to interpret and predict many biological properties of potential drug compounds. Cytokines and related molecules have been implicated in inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, asthma, inflammatory bowel disease and autoimmune diseases as well as cancer and infectious diseases. These assays can be used in a sequential approach and have the ability to determine a compound's effect on cell proliferation, cytokine production, effects on cellular responses as well as molecular mode of action.

General Anti-inflammatory screen:
Human PBMCs are stimulated with a stimulant such as LPS or PHA plus various concentrations of compound. Cytokine levels are then evaluated using multiplex technology. Gene expression can also be evaluated using branched DNA (bDNA) signal amplification.

T-cell activation assay:
Human PBMCs are stimulated with a T cell activation cocktail plus various concentrations of a compound. T cell activation and cell proliferation are then evaluated providing information on a compound's effect upon T cell activation.





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immuneprofiler 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-3β, HSP27, IκBα, 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 NFκB, 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 technology[1], 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.

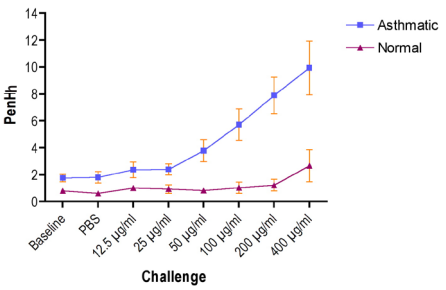
Although much research on this topic has been carried out, we still do not fully understand why the AHR response occurs. Airway inflammation involving cytokines such as IL-4, IL-5 and IL-13 and cells such as mast cells and eosinophils as well as neurogenic abnormalities are believed to contribute to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

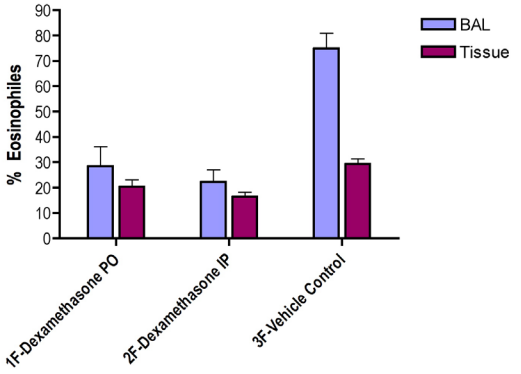
There are publications showing strong correlations between Penh and airway resistance and that Penh correlated to eosinophil numbers (1) as well as publications indicating that Penh is not a relevant readout for lung function plethysmography in mice (2).

Graph 1

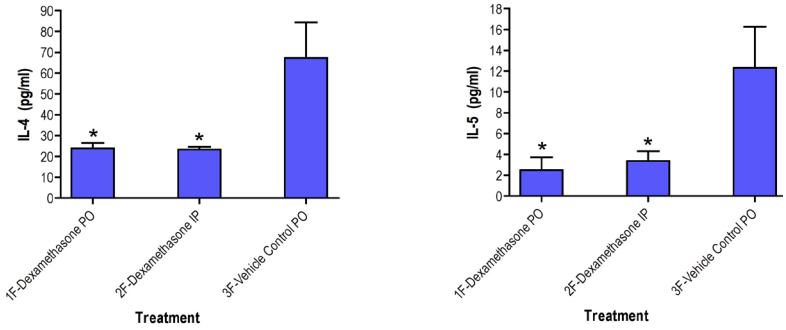


Generally several different concentrations of the bronchoconstrictive agent are used in order to generate a curve for Penh as can be seen below. In this graph the nonselective adenosine receptor agonist 5'-N-ethylcarboxamido adenosine (NECA) was nebulised and administered to the mice in

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.mdbioscienes.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

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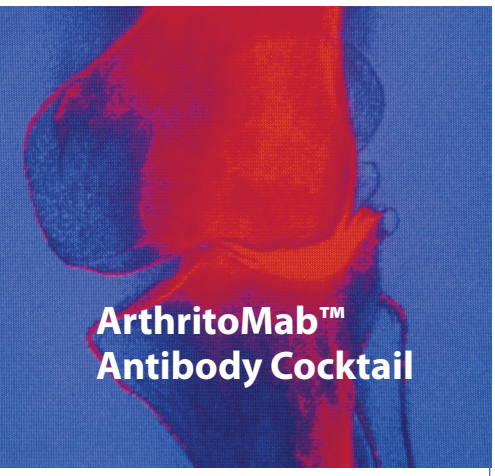

The ability to induce arthritis using this arthritogenic antibody cocktail provides an efficient protocol for the induction of antibody-mediated arthritis that can be used as a shorter, more synchronized alternative to the CIA model.

Catalog #CIA-MAB-50

- **Rapid Model:** results in as little as 7 days reducing costs associated with expensive compounds, controls, scoring and administration periods
- **Synchronized Disease Onset:** animals develop arthritis at the same time, eliminating complicated administration schedules based on disease onset of each mouse
- **Histology Results in Just 18 Days:** provides researchers valuable data quickly compared to months in CIA model
- **Susceptibility:** Arthritis is induced not only in CIA-susceptible DBA/1, B10.RIII and Balb/c mice, but also in some CIA-resistant mice, such as C57Bl/6 mice.

To place an order or download the whitepaper, log on to www.mdbiosciences.com

Inflammation and Neurology Research

Induction Reagents

Species	Catalog #	Form	Size
Bovine Collagen II	804001-Lyo	Lyophilized	10 mg/vial
Bovine Collagen II	804001-Sol	Solution	10 mg/vial
Chicken Collagen II	804002-Lyo	Lyophilized	10 mg/vial
Rat Lathyritic Collagen II NEW!	8041001	Solution	0.5 mg or 1.0 mg/vial
Rat Pepsin-digested Collagen II NEW!	8041002	Solution	0.5 mg or 1.0 mg/vial
ArthritoMab™ Antibody Cocktail	CIA-MAB-50	Liquid	50/mg vial
Myelin Oligodendrocyte Glycoprotein (MOG 25-55)	3038001		25/mg vial
Myelin Proteolipid Protein (PLP 139-151)	301008		15/mg vial

ELISAs

Analyte	Catalog #	Species	Sample Size
Animal COMP ELISA	A-COMP.96	rat/mouse/sheep/bovine/canine	100 µL
Aggrecanase Activity ELISA	ACT-AGG.96	human	100 µL
Sensitive Aggrecanase Activity ELISA	SEN-AGG.96	human	100 µL
active MMP-13	ACT-MMP13.96	human	100 µL
Mouse OVA-IGE ELISA	OVA-IGE96	mouse	100 µL
Substance P ELISA	SUBP.96	rat/mouse/human	100 µL
β-endorphin ELISA	EDRF.96	rat/bovine/equine/human/ovine/camel/porcine	100 µL

Collagen Kits

Product	Catalog #
Collagen II Staining Kit	CIIST
Collage Type II ELISA	CII96
Mouse IgG anti-Bovine Collagen Type II ELISA NEW!	CIIAB96-B
Mouse IgG anti-Chicken Collagen Type II ELISA NEW!	CIIAB96-C
Mouse IgG anti-Porcine Collagen Type II ELISA NEW!	CIIAB96-P
Mouse IgG anti-Mouse Collagen Type II ELISA NEW!	CIIAB96-M
Mouse IgG anti-Rat Collagen Type II ELISA NEW!	CIIAB96-R

Proteoglycans

Product	Catalog #
Septum Cartilage Extract	5028044
Joint Cartilage Extract	5028045

Adjuvants

Product	Catalog #	Size
Complete Freund's 4 mg/mL	501009	5 ml/vial
Complete Freund's 3 mg/mL	501010	5 ml/vial
Incomplete Freund's	501001	5 ml/vial

Antibodies

Species	Catalog #	Size
Aggrecan mAb to N-terminal neoepitope ARG (clone BC3)	1042001	100 µg
Aggrecan mAb to N-terminal neoepitope DIPEN (clone BC4)	1042002	100 µg
Aggrecan mAb to C-terminal neoepitope NITEGE (clone BC13)	1042003	100 µg
Aggrecan mAb to N-terminal neoepitope FFGV (clone BC14)	1042004	100 µg
BRCA1 mAb (clone 6B4)	1042005	100 µg
TCR, D011.10 mAb (clone KJ1-26)	1042006	200 µg

In Vivo Efficacy Disease Models

Arthritis
- Collagen-induced Arthritis (CIA)
- Anti-collagen-induced Arthritis (ACIA)
- Adjuvant-induced Arthritis (AIA)

Asthma
- OVA-induced Asthma

Diabetes
- Low Dose STZ Diabetes

IBD
- DSS-induced IBD
- TNBS-induced IBD
- SCID IBD Model

Multiple Sclerosis
- PLP-induced EAE
- MOG-induced EAE
- MBP-induced EAE

Pain
- CCI Sciatic Nerve Ligation (Bennet & Zie Model)
- Spinal Nerve Ligation (Chung Model)
- Taxol-induced Neuropathy
- STZ-Diabetic Neuropathy
- Carrageenan-induced Acute Inflammatory Pain
- CFA-induced Acute Inflammatory Pain
- Post-incisional pain in rats (Brennan Model)
- Post-incisional pain in pigs
- Adjuvant-induced Arthritis & Arthritic Pain
- Tail Flick Test
- Visceral Pain (acetic acid writhing test)
- Capsaicin

Parkinson's Disease
- Acute MPTP Model
- Chronic MPTP Model
- 6ODHA Model

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Recent Publications:

Ho *et al. (2007)*
Journal of Leukocyte Biology
Volume 82, December 2007

IL-33 induces IL-13 production by mouse mast cells independently of IgE-Fc{varepsilon}RI signals.

Product Cited: T1/ST2 Antibody
This clone recognizes the membrane anchored murine T1M protein on the surface of T helper 2 cells and mast cells. T1M appears on fetal blood derived mast cell progenitors before they express the Fcε RI, on IL-3-dependent bone marrow derived mast cells and on mature peritoneal mast cells. The antibody detects T1S protein consisting only of the extracellular portion of the protein, which is secreted from growth factor and proinflammatory cytokine stimulated murine fibroblasts.

- Catalog # 101001
  101001F (FITC)
  101001B (Biotinylated)
- Description: Affinity purified monoclonal antibody, Clone DJ8
- Applications: Flow Cytometry, IP, IHC

Other T cell antibodies

Mouse Anti- Human ST2L, monoclonal antibody, affinity purified
- Catalog # 101002
  10102F (FITC conjugated)
  101002B (Biotinylated)

Mouse Anti- Human IL-18R, receptor, polyclonal antibody, affinity purified
- Catalog # 201006

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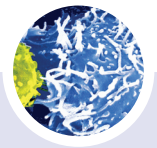
Finding breakthrough therapies in the treatment of chronic pain



Models of Pain

MD Biosciences offers established pre-clinical models for evaluating potential therapies for Nociceptive, Neuropathic and Inflammatory Pain.

Neuropathic Pain:
- CCI Sciatic Nerve Ligation (Bennet & Zie Model)
- Spinal Nerve Ligaton (Chung Model)
- Taxol-induced Neuropathy
- STZ-Diabetic Neuropathy

Inflammatory Pain:
- Carrageenan-induced
- CFA-induced Inflammatory Pain

Post-operative Pain:
- Post-incisional pain in rats
- Post-incisional pain in pigs

Arthritic Pain:
- Adjuvant-induced Arthritis & Arthritic Pain

Nociceptive:
- Tail Flick Test
- Visceral Pain (acetic acid writhing test)
- Capsaicin

To speak to a scientist about evaluating a compound in a model of pain, email info-us@mdbiosciences.com or log on to www.mdbiosciences.com

Pain is associated with the threat or presence of tissue damage and protects the body by warning us to avoid potentially harmful situations. We sense pain through the activation of sensory neurons. The "first-order" sensory neuron resides in the dorsal root ganglion (DRG). Axons from the neurons within these ganglia innervate our skin, organs, and bones. These fibers terminate either in specialized receptors that sense vibration, mechanical forces, heat, etc., or they terminate as free nerve endings that are exposed to the chemical environment of the tissue.

Nociceptors are specialized sensory neurons that preferentially respond to noxious stimuli. Nociceptive or physiological pain refers to the type of pain that we feel on a day-to-day basis. It is directly associated with a noxious stimulus, such as bending our joints too far, and helps us put limits on ourselves to avoid damage. This information is mediated by both small unmyelinated fibers and myelinated fibers, and provides us with an awareness of the environment. Nociceptive pain is pharmacologically treatable.

When pain continues beyond the original insult, it is generally associated with the healing process and reminds us to establish limits which allow our bodies to repair themselves. Under certain conditions, pain can continue long after the initial stimulus or occur even in the absence of any obvious damage. This phenomenon is referred to as chronic pain, a condition that affects millions worldwide.

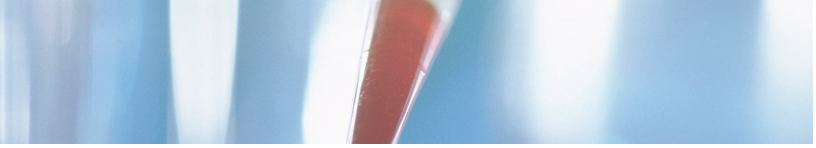
The causes of neuropathic pain are mechanical nerve injury (either to periphery nerve or to the spinal cord) as results of tumor compression, trauma, ischemia, inflammation, diabetes and chemotherapy. It is a condition that develops after the original injury and is manifested by both constant (spontaneous) pain and an abnormal response to sensory stimuli (evoked activity) that is interpreted out of proportion to the intensity of the stimulus (that is, greatly exaggerated).

Depending on the type and cause of pain, it may be treated with conventional analgesics/anti-inflammatory drugs such non-steroidal anti-inflammatory drugs (NSAIDs) or acetaminophen including ibuprofen or naproxen. More intense pain may require stronger treatment like opioid therapy; morphine, the prototypical opiate, is still commonly used to relieve severe pain. In addition, medications such as tricyclic antidepressants and anticonvulsants, initially approved for other conditions, have shown some efficacy in treating chronic neuropathic pain.

Despite many advances in the treatment of pain, some patients continue to struggle to find effective relief. Chronic pain, especially when neuropathic in origin, can become difficult to manage with long-term conventional therapy.

One challenge that arises in the search for an effective pain therapy is inconsistency in patient responsiveness to a given treatment, even among those who present similar symptoms. For example, medications used to treat chronic neuropathic pain typically show no more than a 40% to 60% success rate in providing clinically relevant relief to patients, and complete relief is often achieved in a much smaller proportion [1]. The incidence of negative side effects such as constipation, dizziness, and sedation may also limit treatment options. Additionally, patients may develop tolerance to certain medications after long-term use. Tolerance is a common problem among the some 6 million patients who are on prolonged opioid therapy for severe chronic pain. Because of the risk of abuse, many doctors are reluctant to prescribe high doses of opioids, leaving some patients with inadequate relief.

Ideally, pain therapy would eliminate nociceptive signals without affecting cognitive, motor, or other sensory functions. Unfortunately, none of the currently available treatments meet these criteria. For example, lidocaine, the most commonly used local anesthetic, interferes with neuronal signaling by diffusing into cells and blocking sodium channels from the inside. Although this mechanism of action is very effective in attenuating pain, it



does not allow discrimination between cell types. As a result, anti-nociceptive effects are accompanied by a paralysis, numbness, and disruption of autonomic input.

As basic scientists continue to make discoveries regarding the contribution of particular ion channels to sensory function, more selective targeting of pain signaling will be possible. A recently published study evaluated a novel strategy that may bring us closer to this goal. Researchers combined the administration of capsaicin, the active ingredient in hot peppers, and a lidocaine derivative called QX-314. Capsaicin acts at TRPV1 receptors, which are uniquely expressed in nociceptive neurons. Although QX-314 retains the anesthetic property of lidocaine, it is distinct in its inability to pass through the cell membrane under normal conditions. When capsaicin binds to TRPV1 receptors, a pore large enough for QX-314 to pass through is opened. Co-administration of capsaicin with QX-314 thereby allows the drug to selectively enter and inhibit nociceptors.

In this study, the effect of combined capsaicin and QX-314 treatment on neuronal excitability was first evaluated *in vitro* using cultured sensory neurons. While the application of QX-314 alone had little effect unless injected directly into cells, co-application with capsaicin achieved a nearly complete blockage of evoked sodium current, reflecting a substantial decrease in nociceptor excitability. This effect was observed only in TRPV1-expressing neurons, confirming that the drug's anesthetic actions are dependent on entrance into the cell through TRPV1 receptors.

The researchers then tested whether the selective effects would be maintained *in vivo*. In both heat and mechanical nociceptive tests, injection of capsaicin and QX-314 in combination into the hind paw or sciatic nerve of rats dramatically decreased sensitivity to noxious stimuli. As expected, QX-314 had no significant effect on nociceptive thresholds when injected alone. Furthermore, in contrast to lidocaine injection, the combined treatment resulted in successful maintenance of motor and tactile sensory function. This important difference demonstrates the value of this method in selectively targeting pain transmission without disrupting other functions [2].

This is the first study to exploit a channel expressed by a distinct group of neurons to deliver a drug exclusively to those cells. Building on this exciting new approach of using combination therapy to selectively target nociceptive function may eventually lead to the development of improved pain treatment for a variety of conditions wherein maintenance of cognitive, motor, sympathetic and tactile sensory function is favored. Importantly, this novel technique also holds promise for breakthroughs in the treatment of chronic pain.

References:

1. Markman JD and Dworkin RH. (2006) J Pain, 7(15): S38-47.
2. Binshtok AM, et al. (2007) Nature, 449(7162): 607-10.

MD Biosciences offers several in vivo models for the study of nociceptive, inflammatory and chronic pain. Custom design models and in vitro assays are also available.

Understanding How a Compound Affects Cell Adhesion and Migration

The recruitment and adhesion of leukocytes to areas of inflammation is a highly regulated process. Selectin-carbohydrate interactions slow down circulating leukocytes. Chemokines activate the appropriate cells through chemokine receptors on the tethered leukocytes. Finally, leukocyte integrins interact with components of the extracellular matrix and cell adhesion molecules on endothelial cells to form a tight bond. By understanding how a compound affects the adhesion process, one can predict the specific interactions being disrupted or enhanced. MD Biociences offers the following *in vitro* assays for the study of cell adhesion and migration.

- Leukocyte migration assays. Fluorescently labeled cells (neutrophils, T-cells or monocytes) are incubated on a permeable cell culture insert above a chemoattractant. The number of cells migrating through the insert towards the signal are quantified by fluorescence intensity.

- Endothelial cell adhesion assay. Fluorescently labeled leukocytes are incubated on a monolayer of TNF-a stimulated endothelial cells. After a wash to remove the unbound cells, the bound leukocytes are quantified by fluorescence intensity.

- Integrin-mediated adhesion. Fluorescently labeled leukocytes are incubated in plates coated with various integrin ligands such as collagen I, collagen IV, fibronectin, laminin, vitronectin, ICAM-1, ICAM-2, VCAM-1 and MAdCAM-1. After a wash to remove the unbound cells, the bound leukocytes are quantified by fluorescence intensity.

To speak to a scientist about cell adhesion and migration *in vitro* assays, email info-us@mdbiosciences.com or log on to www.mdbiosciences.com

SCID Transfer Model for IBD

Human Inflammatory Bowel Diseases (IBD), encompassing two illnesses, Crohn's disease and Ulcerative Colitis, are chronic wasting conditions of unknown etiology for which there is still no cure. They are characterized clinically by diarrhea and body weight loss. Histopathogical signs include leukocyte infiltration, pronounced epithelial hyperplasia, depletion of mucin secreting goblet cells and finally ulceration.

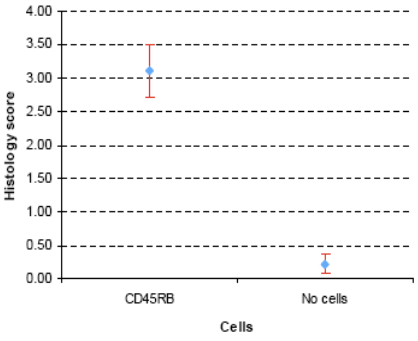
The study of IBD has been greatly advanced in the past ten years by the availability of a number of animal models facilitating the preclinical evaluation of drugs. The most commonly used are either chemically induced colitis models such as those induced by dextran sodium sulfate (DSS) or trinitrobenzene sulfonic acid (TNBS) or those resulting from an inadequate regulatory response exemplified by the SCID transfer model.

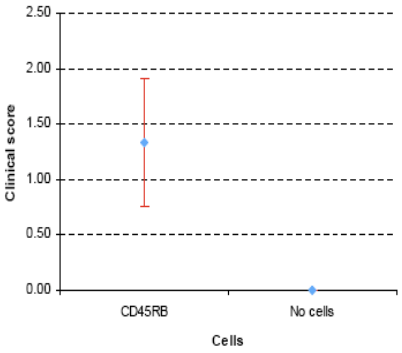
The SCID transfer model was pioneered by Fiona Powrie and colleagues, who observed that the transfer of naïve CD4+CD45RBhi cells from BALB/c mice into SCID (severe combined immunodeficient) mice resulted in the induction of a human-like colitis in the immuno-compromised recipients. The disease mechanism appears to be driven by the reaction of CD4+ T cells to the normal intestinal commensals present in the gut and is IL-12 and IFN-g dependent. The underlying mechanism is due to the depletion of the natural regulatory T cell population characterized by surface CD25 and low CD45RB expression, which in the normal animal, keep the potential disease inducing CD4+ T cells in check through TGFb and IL-10 production.

The advantage of the SCID transfer model over the more commonly available chemically induced colitis models is that it offers a more human-like IBD pathology where the mechanisms of disease induction closely resemble the hypothesized mechanism of colitis induction in humans, where a dysregulation of the immune response to harmless gut bacteria is proposed.

Clinical signs of colitis are expected from 4 weeks after cell transfer and include occult bleeding, body weight loss and diarrhea. The colitis becomes more severe during weeks 6-10. Endpoint readouts include histological scoring of the colon pathology and multiplex analysis of Th1 and pro-inflammatory cytokine production by cultured colon tissue explants.

Data from SCID IBD model showing histology and clinical scores.





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