In vitro model for the prediction of respiratory sensitization of inhaled chemicals and protein allergens.

Chary A.[1,2], Serchi T.[1*], Cambier S.[1], Moschini E.[1], Contal S.[1], Hennen J.[2], Ezendam J.[3], Blömeke B.[2], Gutleb A.C.[1]

[1] Department of Environmental Research and Innovation, Luxembourg Institute of Science and Technology (LIST), Luxembourg. [2] Department of Environmental Toxicology, Trier University, Germany. [3]Centre for Environmental Protection,National Institute for Public Health and the Environment (RIVM), The Netherlands. * tommaso.serchi@list.lu

Introduction

✧Chemical respiratory sensitization resulting from occupational exposure to synthetic compounds has increased over the last decades leading to important occupational health issues.

✧Complex *in vitro* co-culture systems represent valuable tools to understand the mechanisms involved in lung sensitization.

✧No validated *in vitro* model is currently available to assess the respiratory sensitization potential of chemicals.

✧An interesting *in vitro* model developed by Klein *et al*. in 2013 combining alveolar type II epithelial cell line A549, acute monocyte cell line THP-1 cells differentiated into macrophage like cells, endothelial cells EA.hy 926 and human mast cell line HMC-1 in coculture has been developed to assess the toxic effects of particles at the alveolar barrier. The coculture was designed in a 3D-organisation to mimic at best the in vivo histology of the alveolar barrier and cultured at the air liquid interface (ALI) to mimic realistic exposure of inhaled compounds.

✧DCs, which have a crucial role in sensitization, need indeed to be included in such model to study the relevant process of sensitization. The coculture was redesigned to address the sensitizing potential of inhaled compounds in a relevant way, including the possibility of migration of DCs to ensure to have a functional model.

Aim

✧Adapt a 3D-coculture system previously developed by us (Klein *et al.* 2013) allowing the study of respiratory sensitization processes by including dendritic cells, which have a crucial role in sensitization

✧Find markers for the assessment of the respiratory sensitizing potential of inhaled compounds by measuring the activation of DCs as well as the release of cytokines

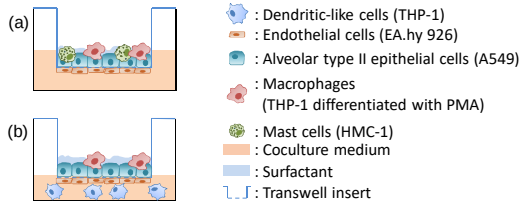


Figure 1: Coculture system (a) mimicking the alveolar barrier to study cell-to-cell communication and inflammatory effects of NPs at the ALI (Klein *et al.* 2013, 2017) and its variant (b) of the system to study the sensitizing potential of chemicals at the ALI (Chary et al. 2017, Manuscript under preparation).

Material and Methods

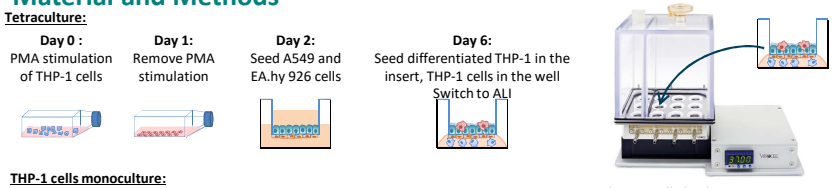
Tetraculture:

Day 0 : PMA stimulation of THP-1 cells

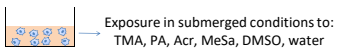
Day 1: Remove PMA stimulation

Day 2: Seed A549 and EA.hy 926 cells

Day 6: Seed differentiated THP-1 in the insert, THP-1 cells in the well Switch to ALI



THP-1 cells monoculture:

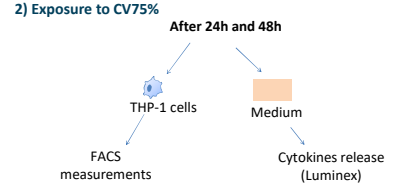


Day 7 : Exposure to the Vitrocell Cloud system to:
- Respiratory sensitizers: Trimellitic anhydride (TMA) and Phthalic anhydride (PA)
- Respiratory irritant: Acrolein (Acr), Methyl Salicylate (MeSa)
- Vehicle control (water for Acr and DMSO for TMA and PA)
- Protein sensitizers: Pollen allergen Bet v1 and House Dust Mites (HDM)

1) Preliminary experiments: viability assessment

→ Selection of the CV75% concentration

2) Exposure to CV75%



References

- Klein, S.G., Serchi, T., Hoffmann, L., Blömeke, B., Gutleb, A.C., 2013. An improved 3D tetraculture system mimicking the cellular organisation at the alveolar barrier to study the potential toxic effects of particles on the lung. Part. Fibre Toxicol. 10, 31. doi:10.1186/1743-8977-10-31
- Chary, A., Hennen, J., Klein, S.G., Serchi, T., Gutleb, A.C., Blömeke, B., 2018. Respiratory sensitization: toxicological point of view on the available assays. Arch. Toxicol. 92, 803–822. https://doi.org/10.1007/s00204-017-2088-5
- Patent LU93401

Acknowledgment and financial support

✧This work was financially supported by the LIST funded projects CHEMSENS and IMPERIS.

✧We thank the LCSB Luxembourg for the help and support with cytofluorimetry analysis.

Results

Tissue viability

Chemical	Monoculture (µg/mL)	Tetraculture (µg/cm²)
Acrolein	1,7	8
MeSa	353*	56*
TMA	240*	90
PA	193*	148

Table 1: summary of calculated CV75 to which both mono and tetraculture are exposed for the next experiments, * indicates when CD75 could not be reached, cells were then exposed to the maximum of solubility of the chemicals.

Cytokines pattern

	Acrolein		PA		TMA	
	24h	*48h*	*24h*	*48h*	*24h*	*48h*
MCP-1	↘	=	=	↗	=	=
MIP-3a	↘	↘	↗	↗	↗	↗
IL-6	↘	↘	↗	↗	↗	↗
IL-7	↗	↗	=	=	=	=
RANTES	=	↗	↗	↗	↗	↗
GM-CSF	↘	↘	↗	↗	↗	↗
IL-10	↘	↘	↗	↗	↗	=

Table 2: Summary of the cytokines released in the coculture after exposure to chemical sensitizers and irritant for 24h and 48h

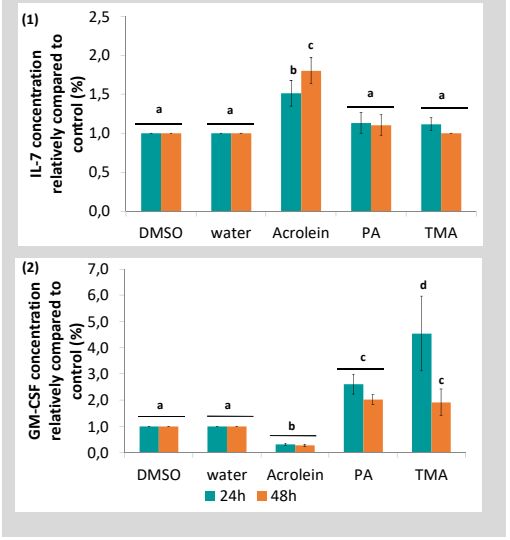


Figure 3: Cytokines released in the coculture after exposure to chemical sensitizers and irritant for 24h and 48h **(1)**: IL-7 basal levels: DMSO basal level for 24h: 18,2 ± 0,0 pg/mL; for 48h: 19,5 ± 1,2 pg/mL; Water for 24h: 18,6 ± 0,3 pg/mL; for 48h: 18,2 ± 0,0 pg/mL **(2)** GM-CSF basal levels: DMSO basal level: DMSO: for 24h: 32,4 ± 6,0 pg/mL; for 48h: 31,0 ± 3,7 pg/mL; water: for 24h: 47,0 ± 9,2 pg/mL; for 48h: 43,1 ± 5,4 pg/mL (Mean +/- SE) (Letters illustrate significant differences (Factorial ANOVA + Fisher LSD post hoc test at P<0,05 level of significance, n=6).

FACS measurements

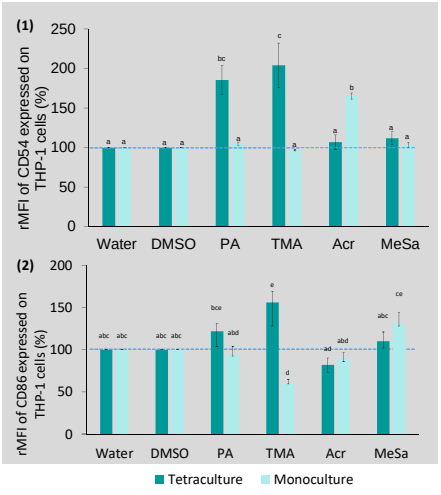


Figure 4: Cell surface marker expression of **(1)** CD54 and **(2)** CD86 on THP-1 cells in the coculture and monoculture after exposure to chemical sensitizers and irritants for 24h (Mean +/- standard error) Letters illustrate significant differences (Factorial ANOVA + Fisher LSD post hoc test at P<0,05 level of significance, n=3).

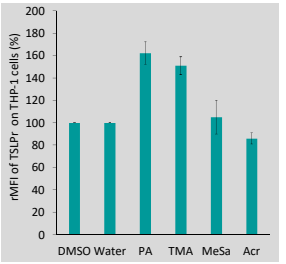


Figure 5 : rMFI of TSLPr expressed on THP-1 cells in tetraculture after 24h exposure to chemical sensitizers ans irritants (Mean +/- SE, n=3)

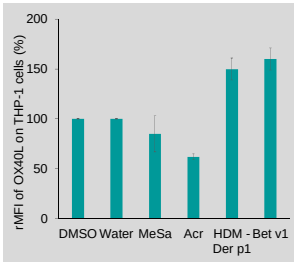


Figure 6 : rMFI of OX40L expressed on THP-1 cells in tetraculture after 24h exposure to chemical irritants and protein allergens (Mean +/- SE, n=3)

Summary and Conclusions

✧Better prediction using THP-1 cells in the tetraculture system compared to monoculture in submerged condition.

✧Increase of CD54 expression after exposure to the chemical respiratory sensitizers TMA and PA and decrease of the CD54 expression after exposure to the irritant acrolein : **CD54 could be used as a marker to discriminate irritants from chemical respiratory sensitizers.**

✧Increase of TSLPr expression after exposure to the chemical respiratory sensitizers TMA and PA : **TSLPr could be used as a marker to identify chemical respiratory sensitizers**

✧**Panel of cytokines able to identify chemical respiratory sensitizers**

✧Increase of OX40L expression after exposure to the protein allergen HDM and Bet v1: **OX40L could be used as a marker to identify protein allergens**