

# Development of a new 3D-Human Airway Epithelium/ Whole-blood Co-culture Model Combined with Multi-Analyte Profile (MAP) Analyses for Assessing Drug Effects

Blum M<sup>1</sup>, Stein GM<sup>1</sup>, Constant S<sup>2</sup>, Wiszniewski L<sup>2</sup>, Huang S<sup>2</sup>, Mapes J<sup>3</sup>, Spain M<sup>3</sup>, Joos TO<sup>1</sup>, Schmolz M<sup>1</sup>



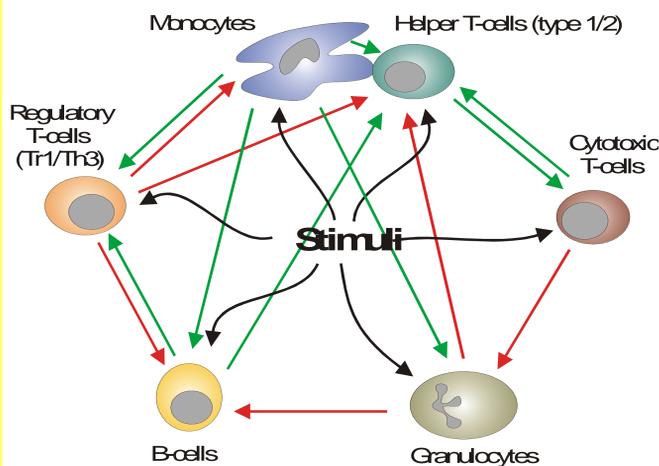
<sup>1</sup> EDI GmbH, Reutlingen, Germany; <sup>2</sup> Epithelix Sàrl, Plan-Les-Ouates, Genève, Suisse

<sup>3</sup> Rules-Based Medicine Inc., Austin, TX, USA



## Background

### Complex interaction of immunocompetent cells



The dialogue between cells of the immune system and cells of various tissues controls immune reactions and is in part mediated by a variety of cytokines, chemokines etc. This network may be strongly influenced by the application of drugs.

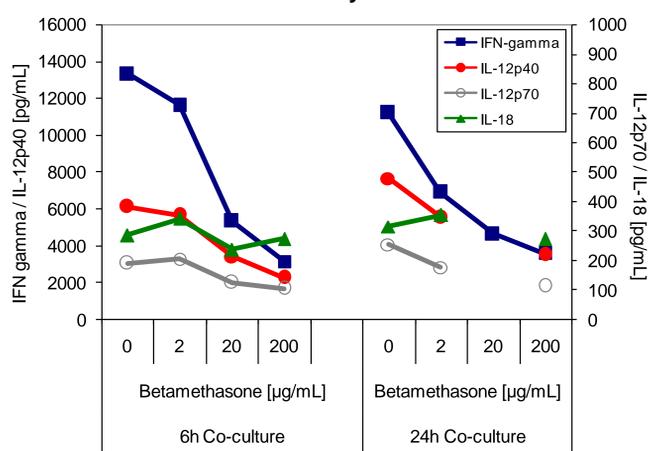
Aim of our investigations was to develop an innovative organo-typical human airway epithelial co-culture model for the analysis of immunopharmacological activities of drugs. A differentiated airway epithelium, MucilAir, was combined with whole-blood cultures in a two-chamber system to study the effects of betamethasone applied onto the epithelium sitting on activated immune cells from a healthy donor mimicking an inflamed tissue environment. 92 mediators and other parameters were tested in the supernatants of the cell cultures by multiplexed bead assays (RBM MAP analysis).

### Multi-Analyte Profiles (MAPs)

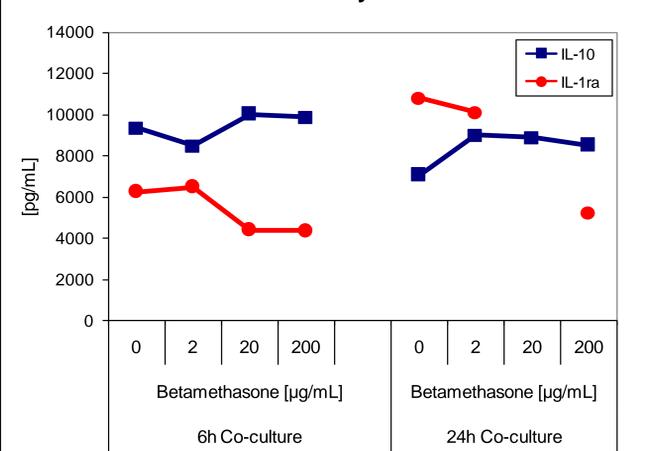
Th1-related	Th2-related	Treg-related	Chemokines	Cancer antigens
Interferon- $\gamma$ Interleukin-12p40 Interleukin-12p70	Interleukin-4 Interleukin-5 Interleukin-13	Interleukin-10	ENA-78 Eotaxin Interleukin-8 Lymphotactin MCP-1 MDC MIP-1 $\alpha$ MIP-1 $\beta$ RANTES	Alpha-Fetoprotein Cancer Antigen 19-9 Cancer Antigen 125 Carcinoembryonic Antigen Prostatic Acid Phosphatase PSA, Free
Monocyte/M $\Phi$ -related	Others		Cardio-/vascular disease related proteins	
G-CSF Interleukin-1 $\alpha$ Interleukin-1 $\beta$ Interleukin-1 $\beta$ Interleukin-6 Tumor Necrosis Factor- $\alpha$	Epidermal Growth Factor FGF-basic GM-CSF IGF-1 Stem Cell Factor Tumor Necrosis Factor- $\beta$	Interleukin-2 Interleukin-3 Interleukin-7 Interleukin-15 Interleukin-16	Apolipoprotein A-1 Apolipoprotein C-III CK-MB Endothelin-1	Fatty Acid BP Lipoprotein (a) Myoglobin PAPP-A
Enzymes	Hormones/BP	Receptors/Ligands	Others	
GST MMP-2 MMP-3 MMP-9 MMP-9 Myeloperoxidase SGOT	Adiponectin Calcitonin Erythropoietin Growth Hormone Insulin Leptin Sex Hormone Binding Globulin Thrombopoietin Thyroid Binding Globulin Thyroid Stimulating Hormone	CD40 CD40Ligand ICAM-1 Tissue Factor Tumor Necrosis Factor RII VCAM-1	Alpha-1 Antitrypsin Alpha-2 Macroglobulin Apolipoprotein H BDNF Beta-2 Microglobulin Complement 3 C-Reactive Protein ENRAGE Ferritin Haptoglobin Serum Amyloid P	Immunoglobulin A Immunoglobulin M Immunoglobulin E VEGF Factor VII Fibrinogen von Willebrand Factor PAI-1 TIMP-1

## Th1-related mediators

### Th1-related cytokines

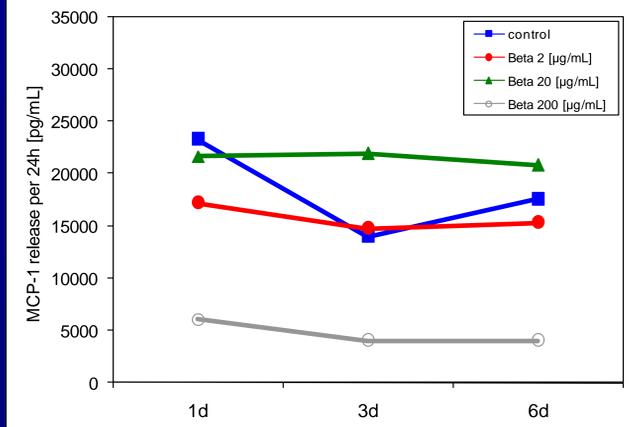


### anti-inflammatory mediators

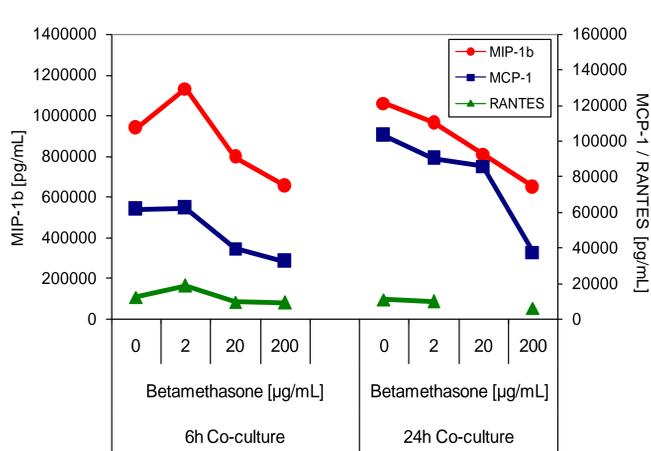


## MucilAir-Compartment

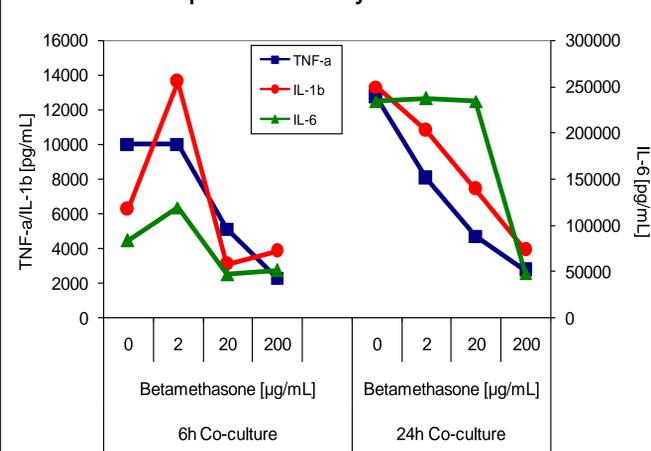
### MCP-1 MucilAir follow-up culture after 24h of Co-culture



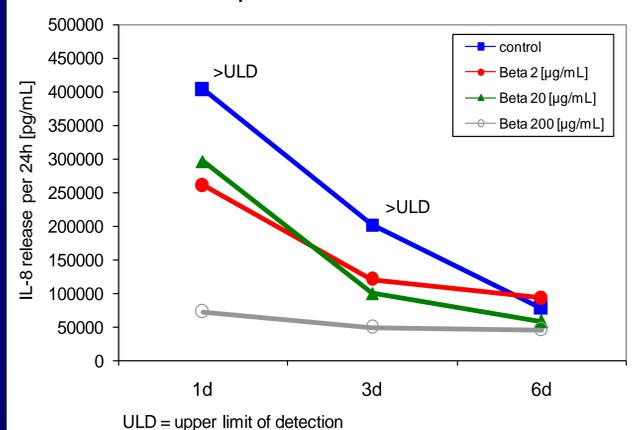
### Th1-related chemokines



### pro-inflammatory mediators

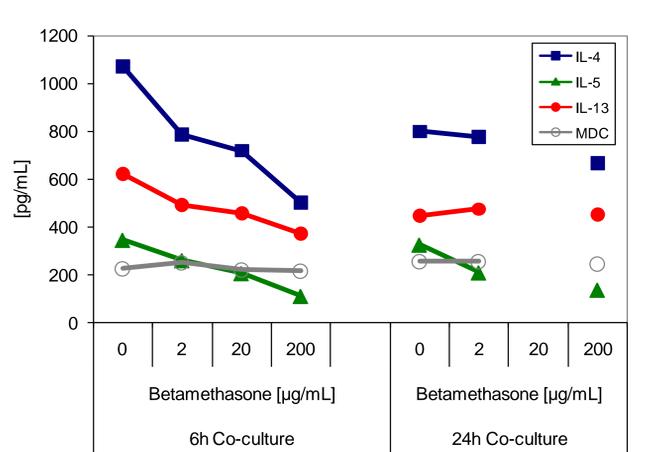


### IL-8 MucilAir follow-up culture after 24h of Co-culture



## Th2-related mediators

### Th2-related mediators



## Results and Conclusions

In this newly developed co-culture model of human airway epithelial cells in combination with whole blood cells, Betamethasone exhibited its typical, strong pharmacological effect profile on both, the immune and the epithelial cells: It dose-dependently inhibited a variety of pro-inflammatory mediators, being either T helper cell type 1- (Th1), Th2-, or macrophage-associated, such as interferon (IFN)-gamma, interleukin (IL)-12p70, IL-4, -5, -13 and tumor necrosis factor (TNF)-alpha, respectively. In contrast, IL-10 as anti-inflammatory mediator was up-regulated after 24h of co-culture. Furthermore, epithelial cells were cultured for another 6 days showing a dose-dependent effect on e.g. the monocyte chemotactic protein-1 (MCP-1) and IL-8.

From the data presented here, it is evident that the highly complex, organo-typical co-culture model provides an excellent tool to study *in vitro*, under *in vivo*-like conditions not only the pharmacokinetics and pharmacodynamics of inhaled drugs, but also the harmful effects of toxicants that get access to the human lung.

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Dr. Manfred Schmolz  
Aspenhaustrasse 25  
D-72770 Reutlingen  
Germany

phone: +49 7121 434103  
fax: +49 7121 491074  
e-mail: info@edigmbh.de  
web: www.edigmbh.de



Dr. Sabine Küsters  
Director of Europe Operations  
Rules-Based Medicine Inc.  
Austin, TX (USA)

phone/fax: +49 211 9894232  
e-mail: sabinek@rulesbasedmedicine.com  
web: www.rulesbasedmedicine.com



Dr. Song Huang  
14 chemin des Aulx  
CH-1228 Plan-Les-Ouates,  
Genève, Suisse

phone: +41 22794 6515  
fax: +41 22794 6517  
e-mail: song.huang@epithelix.com  
web: www.epithelix.com