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# Progress towards novel testing strategies for *in vitro* assessment of allergens

Erwin L Roggen, Hans-Ulrich Weltzien, Helma Hermans
for
The Sens-it-iv Consortium

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# Overview on the project activities and WP1-10 interactions

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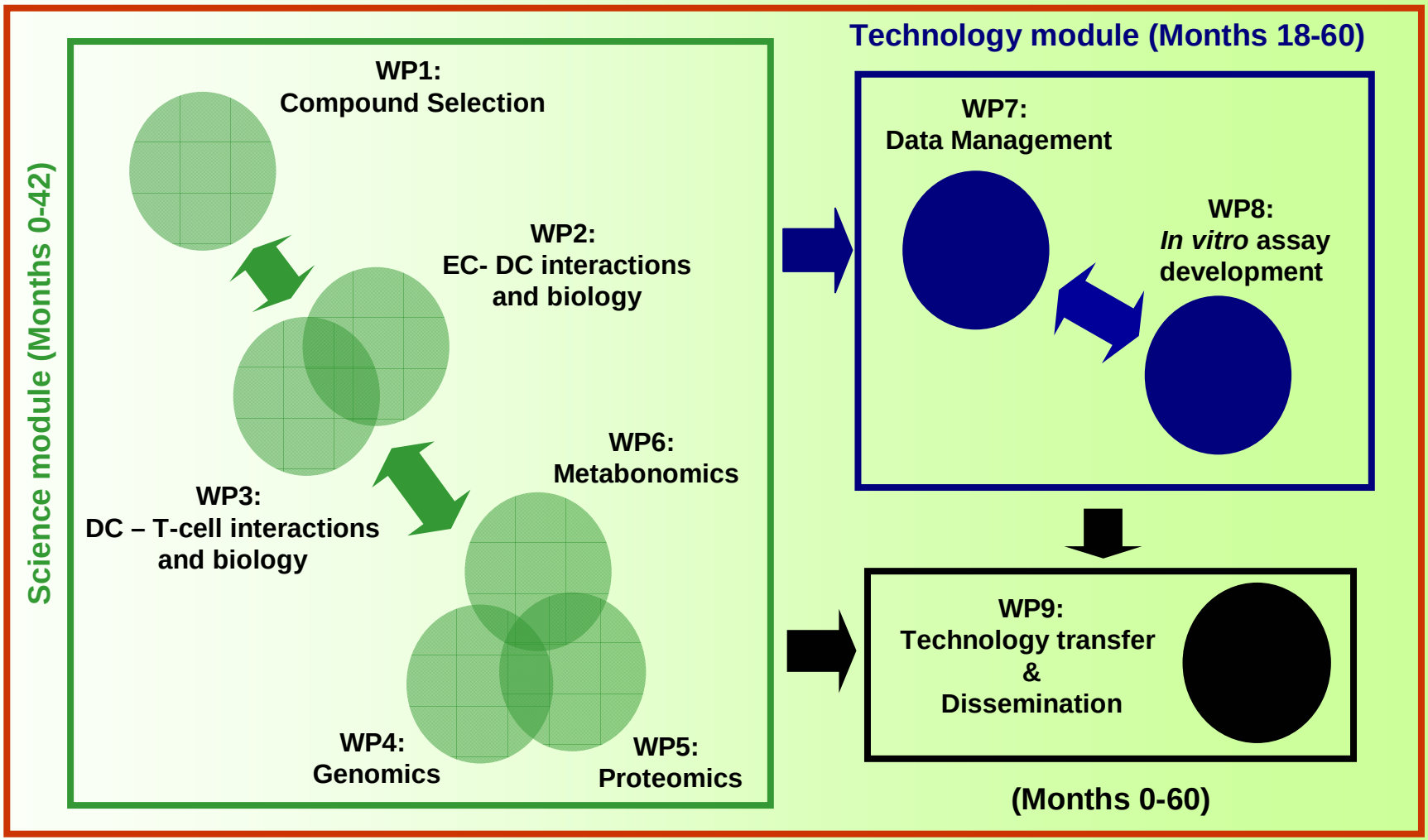

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# Addressed topics

- Precision cut lung slices
- Standardised immunologically and metabolically competent human cell lines for hazard assessment
- Assays addressing bio-activation and hapten formation
- Culture systems suitable for assessing the sensitising potential of compounds
- Relevant markers for the sensitising potency of different classes of chemicals

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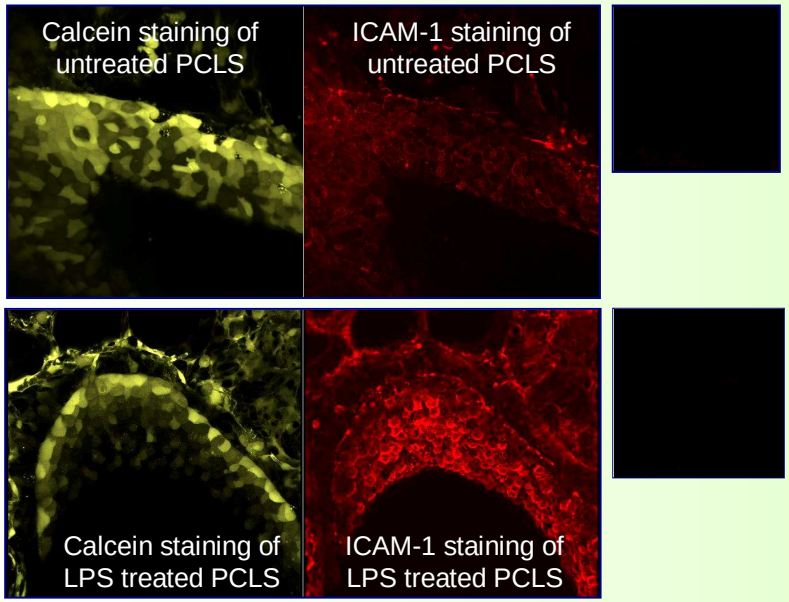
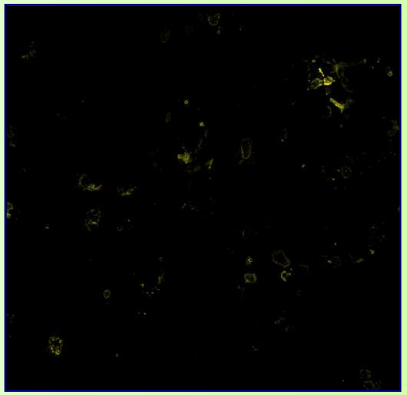
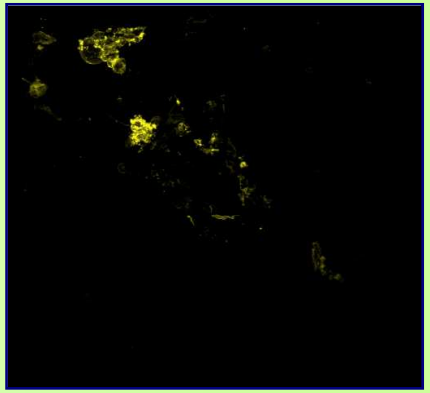
# *Ex Vivo*: Precision-cut lung slices (PCLS)

- PCLS react to immunomodulary compounds, including sensitisers.
- Relevant changes in membrane marker expression are observed.



Untreated mouse PCLS, stained with anti-CD86 antibodies



mouse PCLS incubated with LPS, staining with anti-CD86 antibodies

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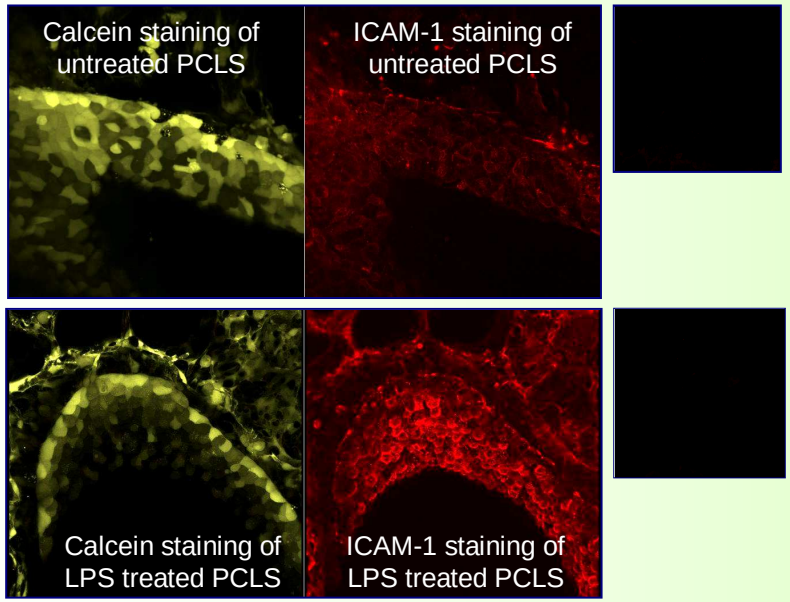
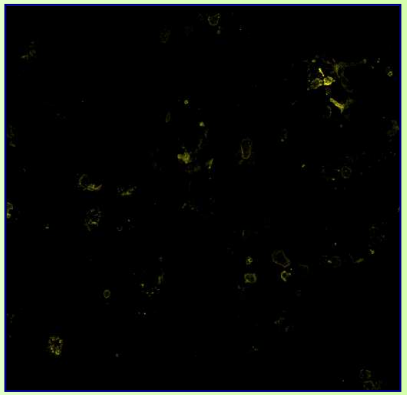
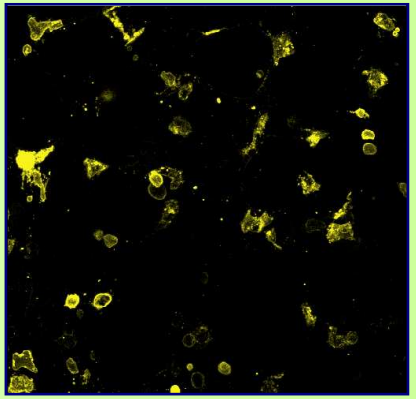


Untreated mouse PCLS, stained with anti-CD86 antibodies



mouse PCLS incubated with ovalbumin-specific T-cells, staining with CD86 after exposure to ovalbumin and LPS

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# *Ex Vivo*: Precision-cut lung slices (PCLS)

- PCLS react to sensitising compounds by up-regulating the expression of cytokines (intra- and extra-cellular).
- PCLS seem to discriminate between respiratory and skin sensitisers.

| | Eotaxin | G-CSF | IFN-γ | IL-1α | IL-5 | IL-8 | IL-10 | IL-12 (p40) | MCP-1 | MIP-1ß | RANTES | TNF- α |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (+)control: LPS | | + | | +++ | + | | | ++ | + | | +++ | ++++ |
| Lung: TMA | | ++ | | ++ | | ++ | | | ++ | + | | |
| HCpt | + | + | | ++ | | + | ++ | + | | + | - | ++ |
| Skin: DNCB | | | | | | | | | | | | |
| CA | | | | | | | | | | - | | |
| (-)control: SA | | | | | | | | | | | | ++ |
| Ph | | | | - | | | | | | - | | |

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# Identification of *in vivo*-like epithelial cell (EC) lines

- Criteria
  - adherance to the surface; } *microscopy, histology*
  - differentiation; } *microscopy, histology*
  - viability/sustainability; → *MTT, Alamar Blue*
  - polarisation; } *retention of macromolecules, TEER, presence of TJ proteins*
  - tight-junction formation (TJ): } *retention of macromolecules, TEER, presence of TJ proteins*
  - metabolic activity (constitutive, inducible); → *substrate cocktail + MS*
  - responsiveness to immuno-modulating stimuli.. → *ELISA*

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# Identification of *in vivo*-like epithelial cell (EC) lines

- Cell culture engineering
  - soluble factors;
    - growth factors
  - interaction with matrix constituents;
    - collagen IV, hyaluronic acid
  - enhanced cell-cell interaction;
  - position, shape and polarity;
  - biomaterials

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# Identification of *in vivo*-like epithelial cell (EC) lines

| | Primary cells | Cell lines |
|---|---|---|
| **Skin** | **keratinocytes** | NCTC 2544 |
| **Alveolar** | alveolar EC (type 1 and type 2) | A427, **A549**, H292 |
| **Bronchial** | tracheobronchial and **bronchial EC** | 9HTE16o-, HBE14o-, 1HAEo-, BEAS-2B, CF/T43, CFBE41o-, CFBE45o- |
| | | Calu-1, **Calu-3**, Calu-6, *H441*, HBE1, IB3-1 |

*Steimer, Haltner and Lehr, Journal of Aerosol Medicine 18; 137-182 (2005)*

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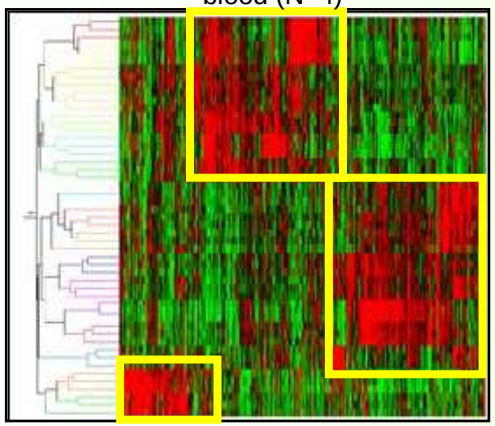
# Identification of *in vivo*-like dendritic cell (DC) lines

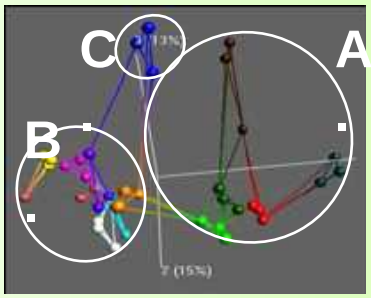
- Hierarchical clustering and principle component analysis (PCA) revealed population-specific profiles.
- PCA of 80 DC marker genes revealed subtype similarities.

A. primary DC from tonsils (N=3) and blood (N=4)



B. DC derived from precursor cells (N=8)

C. primary DC from skin (N=2)




***MUTZ-3***
***- Cell line***
***- Expressing most characteristic markers***
***- Most in vivo-like functionality:***
***- differentiation***
***- maturation***
***- antigen-presentation***

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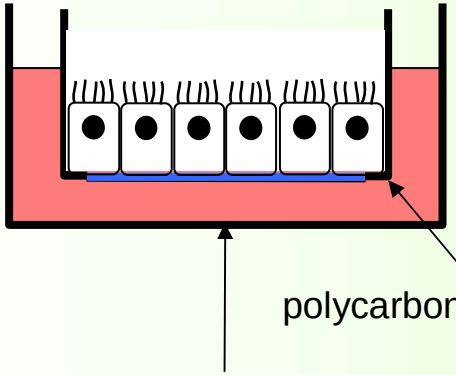
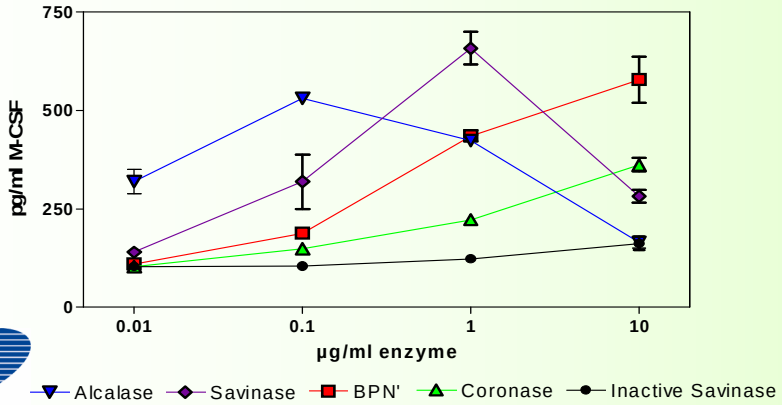
# *In Vitro*: Air/liquid phase A549 models for sensitisation




6-well tissue culture plate




Protein-specific cytokine profiles (33 tested):

| | Protease | Lipase | BSA |
|---|---|---|---|
| G-CSF | + | + | - |
| GM-CSF | + | - | - |
| M-CSF | + | - | - |
| I-309 | + | - | - |
| IFN-γ | + | - | - |
| IL-1β | + | - | + |
| IL-6 | + | + | + |
| IL-8 | + | + | - |
| MCP-1 | + | - | - |
| RANTES | - | + | - |

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# *In Vitro*: Executive summary of sensitiser induced cytokine responses in A549 EC

PCLS

| | Eotaxin | G-CSF | GM-CSF | IFN-γ | IL-1 | IL-5 | IL-6 | IL-8 | IL-10 | IL-12 (p40) | MCP-1 | M-CSF | MIP-1ß | RANTES | TNF- α |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (+) control) | | + | n.d. | | +++ | + | n.d. | | | ++ | + | n.d. | | +++ | ++++ |
| Lung | + | ++ | n.d. | | ++ | | n.d. | ++ | ++ | + | ++ | n.d. | + | - | ++ |
| Skin | | | n.d. | | | | n.d. | | | | | n.d. | | | |
| (-) control) | | | n.d. | | - | | n.d. | | | | | n.d. | - | | ++ |

A549 EC line

| | Eotaxin | G-CSF | GM-CSF | IFN-γ | IL-1 | IL-5 | IL-6 | IL-8 | IL-10 | IL-12 (p40) | MCP-1 | M-CSF | MIP-1ß | RANTES | TNF- α |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (+) control) | | n.d. | | | + | | + | + | | | + | | | + | + |
| Lung | | + | + | | + | | + | ++ | | | + | + | | | |
| Skin | | | | | | | | | | | | | | - | |
| (-) control) | | | | | | | | | | | | | | | |

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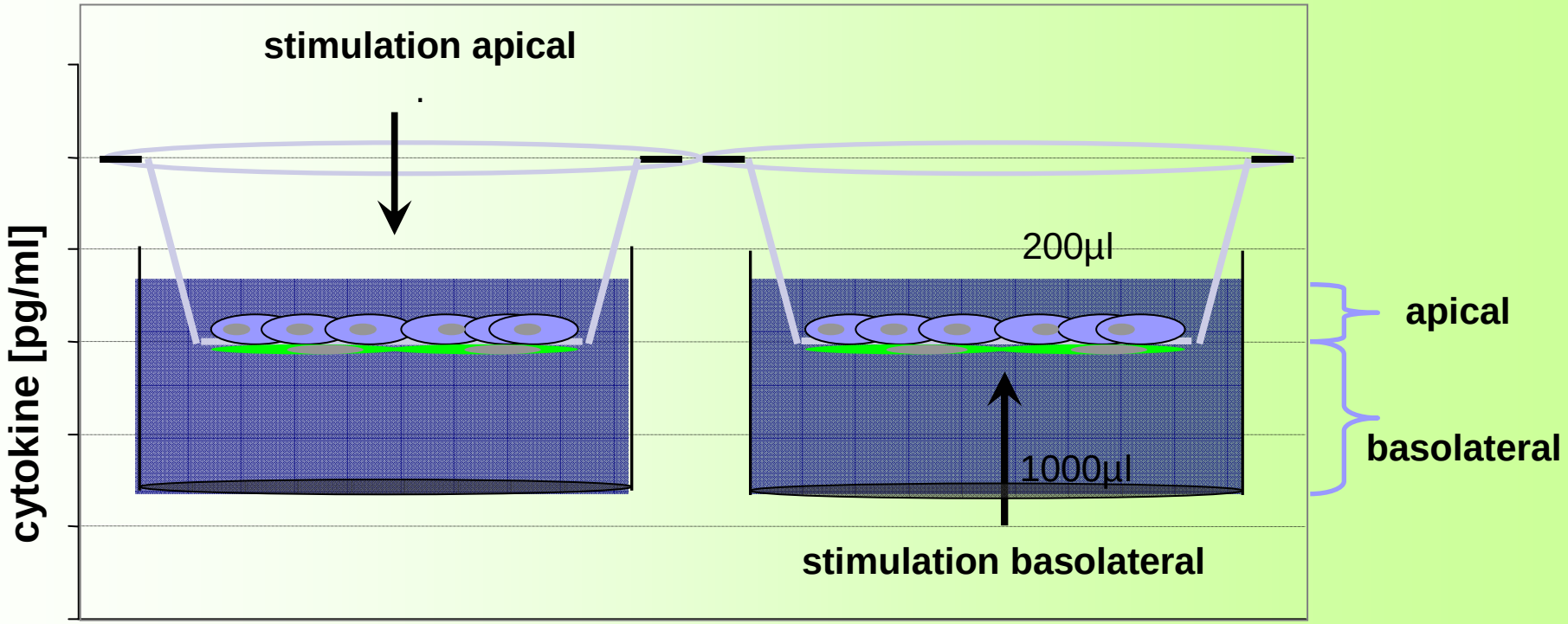
# An *in vitro* co-culture model of the human alveolo-capillary barrier using the H441 cell line




*Iris Hermanns, Johannes Gutenberg University, Mainz, Germany*

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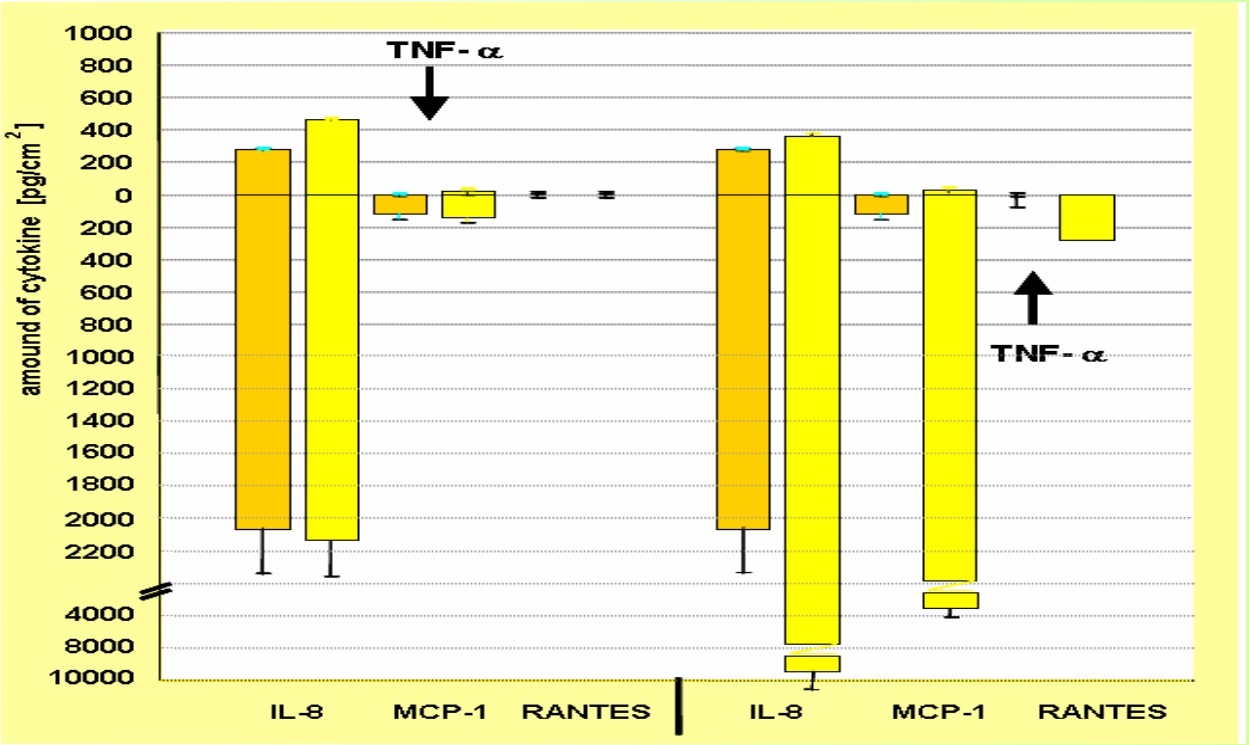


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# *In Vitro*: Air/liquid phase model using 'primary' human lung EC (Epithelix)

After 1-2 months:
1. Appearing of ciliated, mucus and basal cells
2. Establishment of absorption / secretion properties
3. Stabilisation of electrophysiological properties

Exposure regimes:

- Basal:
  - the cells can be incubated during 24, 48 or 72 h
- Apical:
  - only a short time exposure
  - immersion (> 1 hr) induced inflammatory response
  - chronic exposure (10x 1hr/day)
- Read-outs:
  - viability
  - cytokine profiles (IL1, IL-6 and IL-8)

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# *In Vitro*:
# The Sens-it-iv epidermal equivalent
## penetration/cytokine release

| | high barrier competency | impaired barrier | sumerged |
|---|---|---|---|
| **MDI** | no/no | no/no | n.a./yes |
| **TMA** | no/no | no/no | n.a./yes |
| **HCPt** | no/no | no/no | n.a./yes |
| **DNCB** | yes/yes | yes/yes | n.a./yes |
| **CIN** | yes/yes | yes/yes | n.a./yes |
| **TMDT** | no/no | no/no | n.a./yes |
| **SLS** | poor/poor | yes/yes | n.a./yes |
| **SA** | | | |
| **Phe** | | | |

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# *In Vitro*: DC models for sensitisation

- MUTZ-3, THP-1, U937, MoDC
  - SOPs
  - IL-1β and IL-8 (ELISA and Q-PCR)
  - CD54 and CD86 (flow cytometry)
- Evaluation in progress

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# *In Vitro*: EC - DC cultures supporting *in vivo*-like cell-cell interactions

- Lung
  - Development of EC-DC airlifted assays is experiencing problems:
    - Non-polarising cell lines (A549, BEAS-2B) do not form the tight-junctions (TJ) required for assay development (EC-DC)
    - Calu-3 form TJ but shows weak responsiveness to stimuli.
  - tTert immortalised human bronchial cells available

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# *In Vitro*: EC - DC cultures supporting *in vivo*-like cell-cell interactions

- Skin
  - keratinocyte-MUTZ-3 interactions do not affect viability nor phenotype (preliminary)
  - Progenitor MUTZ-3 cells differentiate into MUTZ-LC in epidermis and MUTZ-DC in the dermis
  - keratinocytes required for stimulation (IL-8 release) of DC by pro-haptens
  - progenitor DC respond stronger than differentiated MUTZ-3 are responsive.

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# *In Vitro*: EC - DC cultures supporting *in vivo*-like cell-cell interactions

- Any relevance for EC-DC based assays?
- Learnings from the skin sensitisation area (TeSens):
  - loose-fit co-culture of activated keratinocytes and DC-related cells
  - sensitivity allows for testing without general cytotoxicity
  - discrimination of sensitisation and inflammation
- Co-cultures of A549 and MUTZ-3 demonstrated an intensive communication between these cells.
  - no sensitiser-induced changes

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# Identification of specific endpoints and *in vivo* relevant markers (EC)

- Genomic analysis:
  - immune reponses
    - IL-6, IL-6R, IL-16, IL-16R, IL-27R, IL-28R
  - cell cycle genes
  - energy (phosphate) metabolism

- Proteomic analysis:
  - G-CSF, GM-CSF, IL-1, IL-6, IL-8, M-CSF, RANTES

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# Identification of specific endpoints and *in vivo* relevant markers (EC)

- Signaling pathway analysis:
  - Jak-Stat signaling
  - SAPK/JNK signaling
  - PI3K/AKT signaling
  - Notch-Jagged signaling
  - integrin-mediated signaling
  - leukocyte extravasation signaling
  - axonal guidance signaling
  - neuregulin signaling
  - IGF-1 signaling
  - Toll-like receptor
  - cAMP-mediated signaling

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# Identification of specific endpoints and *in vivo* relevant markers (DC)

- Proteomics
  - IL-1β, IL-8 (CXCL8)
  - CD11c, CD40, CD54, CD86
  - HLA-DR

  - IL-8 (CXCL8) and CD86 induced by most sensitisers

  - progenitor MUTZ-3 cells > differentiated MUTZ-3 and DC

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# Identification of specific endpoints and *in vivo* relevant markers (DC)

- PepChip Kinase array
  - p38 signaling pathway
- CD analysis
  - Proteins: CD30, CD49d, CD71, CD81, **CD86**, CD95, CD123
  - Chemicals:CD40, CD43, CD54, CD80, CD83, **CD86**

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# Status

- An *in vivo*-like lung EC was not yet identified:
  - EC lines discriminate lung from skin sensitisers
  - A catalogue of biomarkers
- An *in vivo*-like DC line was identified (MUTZ-3):
  - A catalogue of biomarkers
- Development of EC-DC airlifted assays experiences problems:
  - Lung: non-polarising cell lines do not form the tight-junctions (TJ) required for EC-DC assay development.
  - Skin: model established.
  - Submerged co-cultures under investigation

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# … to be continued

- www.sens-it-iv.eu
- IVTIP, ECOPA, ECVAM, COLIPA
- Monthly newsletters
- Meetings, Congresses
- Workshops/Courses (Hogeschool Utrecht, Poster 68)
- Publications
  - 2nd year: 11 manuscriptes published, accepted or in press

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