Epithelix

in vitro Respiratory Solutions

Development of immuno-competent human airway epithelial models with macrophages for inhalation toxicity evaluation of airborne substances

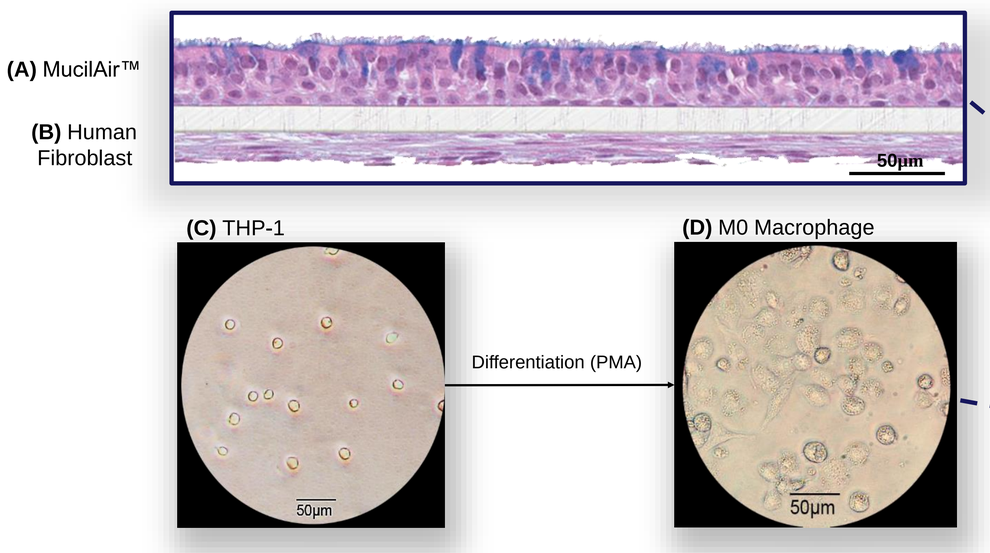
Bernadett Boda, Ina Fureraj, Imen Larafa, Xiao-Yann Huang, Christophe Mas, Song Huang, Samuel Constant

Epithelix, 18 chemin des Aulx, Plan-les-Ouates, CH-1228, Geneva, Switzerland

Introduction:

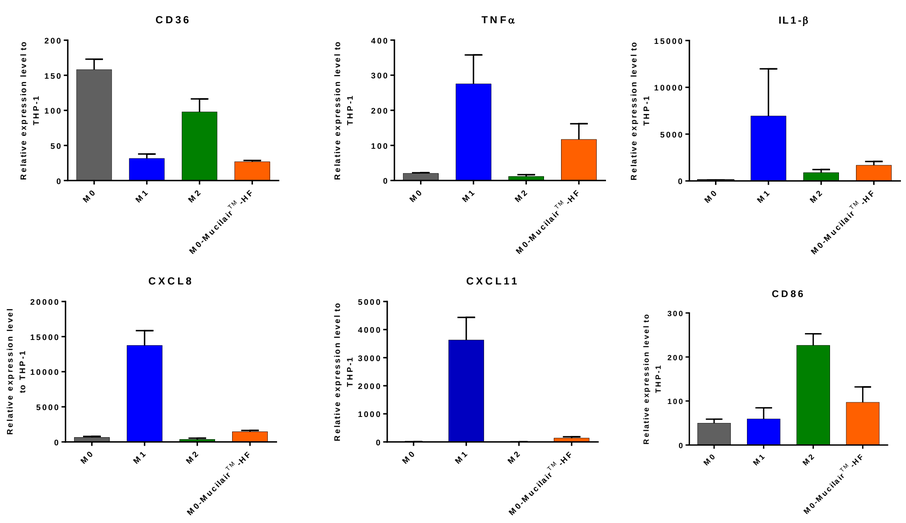
The human airway epithelium reconstituted *in vitro* from primary cells such as the MucilAir™ model is a powerful platform to support research on respiratory diseases and toxicity testing of inhaled compound. Although MucilAir™ recapitulates almost completely the inherent natural defenses mechanisms of the airway epithelia (mucociliary clearance, secretion of cytokines and antimicrobial peptides ect...), it lacks one of the major defense system against external threat: the immune cells. Epithelix developed **a novel co-culture media: the ImmunoAir™ medium, which is adapted for the culture of both MucilAir™ and Macrophages**. We hereby present preliminary data on a co-culture model that integrates MucilAir™, human fibroblast (MucilAir™ -HF) and THP-1 acute monocytic leukemia cell line derived Macrophages (M0 like) in ImmunoAir™ medium. The model (MucilAir™-HF-M0) is here challenged with reference inflammatory and anti-inflammatory compounds.

1- Co-culture strategy



Schematic representation of the co-culture model and compound testing. *Phorbol 12-myristate 13-acetate (PMA) is used for In vitro differentiation of THP-1(C) toward M0 cells like macrophages(D) ,during 72 hours in the ImmunoAir™ medium directly in a 24 well plate. MucilAirTM (A) co-cultured with Human fibroblasts(B) are then plugged into the well, and incubated at 37°C, 100% humidity, 5% CO_2 for 4 days (resting period). The co-culture system (*MucilAir™-HF-M0) *is then ready to use for testing (3) (4) (5). Additionally, characterization of the M0 phenotype was invastigated(2).*

2 - Macrophages characterization by RNA expression



Selected genetic markers for characterizing M0, M1, M2 and co-cultured M0 relative to THP-1. *CD36 is highly expressed in M0 while M1 has high expression levels of pro-inflammatory genes which are TNF-α, IL-1β, CXCL8 and CXCL11. CD86 and CD206 (data not shown) were selected as the best markers for M2. Co-cultured macrophages have high levels of pro-inflammatory cytokines suggesting their evolution toward M1 macrophages like phenotype. (Data shown for RTq-PCR are the means +/- SEM errors from 3 biological replicates).*

3 - MucilAir™-HF-M0 is sensitive for the detection of TNF-α pro-inflammation

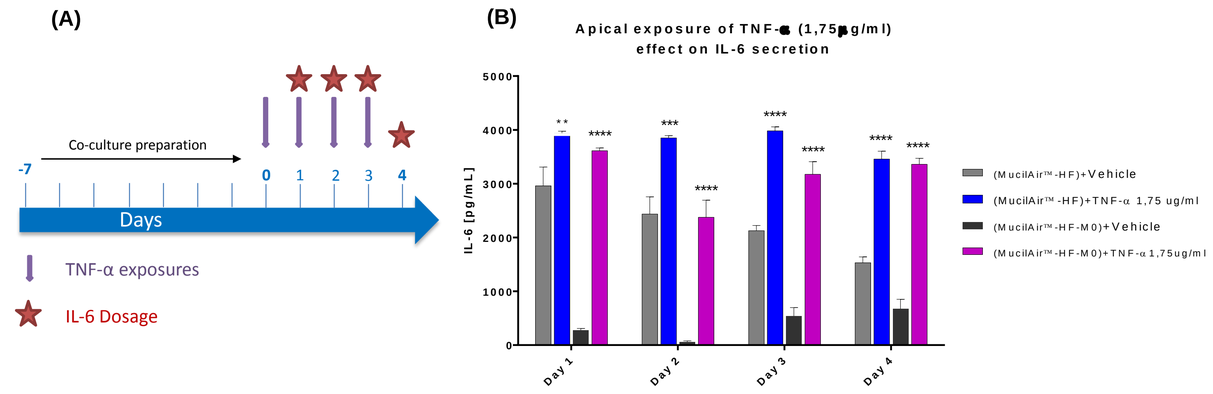


Figure A*: MucilAir™-HF co-cultured with macrophages (MucilAir™-HF-M0) are prepared following our standards protocol. Both MucilAir™-HF and MucilAir™-HF-M0 are exposed to 20µL of TNF-α in NaCl 0.9% vehicle (1.75µg/mL) every days during 4 days. Basal media is sampled on day 1, 2 3 and 4 to dose the IL-6 Level.*
Figure B* : *IL-6 basal secretion delta between the vehicle and TNF-α treated conditions are greater in the MucilAir™-HF-M0 than in the MucilAir™-HF conditions suggesting that MucilAir™-HF-M0 is potentially more sensitive than MucilAir ™-HF to detect TNF-α induced inflammation.*

4 – MucilAir™-HF-M0 detect transient $CdCl_2$ induced irritation

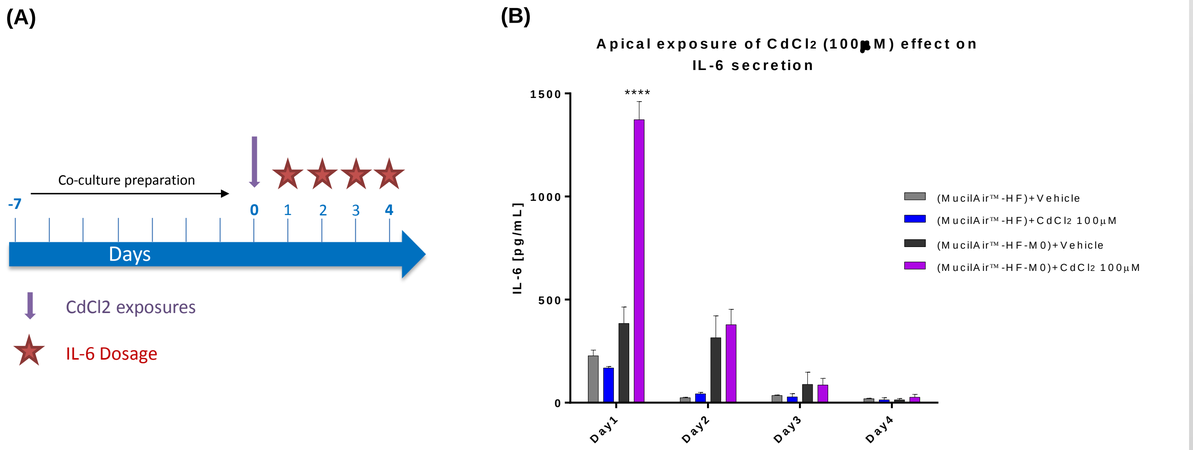


Figure A*: MucilAir™-HF co-cultured with macrophages (MucilAir™-HF-M0) are prepared following our standards protocol. Both MucilAir™-HF and MucilAir™-HF-M0 are exposed to 20µL of 100µM $CdCl_2$ in NaCl 0.9% vehicle on day 1. Basal media is sampled on days 1, 2 3 and 4 to dose IL-6 Level.*
Figure B*: *IL-6 basal secretion: $CdCl_2$ induce high stimulation of IL-6 at day 1 using MucilAir™-HF-M0. The co-culture model is able to detect in a sensitive way $CdCl_2$ induced transient inflammation.*

5 – Anti-inflammatory effect of Budesonide is detected using MucilAir™-HF-M0

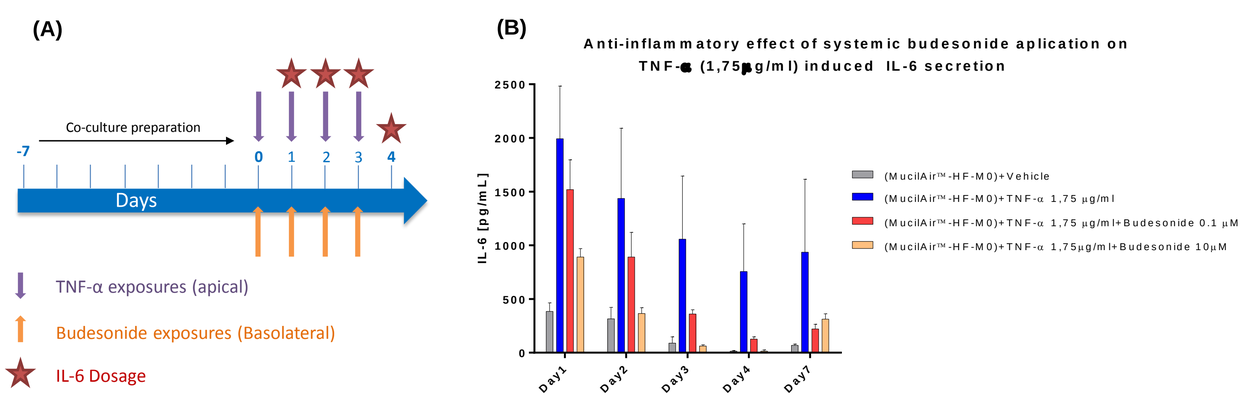


Figure A*: MucilAir™-HF co-cultured with macrophages (MucilAir™-HF-M0) are prepared following our standard protocol. Both MucilAir™-HF and MucilAir™-HF-M0 are exposed to 20µL of TNF-α in NaCl 0.9% vehicle (1.75µg/mL) cocomitantly with 0.1µM or 10µM of Budesonide*, in the ImmunoAir™ media, *every days during 4 days. Basal media is sampled on day 1, 2 3 and 4 to dose the IL-6 Level.*
Figure B* : *IL-6 basal secretion: TNFα induced inflammation could be attenuated using low doses of Budesonide.*

** Data shown for ELISA (ELÏSA kit BD OptEIA™Human IL-6) are the means +/- SEM errors from 3 biological replicates, statistical comparison one-way or two-way analysis of variance were performed with multiple comparison tests using GraphPad Prims software (version 6.01, La Jolla, USA) (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).*

Conclusion:

A human *in vitro* immuno-competent 3D respiratory epithelial model was successfully developed, combining MucilAir™, human fibroblasts (HF) and M0 cell like macrophages derived from THP-1 acute monocytic leukemia in an adequate culture medium (ImmunoAir™). This model (MucilAir™-HF-M0) is efficient for detecting TNF-α induced inflammation, $CdCl_2$ induced irritation and anti-inflammatory effects of Budesonide.