

# Inflammatory response modulation of airway epithelial cells exposed to formaldehyde

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## ABSTRACT

The two main difficulties when assessing the role and action mechanism of environmental pollutant exposure on the respiratory tract using in vitro methodology are firstly to create exposure conditions that closely mimic the human situation, and secondly to choose an experimental model that accurately represents lung compartment complexity, with different types of cell interaction.

The aim of this study was to resolve these two challenges. The first of our difficulties was to find the closest experimental conditions to mimic respiratory environmental pollutant exposure.

We compared the effects of formaldehyde (FA) on two cellular models, alveolar and bronchial cell lines, respectively A549 and BEAS-2B. The cells were exposed for 30 min to an environmental dose of gaseous FA (50 µg/m<sup>3</sup>) at the air–liquid interface.

In order to mimic macrophage–epithelial cell cooperation, sensitizations (with TNFα or with conditioned medium from macrophages – CM) prior to gas exposure were applied. After toxicity evaluation, local inflammation was assessed by IL-8 and MCP-1 production 24 h after exposure.

In our experimental conditions FA had no effects on alveolar and bronchial epithelial cells without any sensitization. FA exposure after TNFα sensitization alone induced a moderate increase of IL-8 by A549 cells. After sensitization with CM, FA exposure induced a strong increase of IL-8 production by A549 cells in comparison to air, whereas a decrease of MCP-1 production was observed on BEAS-2B cells.

It appears that the response of alveolar and bronchial epithelial cells to FA was moderate and that complex sensitization refines the inflammatory response to environmental stresses. When sensitized with CM, these cell lines responded differently to FA exposure. Finally by interacting with the respiratory epithelium, FA could exacerbate the inflammation of airways that occurs in severe asthma, and even synergize the effects of other air pollutants such as allergens. Evaluation of nasal cell inflammatory response could shed further light on the effects of FA on respiratory epithelium.

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## 1. Introduction

Air pollution is known to be associated with health effects on the respiratory tract, such as asthma symptoms and bronchial hyper reactivity, even if the relation between specific health effects and indoor air pollutants needs to be specified (Bernstein et al., 2008). In order to clarify the relation between indoor pollution and lung inflammation, experimental data are needed in addition to epidemiological evidence.

Experimental in vitro studies have contributed wide ranging information on the cellular effects of environmental pollutants

(BéruBé et al., 2009). However this type of study requires that the most relevant air–liquid exposure model is chosen in order to reach the most realistic conclusions (Roggen et al., 2006). The choice of model must integrate different parameters, such as cell type (epithelial bronchial, and alveolar cells, dendritic cells, macrophages, etc.) and the cellular environment related to the surrounding cells, and pollutant levels, which must be consistent with those observed in the environment.

Epithelial cells are present all along the respiratory tract: nasal, tracheal, bronchial and the deepest, alveolar cells. Cell lines have been developed for all lung cell types (Roggen et al., 2006) but the classical model in respiratory toxicology is the unique human alveolar cell line. In particular, a large majority of in vitro studies into the effects of environmental pollutants on lung have been performed with the alveolar A549 cell line (Feltens et al., 2010; Koehler et al., 2010).

As described in a review by Roggen et al. (2006), this cell line is representative for the lung compartment that is very likely

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involved in immunotoxicity, but its relevance for in vitro respiratory toxicology experiments is a matter of debate. The epithelial cells playing the greatest role in lung hyper reactivity in response to environmental exposure are located above the alveolar level in the respiratory tract, at the tracheal and bronchial level. Bronchial cells could therefore be more relevant, since environmental pollutants (such as ozone or formaldehyde) might not reach the alveolar epithelium due to their strong chemical reactivity through the respiratory tract. Nevertheless the bronchial cell lines have been much less used than the A549 cell line in in vitro experiments to evaluate the cellular effects of environmental pollutant exposure.

Beside the cellular model, studies into the effects of environmental pollutants on respiratory tract cells have mainly reported high levels of these pollutants which are largely out of the environmental range, only relevant for environmental toxicology (Pariselli et al., 2009; Rager et al., 2011; Wolkoff and Nielsen, 2010).

We previously developed an in vitro model to assess gaseous pollutant effects on lung epithelial cells (Persoz et al., 2010). This model was established using A549 cell line and tested the inflammatory response to air–liquid interface exposure to gaseous formaldehyde (FA) at environmental levels.

The aim of this study is firstly to extend this model to bronchial BEAS-2B cell line to compare the effects of FA-exposure on inflammatory response with those from the alveolar A549 cell line, and secondly to bring our cellular model closer to the respiratory environment mimicking macrophage–epithelial cells cooperation, by sensitization applied prior to gas exposure.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Ham's F12 medium with GlutaMAX®, RPMI 1640 with Hepes, Penicillin–Streptomycin, Hepes and TrypLE® were obtained from Invitrogen (WI, USA). TNF $\alpha$  was purchased from Sigma–Aldrich (MO, USA) and the fetal calf serum (FCS), from Biochrom (Germany). The LDH assay kit (K2054) was from Siemens and used with an automaton Dimension Vista 1500 (Siemens Healthcare Laboratories). The DuoSet kits for ELISA assays were from R&D Systems (MN, USA).

### 2.2. Atmospheres generation and cell exposure device

The Vitrocell® device used for exposure has been previously described by Persoz et al. (2010). Briefly, this device consists of three exposure chambers which can hold one separate insert each. The module is equipped with a heated water jacket to keep cells and culture medium at a steady temperature of 37 °C.

Air and FA (50  $\mu\text{g}/\text{m}^3$ ) atmospheres were generated as previously described (Persoz et al., 2010) and were delivered on the apical side of cells during 30 min at a flow rate of  $5 \pm 0.1$  ml/min.

### 2.3. Cell lines and culture conditions

The human epithelial cells, bronchial: BEAS-2B (Reddel et al., 1988) and alveolar: A549 cells (Lieber et al., 1976), were a generous gift from the Laboratoire de Parasitologie et Mycologie, Faculté Xavier Bichat (Paris, France). The two cell lines were cultured in Ham's F12 medium supplemented with 5% FCS, 100 UI Penicillin and 100  $\mu\text{g}/\text{ml}$  Streptomycin, and 10 mM Hepes.

For all gas-exposure experiments cells were grown on inserts (Greiner Bio-one, Germany), a membrane permeable to culture medium with a growth area of 1.13 cm<sup>2</sup> placed in a 12-well culture Plate 72 h before exposure. Culture medium was added to both sides: 300  $\mu\text{l}$  on the apical side and 1000  $\mu\text{l}$  on the basolateral side.

56 h after cellular seeding, sensitizers were added to the culture medium of BEAS-2B and A549 cells 16 h before gas-exposure. Following this pre-sensitization, the culture medium was removed from the apical side of the cellular monolayer and the cells were placed in exposure-chambers containing culture medium with 1% FCS only on the basolateral side.

Cells were exposed during 30 min to synthetic clean air or to FA at 50  $\mu\text{g}/\text{m}^3$ .

After exposure, inserts containing cells were replaced in a 12-well culture plate with fresh culture medium (5% FCS) on both sides and incubated at 37 °C in a humidified incubator for 24 h. Supernatants were then collected and stored until analysis at –20 °C.

Each experimental exposure with or without sensitization was replicated at least three times.

To control the ability of cells to react to a stimulus in terms of inflammatory response, constantly submerged unexposed cells were treated 72 h after seeding with 2 ng/ml of TNF $\alpha$  for 24 h.

### 2.4. Sensitizations

The modulation of cytokine production in the gas-exposure experiments was tested with two sensitizers: TNF $\alpha$  (1 ng/ml, Sigma–Aldrich) or conditioned medium from macrophages (CM).

CM production was obtained from human monocyte cell line THP-1 (ATCC, USA, ref. TIB-202) (Tsuchiya et al., 1980) (a generous gift from Institut National de l'Environnement Industriel et des Risques, Verneuil-en-Halatte, France) cultured in RPMI 1640 (with 25 mM Hepes) medium supplemented with 10% FCS, 2 mM GlutaMAX®, 100 UI Penicillin and 100  $\mu\text{g}/\text{ml}$  Streptomycin. The medium was changed every 3 days to ensure constant cell growth.

The differentiation of THP-1 cells in macrophages was obtained by seeding cells (10<sup>6</sup> cells/ml) in culture flasks (75 cm<sup>2</sup>, 15 ml/flask) with 30 ng/ml (50 nM) of phorbol 12-myristate 13-acetate (PMA) (Sigma–Aldrich, MO, USA). Cells were incubated for 72 h at 37 °C leading to cell adhesion and spreading, which are hallmarks of macrophages noticed using a light microscope (Daigneault et al., 2010). 72 h after treatment, the RPMI medium was replaced by the Ham F12 with 5% FCS for 24 h, a compatible medium for the both A549 and BEAS-2B cultures.

Then, the culture medium was withdrawn, centrifuged to eliminate cells and aggregates, and stored at –20 °C prior to use for cell sensitization.

Diluted CM in Ham F12 with 5% FCS was used for sensitization of the two types of epithelial cells.

### 2.5. Biological activity assessment

#### 2.5.1. LDH assay

Membrane integrity, evaluated by intracellular lactate dehydrogenase (LDH) release, was used as a viability test.

LDH was measured using an in vitro assay kit in which LDH oxidizes the substrate L-lactate buffered at a pH of 9.4 in the presence of NAD<sup>+</sup> to yield pyruvate and NADH, H<sup>+</sup> which absorbs light at 340 nm. Concentrations were initially expressed in unity of consumed substrate per liter (U/l) and cellular viability, expressed in %, was calculated with maximal LDH activity, obtained after 2 h Triton X-100 1% treatment. Maximal LDH activity was  $664 \pm 43$  U/l for BEAS-2B cells and  $1968 \pm 150$  U/l for A549 cells.

#### 2.5.2. Cytokine assay

The levels of cytokines produced by the macrophages in CM (IL-1 $\beta$ , TNF $\alpha$  and IL-8) and by the epithelial cells (IL-8 and MCP-1) were assessed by ELISA kits as previously reported (Persoz et al., 2011).

The levels of IL-1 $\beta$ , TNF $\alpha$  and IL-8 contained in the CM were respectively equal to  $6 \times 10^{-3}$  ng/ml,  $3 \times 10^{-3}$  and 0.33 ng/ml after dilution.

Quantification of IL-8 and MCP-1 was performed in the apical culture supernatants of BEAS-2B and A549 cells. Results were expressed in pg/ml.

Limits of quantification were 3.9 pg/ml for IL-1 $\beta$ , 15.6 pg/ml for TNF $\alpha$  and MCP-1 and 31.2 pg/ml for IL-8.

### 2.6. Statistics

Results were expressed as arithmetic mean  $\pm$  standard deviation of at least three experiments realized in triplicate. Statistical analyses were performed using R version 2.12.0 software ([www.r-project.org](http://www.r-project.org)). The LDH activity and cytokine concentrations of exposed cells were compared to the specific control with Student's *t*-test and confirmed with Mann-Whitney *U* test. Difference was significant when *p* < 0.05.

## 3. Results

The same exposure device (Vitrocell module) and protocol were used in this study to compare the effects of FA exposure at 50  $\mu\text{g}/\text{m}^3$  on inflammatory response of the two human epithelial cell lines, A549 and BEAS-2B, in absence or in presence of two sensitization conditions (TNF $\alpha$  and CM).

The experimental conditions of air–liquid exposure previously validated for A549 cells (Persoz et al., 2010) were applied for BEAS-2B cells. Briefly after seeding at 40,000 cells/insert, and an incubation at 37 °C during 72 h, a homogeneous cellular monolayer with a confluence of 80–90% was obtained for BEAS-2B cells, as previously described for A549 cells.

After 24 h of TNF $\alpha$  (2 ng/ml) treatment in submerged condition, IL-8 and MCP-1 were significantly increased compared to control

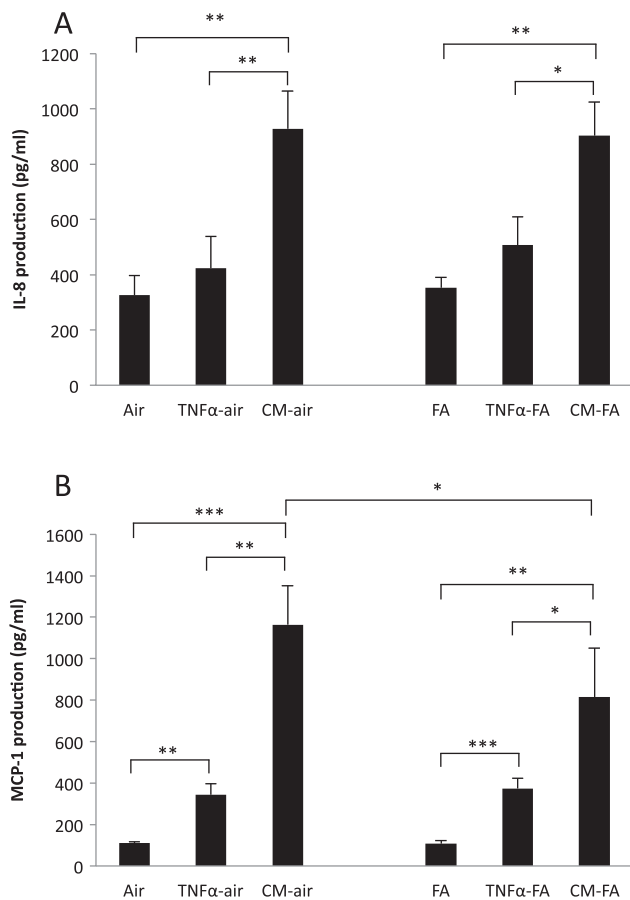


**Fig. 1.** IL-8 (A) and MCP-1 (B) production by bronchial epithelial cells BEAS-2B after air or formaldehyde exposure without or following TNF $\alpha$  or macrophage-conditioned medium (CM) sensitization.

cells which proved the reactivity of these cells in our culture conditions (data not shown).

After air or FA (at 50  $\mu\text{g}/\text{m}^3$ ) exposure for 30 min, no toxicity was observed (data not shown) and the production of the two cytokines was similar for air and FA exposure (Fig. 1). Using the same protocol the cellular effects on bronchial and alveolar epithelial cells were comparable.

### 3.1. Cytokines production after gas exposure on TNF $\alpha$ -sensitized alveolar and bronchial epithelial cells

First, lung epithelial cells (BEAS-2B and A549) were exposed to gas (air or FA) after a pre-sensitization period with only a single potential activator: TNF $\alpha$ .

TNF $\alpha$  sensitization before air or FA exposures on BEAS-2B cells induced an increase of MCP-1 production compared to non-sensitized cells (Fig. 1A) whereas no effect was observed on IL-8 production (Fig. 1B). On A549 cells, this sensitization induced a decrease of IL-8 and MCP-1 production compared to non-sensitized cells only after air exposure (Fig. 2). In these conditions, FA induced a significant increase of IL-8 by A549 cells after TNF $\alpha$  sensitization, compared to air. The IL-8 production by TNF $\alpha$  sensitized BEAS-2B cells tended to be increased after FA exposure compared to air ( $p=0.07$ ). MCP-1 production was not modified by FA exposure on TNF $\alpha$  sensitized A549 and BEAS-2B cells.

Globally, sensitization with TNF $\alpha$  alone induced only a modest modification of IL-8 and MCP-1 production, depending on the cell type, respectively, in response to air or FA.

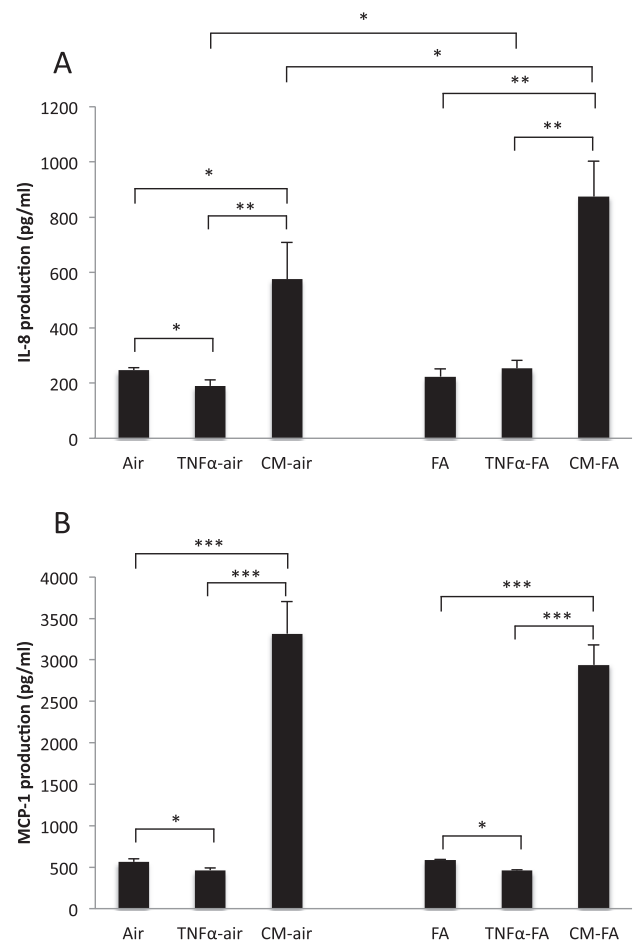


**Fig. 2.** IL-8 (A) and MCP-1 (B) production by alveolar epithelial cells A549 after air or formaldehyde exposure without or following TNF $\alpha$  or macrophage-conditioned medium (CM) sensitization.

### 3.2. Cytokines production after gas-exposure on conditioned medium sensitized alveolar and bronchial epithelial cells

After testing the effect of sensitization using a single activator, we tested the effect of sensitization based on the macrophage secretion medium (CM) containing this inflammatory mediator. This sensitization induced a high increase of IL-8 and MCP-1 for both the bronchial and alveolar epithelial cells (Figs. 1A, B and 2A, B). The increase was markedly significant compared to the non-sensitized cells, and to the TNF $\alpha$  sensitized cells.

On BEAS-2B cells, FA induced a decrease of MCP-1 production after CM sensitization whereas IL-8 production was not modified in comparison to air exposure. Against this, A549 cells sensitized with CM and exposed to FA had an increase of their IL-8 production and MCP-1 was not modified.

It appeared that CM sensitization induced a strong increase of IL-8 and MCP-1 compared to TNF $\alpha$  alone and that A549 and BEAS-2B cells responded differently to FA in this condition of sensitization.

## 4. Discussion

Toxicological risk assessment is now being implemented as a systematic and scientific approach for hazard identification and health risk characterization after human chemical exposures (Bakand and Hayes, 2010). Valuable data useful for risk assessment can be obtained by choosing model parameters adapted to the investigated health effects.

Our aim was to find the closest experimental conditions to copy respiratory environmental pollutant exposure, in order to assess early events resulting from environmental pollution exposure, in relation to respiratory diseases such as asthma.

After setting up an *in vitro* model with A549 cells, we tested the effects of an inflammatory micro-environment on lung epithelial cells using or not CM from human THP-1 macrophages, and A549 and BEAS-2B cells. Macrophage-secreted factors modulated cytokine production by both cell lines, although in a different manner, contrarily to the absence of conditioned medium.

In this study using the same protocol, i.e. same exposure system and protocol for both (30 min of exposure, 24 h of post-incubation, etc.), we tested in parallel the response of bronchial and alveolar epithelial cells to a common indoor air pollutant, FA. Our model of air–liquid exposure to pollutants at environmental doses was initially developed and applied to alveolar epithelial A549 cells (Persoz et al., 2010, 2011). This cell line is the most frequently used for assessment of pulmonary effects of environmental pollutants (Roggen et al., 2006) but is from the lower level of the respiratory tract. FA at environmental level ( $50 \mu\text{g}/\text{m}^3$ ) had no effects on A549 cell viability or on cytokine production in comparison to air.

Environmental FA reacts rapidly in the upper respiratory tract after inhalation leading to a lower concentration than atmospheric levels when it comes in contact to the alveolar epithelium (Wolkoff and Nielsen, 2010). Gas-exposure of a cellular type located upper in the respiratory tract, such as bronchial cells, is probably more relevant for studying the effect of FA exposure in the respiratory tract, and why we selected bronchial BEAS-2B cell line, which has already been tested in toxicology (Roggen et al., 2006).

Only few authors have compared the response of A549 and BEAS-2B cells after exposure to environmental pollutants and their production of IL-8 and MCP-1. Two studies have reported similar IL-8 production by both cell lines in response to MMP-12 (a metalloprotease) and environmental particles (Gualtieri et al., 2010; Le Quément et al., 2008). In addition, Ekstrand-Hammarström et al. (in press) showed that both cell lines also reacted similarly (mediators assayed: IL-8, IL-6, IL-1 $\alpha$  and  $\beta$ , G-CSF, VEGF) to several types of titanium dioxide nanoparticles. However, MCP-1 production by BEAS-2B cells increased in response to one crystalline structure of TiO<sub>2</sub> while the production of this chemokine by A549 cells remained unchanged.

To compare the FA effect on these two cell lines, the exposure level we chose for these experiments was  $50 \mu\text{g}/\text{m}^3$ , the highest level within the environmental range without any cytotoxicity (Persoz et al., 2010, personal data), and we used comparable culture conditions. In these experimental conditions no modification of cytokine production was observed after FA exposure in comparison to air. In these conditions, alveolar and bronchial cells seemed to be insensitive to FA exposure at levels compatible with environmental exposure.

One of the main difficulties in representing environmental exposure *in vitro* is the complexity of the lung compartment, with different types of cells in the respiratory tract cooperating together. Among the models recently developed to perform air–liquid exposure to environmental pollutants, most were performed on epithelial cell without simulation of macrophage–epithelial cell cooperation, just as in toxicological studies, i.e. with high levels of pollutants (Anderson et al., 2010; Bakand et al., 2006; Gminski et al., 2010; Koehler et al., 2010; Switalla et al., 2010; Volckens et al., 2009). Few studies have reported cell sensitization, and in this case single activators, such as TNF $\alpha$  (Fischader et al., 2008), were used. We therefore tried to mimic the continuous cooperation between epithelial cells and macrophages, which is partly based on epithelial cell activation by the main pro-inflammatory cytokines IL-1 $\beta$  and

TNF $\alpha$  (Chung, 2009). Consequently, we first tested sensitization of the epithelial cells with TNF $\alpha$ .

This type of sensitization applied to A549 cells during environmental VOCs (styrene, m-xylène, chlorobenzene) air–liquid exposure enabled modification of cytokine production (IL-6, IL-8 and MCP-1) whereas no effects were observed without any sensitization (Feltens et al., 2010; Fischader et al., 2008). In our experimental conditions, this sensitization with a single activator induced a similar moderate modification of cytokine production when exposure to air for each cell line was performed.

In order to represent a more complex and relevant simulation of the lung microenvironment and its physiology, we refined the sensitization of the epithelial cells with conditioned medium from THP1 macrophages: conditioned medium produced by macrophages contains biological compounds at physiological concentration and ratios as in the lung compartment. We diluted the initial conditioned medium, prior to using it on the cells, to obtain relevant levels of produced cytokines in the same order of magnitude as those reported in induced sputum of healthy subjects (Jatakanon et al., 1999). This medium induced a strong increase of MCP-1 and IL-8 production by A549 and BEAS-2B cells, for air exposure, compared to the results with TNF 1 ng/ml or without any sensitization, although the concentration of conditioned medium TNF $\alpha$  was  $3 \times 10^{-3}$  ng/ml and thus significantly lower than 1 ng/ml, the concentration used for sensitization with only TNF $\alpha$ . In addition CM sensitization prior to FA exposure revealed a decrease in the production of MCP-1 by BEAS-2B cells and an increase of IL-8 by A549 cells, contrary to the other conditions. Decrease of MCP-1 in response to an environmental stress such as particle exposure has been previously described in parallel to the increase of other inflammatory markers (IL-6, IL-8 and IL-1 $\beta$ ) (Fuentes-Mattei et al., 2010). The significance of difference of inflammatory behavior between these cell lines needs further investigations.

With regard to the protocol we used, epithelial cells were sensitized to CM prior to pollutant exposure. Other experimental sequences have also been proposed, such as applying the medium produced by macrophages exposed to pollutants to epithelial cells (Brown et al., 2007; Jiménez et al., 2002). This protocol also resulted in enhancement of IL-8 release by lung epithelial cells.

We can draw two main conclusions from our study. Firstly, that in our exposure conditions the response of human alveolar and bronchial epithelial cells to FA was moderate, and merits further investigation. Considering the high intrinsic effect of the conditioned medium and the low differences induced by FA exposure, whatever the cell line, our results suggest that experimental exposure to FA should be extended to better mimic the long term and at low dose environmental exposure. Secondly, that complex sensitization refines the inflammatory response to environmental stresses: when sensitized with CM, these cell lines respond differently to FA exposure.

Formaldehyde can exacerbate airway inflammation occurring in severe asthma by interacting with the respiratory epithelium, and even synergize the effects of other air pollutants such as allergens. Further information about the effects of FA on the respiratory epithelium could be gathered by evaluating the inflammatory response of upper respiratory tract cells at nasal level.

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## References

- Anderson, S.E., Jackson, L.G., Franko, J., Wells, J.R., 2010. Evaluation of dicarbonyls generated in a simulated indoor air environment using an in vitro exposure system. *Toxicol. Sci.* 115, 453–461.
- Bakand, S., Hayes, A., 2010. Troubleshooting methods for toxicity testing of airborne chemicals in vitro. *J. Pharmacol. Toxicol. Methods* 61, 76–85.
- Bakand, S., Winder, C., Khalil, C., Hayes, A., 2006. An experimental in vitro model for dynamic direct exposure of human cells to airborne contaminants. *Toxicol. Lett.* 165, 1–10.
- Bernstein, J.A., Alexis, N., Bacchus, H., Bernstein, I.L., Fritz, P., Horner, E., Li, N., Mason, S., Nel, A., Oullette, J., Reijula, K., Reponen, T., Seltzer, J., Smith, A., Tarlo, S.M., 2008. The health effects of non-industrial indoor air pollution. *J. Allergy Clin. Immunol.* 121, 585–591.
- BéruBé, K., Aufderheide, M., Breheny, D., Clothier, R., Combes, R., Duffin, R., Forbes, B., Gaca, M., Gray, A., Hall, I., Kelly, M., Lethem, M., Liebsch, M., Merolla, L., Morin, J.P., Seagrave, J., Swartz, M.A., Tetley, T.D., Umachandran, M., 2009. In vitro models of inhalation toxicity and disease. The report of a FRAME workshop. *Altern. Lab. Anim.* 37, 89–141.
- Brown, D.M., Hutchison, L., Donaldson, K., Stone, V., 2007. The effects of PM10 particles and oxidative stress on macrophages and lung epithelial cells: modulating effects of calcium-signaling antagonists. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 292, L1444–L1451.
- Chung, K.F., 2009. Cytokines. *Asthma and COPD*, vol. 524., 2nd ed. Academic Press, Oxford, pp. 327–341.
- Daigneault, M., Preston, J.A., Marriott, H.M., Whyte, M.K., Dockrell, D.H., 2010. The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages. *PLoS One* 5, e8668.
- Ekstrand-Hammarström, B., Akfur, C.M., Andersson, P.O., Lejon, C., Osterlund, L., Bucht, A. Human primary bronchial epithelial cells respond differently to titanium dioxide nanoparticles than the lung epithelial cell lines A549 and BEAS-2B. *Nanotoxicology*, in press.
- Feltens, R., Mogel, I., Roder-Stolinski, C., Simon, J.C., Herberth, G., Lehmann, I., 2010. Chlorobenzene induces oxidative stress in human lung epithelial cells in vitro. *Toxicol. Appl. Pharmacol.* 242, 100–108.
- Fischader, G., Roder-Stolinski, C., Wichmann, G., Nieber, K., Lehmann, I., 2008. Release of MCP-1 and IL-8 from lung epithelial cells exposed to volatile organic compounds. *Toxicol. In Vitro* 22, 359–366.
- Fuentes-Mattei, E., Rivera, E., Gioda, A., Sanchez-Rivera, D., Roman-Velazquez, F.R., Jimenez-Velez, B.D., 2010. Use of human bronchial epithelial cells (BEAS-2B) to study immunological markers resulting from exposure to PM(2.5) organic extract from Puerto Rico. *Toxicol. Appl. Pharmacol.* 243, 381–389.
- Gminski, R., Tang, T., Mersch-Sundermann, V., 2010. Cytotoxicity and genotoxicity in human lung epithelial A549 cells caused by airborne volatile organic compounds emitted from pine wood and oriented strand boards. *Toxicol. Lett.* 196, 33–41.
- Gualtieri, M., Øvrevik, J., Holme, J.A., Perrone, M.G., Bolzacchini, E., Schwarze, P.E., Camatini, M., 2010. Differences in cytotoxicity versus proinflammatory potency of different PM fractions in human epithelial lung cells. *Toxicol. In Vitro* 24, 29–39.
- Jatakanon, A., Laloo, U.G., Lim, S., Chung, K.F., Barnes, P.J., 1999. Increased neutrophils and cytokines, TNF-alpha and IL-8, in induced sputum of non-asthmatic patients with chronic dry cough. *Thorax* 54, 234–237.
- Jiménez, L.A., Drost, E.M., Gilmour, P.S., Rahman, I., Antonicelli, F., Ritchie, H., MacNee, W., Donaldson, K., 2002. PM(10)-exposed macrophages stimulate a proinflammatory response in lung epithelial cells via TNF-alpha. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 282, L237–L248.
- Koehler, C., Ginzkey, C., Friehs, G., Hackenberg, S., Froelich, K., Scherzed, A., Burghartz, M., Kessler, M., Kleinsasser, N., 2010. Aspects of nitrogen dioxide toxicity in environmental urban concentrations in human nasal epithelium. *Toxicol. Appl. Pharmacol.* 245, 219–225.
- Le Quément, C., Guénon, I., Gillon, J.Y., Lagente, V., Boichot, E., 2008. MMP-12 induces IL-8/CXCL8 secretion through EGFR and ERK1/2 activation in epithelial cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 294, L1076–L1084.
- Lieber, M., Smith, B., Szakal, A., Nelson-Rees, W., Todaro, G., 1976. A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. *Int. J. Cancer* 17 (1), 62–70.
- Pariselli, F., Sacco, M.G., Rembges, D., 2009. An optimized method for in vitro exposure of human derived lung cells to volatile chemicals. *Exp. Toxicol. Pathol.* 61, 33–39.
- Persoz, C., Achard, S., Leleu, C., Momas, I., Seta, N., 2010. An in vitro model to evaluate the inflammatory response after gaseous formaldehyde exposure of lung epithelial cells. *Toxicol. Lett.* 195, 99–105.
- Persoz, C., Leleu, C., Achard, S., Fasseu, M., Menotti, J., Meneceur, P., Momas, I., Derouin, F., Seta, N., 2011. Sequential air–liquid exposure of human respiratory cells to chemical and biological pollutants. *Toxicol. Lett.* 207, 53–59.
- Rager, J.E., Smeester, L., Jaspers, I., Sexton, K.G., Fry, R.C., 2011. Epigenetic changes induced by air toxics: formaldehyde exposure alters miRNA expression profiles in human lung cells. *Environ. Health Perspect.* 119, 494–500.
- Reddel, R.R., Ke, Y., Gerwin, B.I., McMenamin, M.G., Lechner, J.F., Su, R.T., Brash, D.E., Park, J.B., Rhim, J.S., Harris, C.C., 1988. Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus-12 SV40 hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. *Cancer Res.* 48, 1904–1909.
- Roggen, E.L., Soni, N.K., Verheyen, G.R., 2006. Respiratory immunotoxicity: an in vitro assessment. *Toxicol. In Vitro* 20, 1249–1264.
- Switalla, S., Knebel, J., Ritter, D., Krug, N., Braun, A., Sewald, K., 2010. Effects of acute in vitro exposure of murine precision-cut lung slices to gaseous nitrogen dioxide and ozone in an air–liquid interface (ALI) culture. *Toxicol. Lett.* 196, 117–124.
- Tsuchiya, S., Yamabe, M., Yamaguchi, Y., Kobayashi, Y., Konno, T., Tada, K., 1980. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int. J. Cancer* 26, 171–176.
- Volckens, J., Dailey, L., Walters, G., Devlin, R.B., 2009. Direct particle-to-cell deposition of coarse ambient particulate matter increases the production of inflammatory mediators from cultured human airway epithelial cells. *Environ. Sci. Technol.* 43, 4595–4599.
- Wolkoff, P., Nielsen, G.D., 2010. Non-cancer effects of formaldehyde and relevance for setting an indoor air guideline. *Environ. Int.* 36, 788–799.