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Identification of cell responses induced by a sensitizer in a

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Introduction

- It is possible to discriminate irritants and sensitizers based on IL-1α and IL-8 release from Reconstructed Human Epidermis (RHE)

PERGAMON

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Toxicology in Vitro

www.elsevier.com/locate/toxinvit

Analysis of interleukin-1α (IL-1α) and interleukin-8 (IL-8) expression and release in in vitro reconstructed human epidermis for the prediction of in vivo skin irritation and/or sensitization

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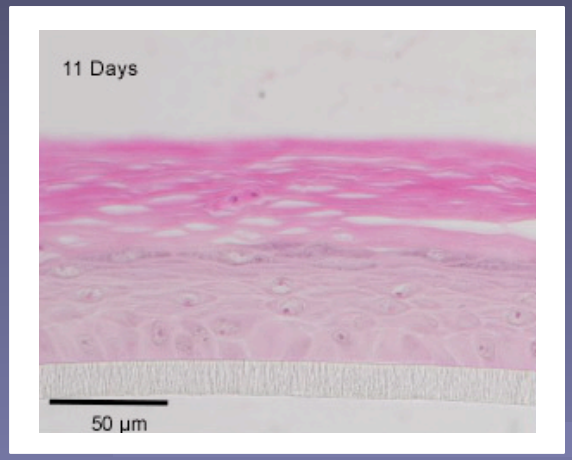
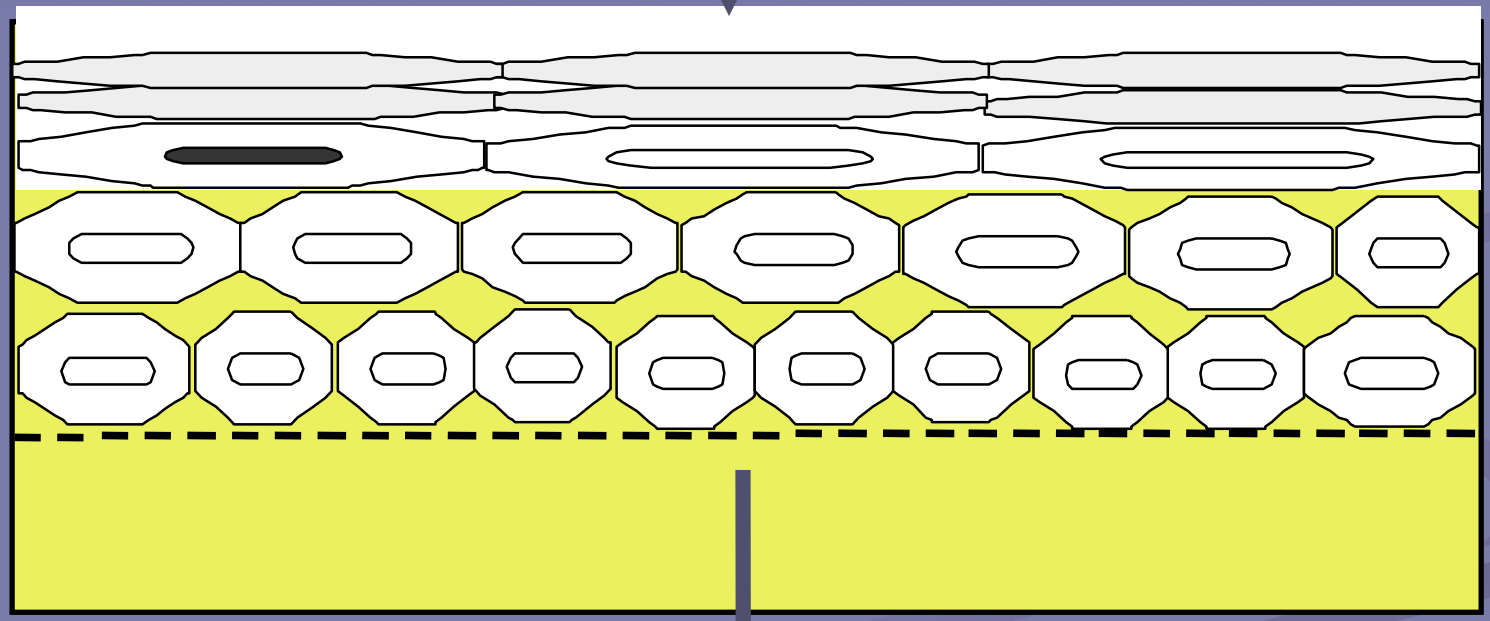
- Following the previous study based on cellular release of cytokines, we investigated several cellular mechanisms possibly responsible for the differences in IL-1α and IL-8 release



Chemicals



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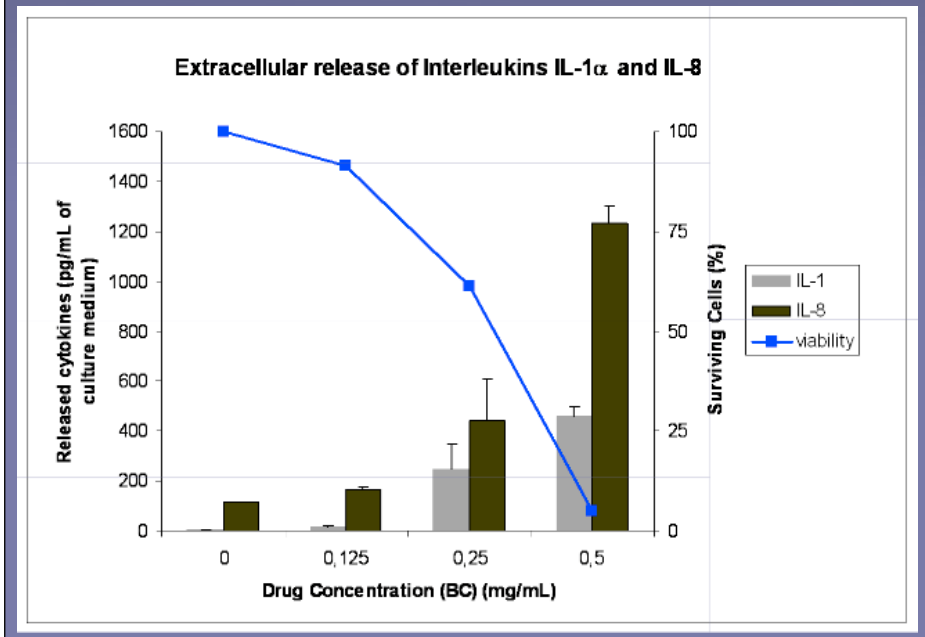
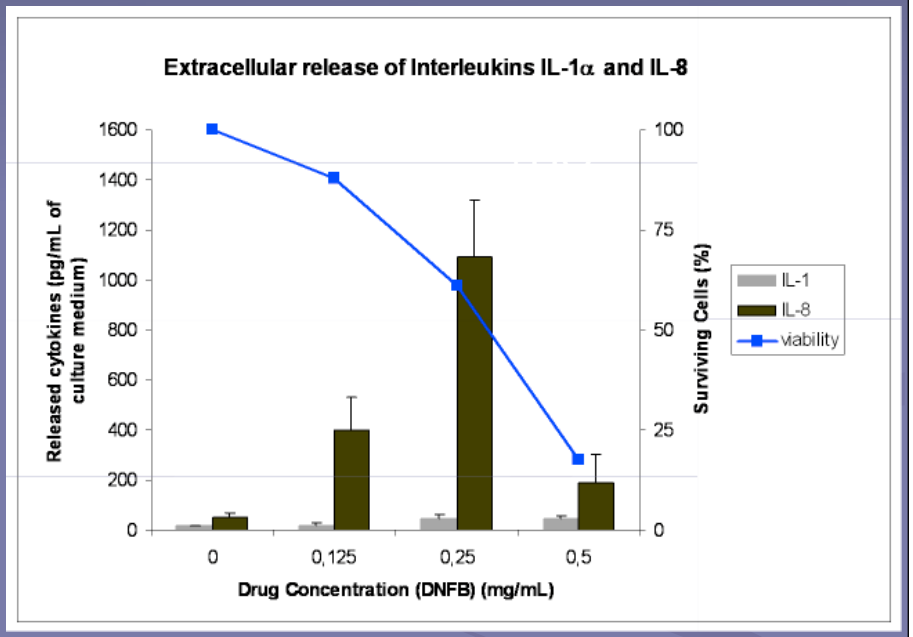
Profile of IL-1α and IL-8 release → Discrimination between irritant and sensitizer

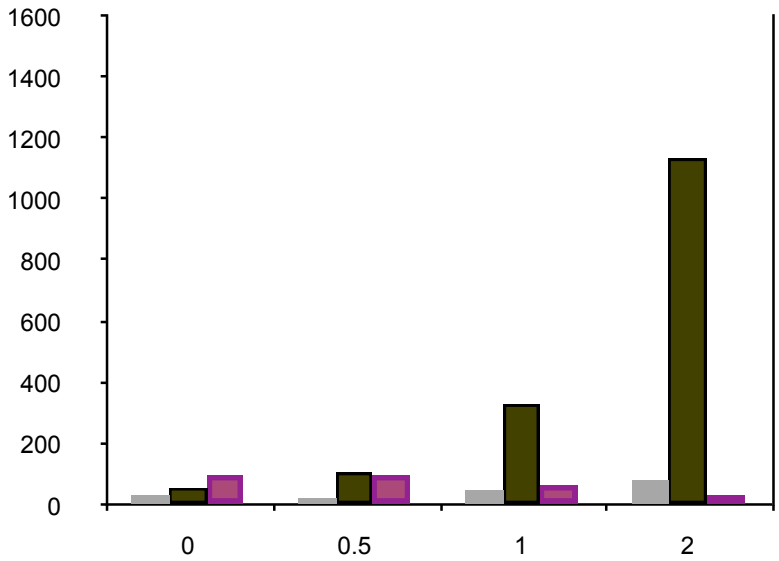




For irritant **benzalkonium chloride** (BC) → At MTT_{50} → Ratio IL-8/IL-1α → low

For sensitizer **2,4-dinitrofluorobenzene** (DNFB) → At MTT_{50} → Ratio IL-8/IL-1α → high

Which mechanisms can be involved in cytokines release by RHE treated with DNFB?



In RHE → H_2O_2 in contact **with the basal layer** induces the release of IL-8 in extracellular medium

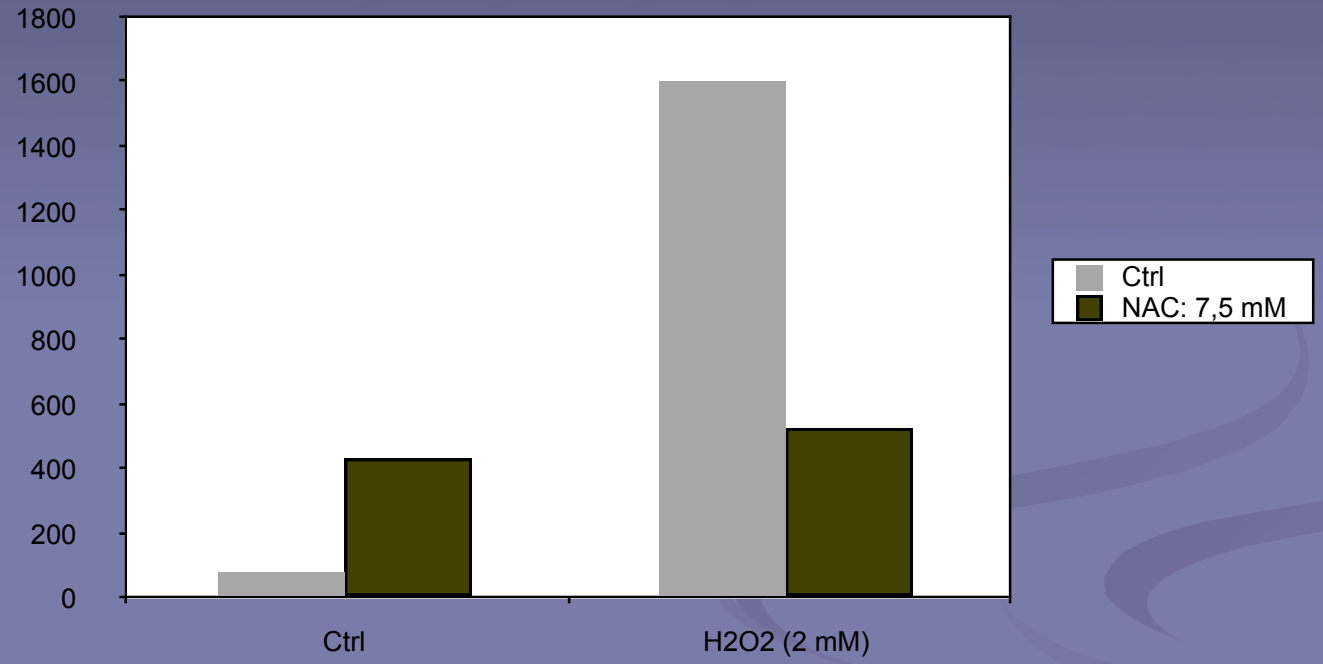
Hypothesis

→ H_2O_2 could be an intermediate in the response of RHE towards DNFB?

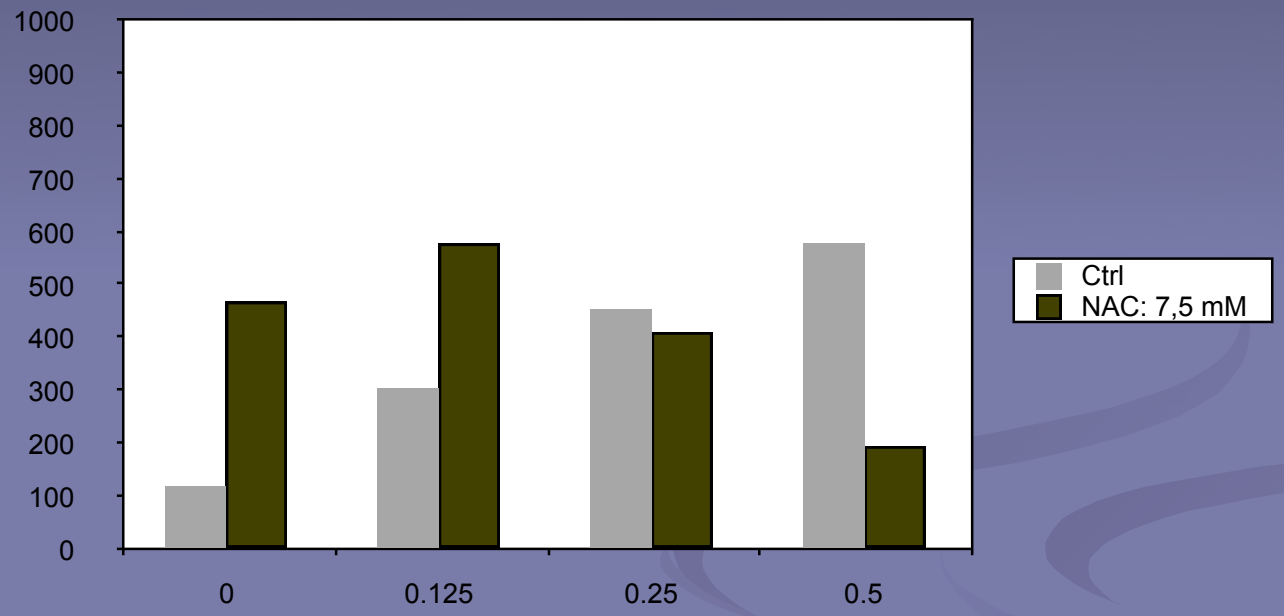
↓

RHE were co-treated with DNFB/H_2O_2 and with an antioxidant agent:

N-acetylcysteine (**NAC)**



NAC reduces the IL-8 release by RHE treated with H_2O_2

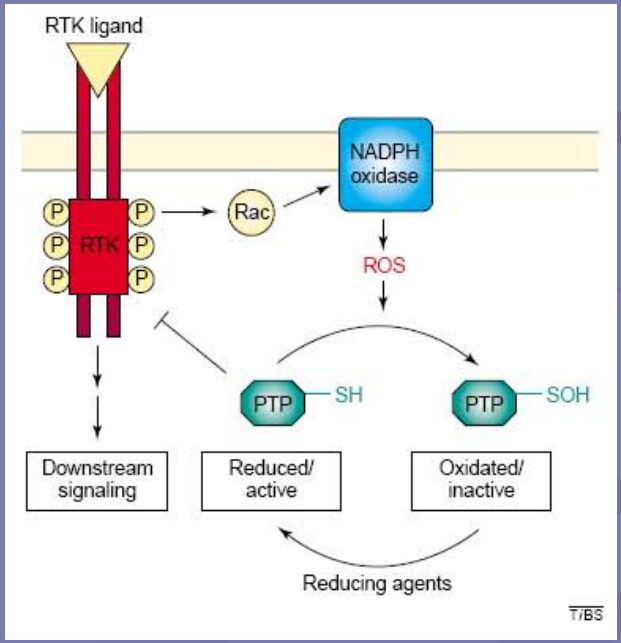


NAC reduces the IL-8 release by RHE treated with DNFB

H_2O_2 seems an intermediate in the response of RHE towards DNFB

H_2O_2 is also an important **intracellular messenger** that regulates protein dephosphorylation of tyrosine residues

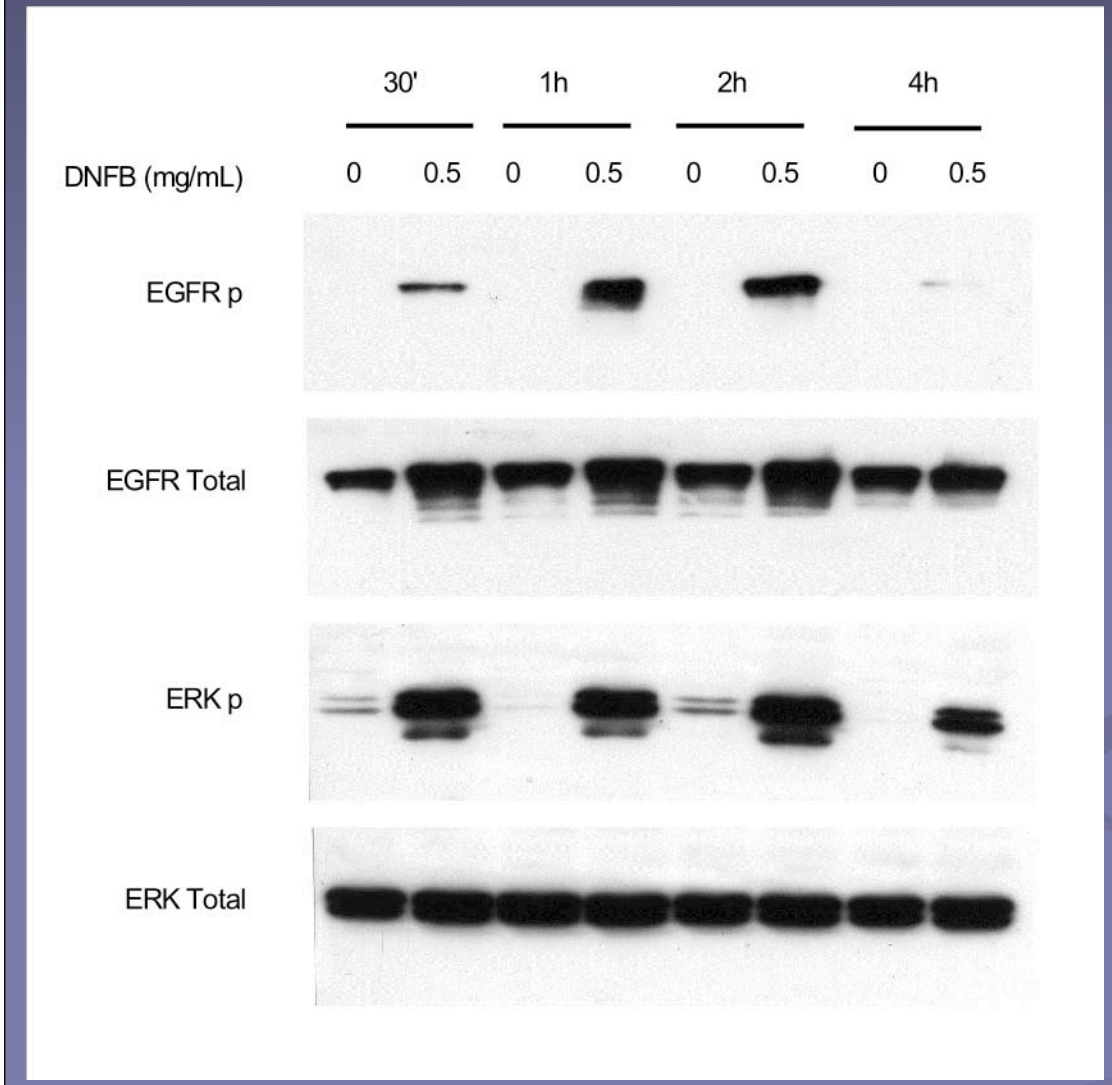
Why? ⟶

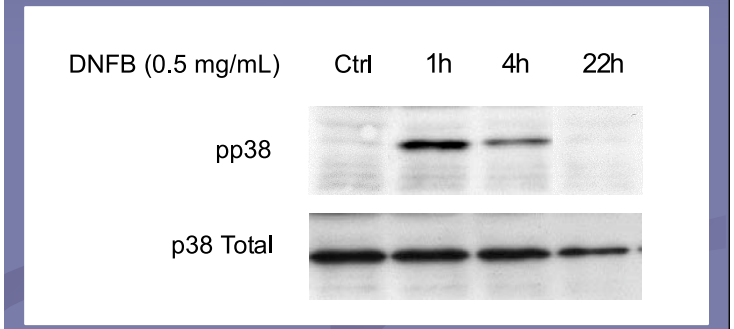


(Chiarugi and Cirri, 2003)

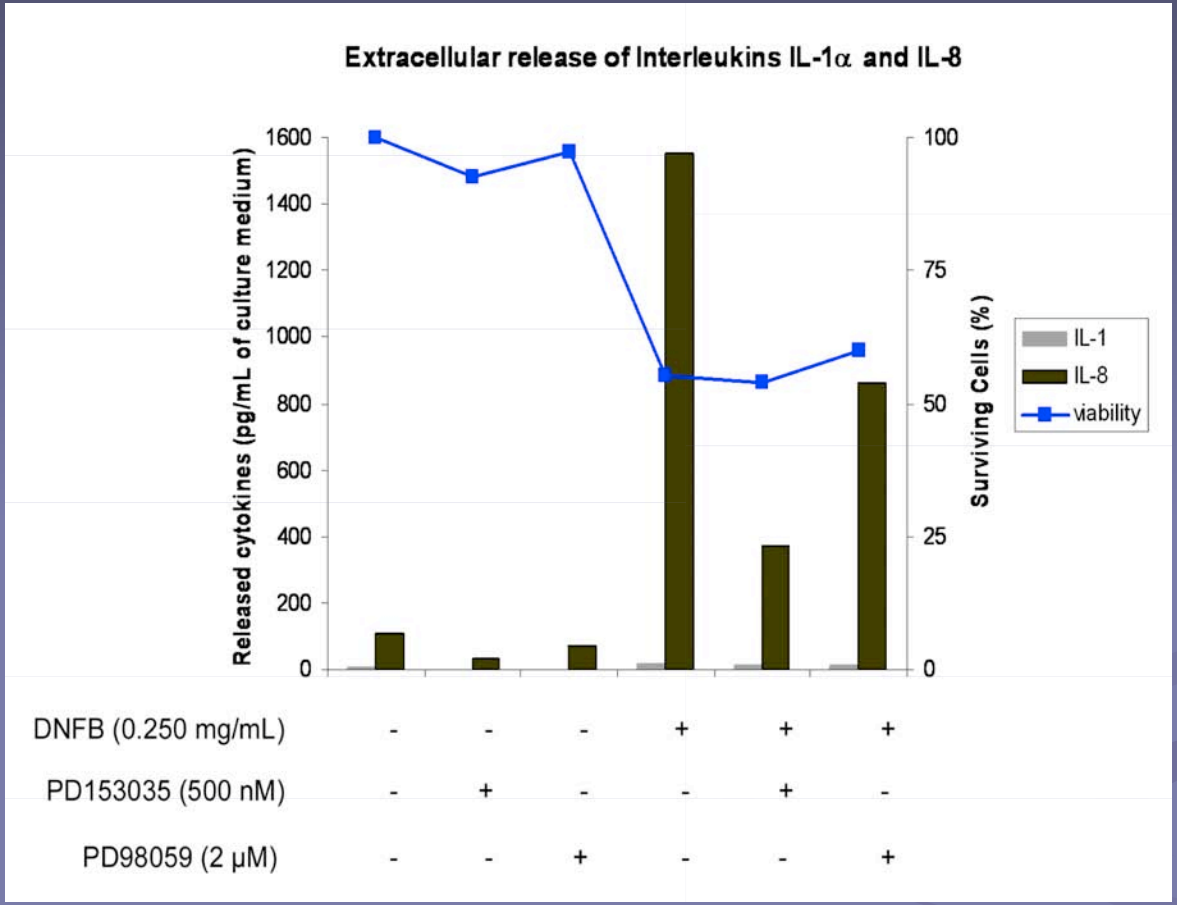
study of cell signaling pathways

Which signaling could be involved in cytokines release by RHE treated with DNFB?





⟶ Phosphorylation of EGFR, ERK 1/2 MAPK and p38 MAPK

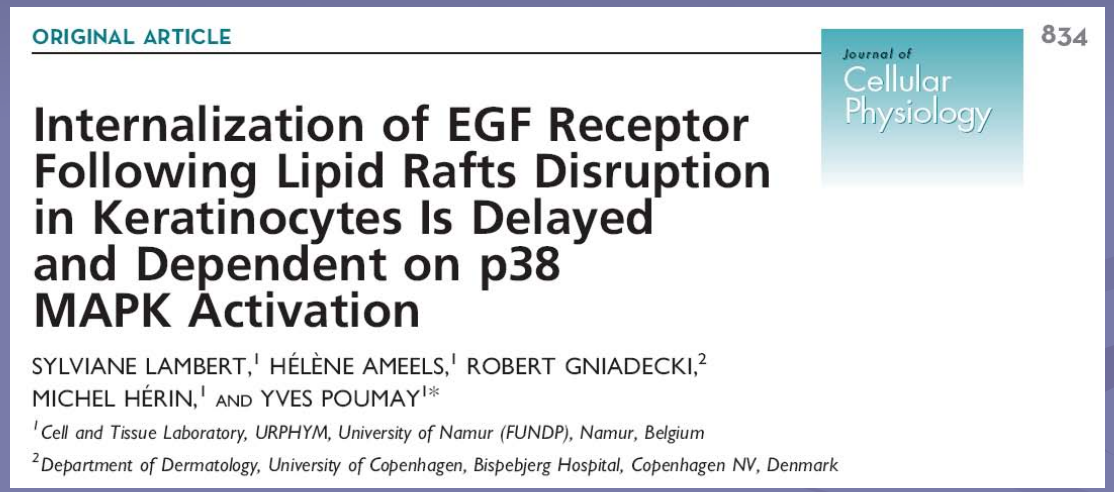


Pharmacological inhibitors of EGFR and MEK 1 reduce the release of IL-8

Involvement of EGFR and ERK 1/2 MAPK in the IL-8 release of RHE treated with DNFB

We observed on monolayers of keratinocytes in stress conditions (disruption of lipid raft, H_2O_2)

- a ligand independent activation
- internalization of the EGFR



ORIGINAL ARTICLE

834

Journal of Cellular Physiology

Internalization of EGF Receptor Following Lipid Rafts Disruption in Keratinocytes Is Delayed and Dependent on p38 MAPK Activation

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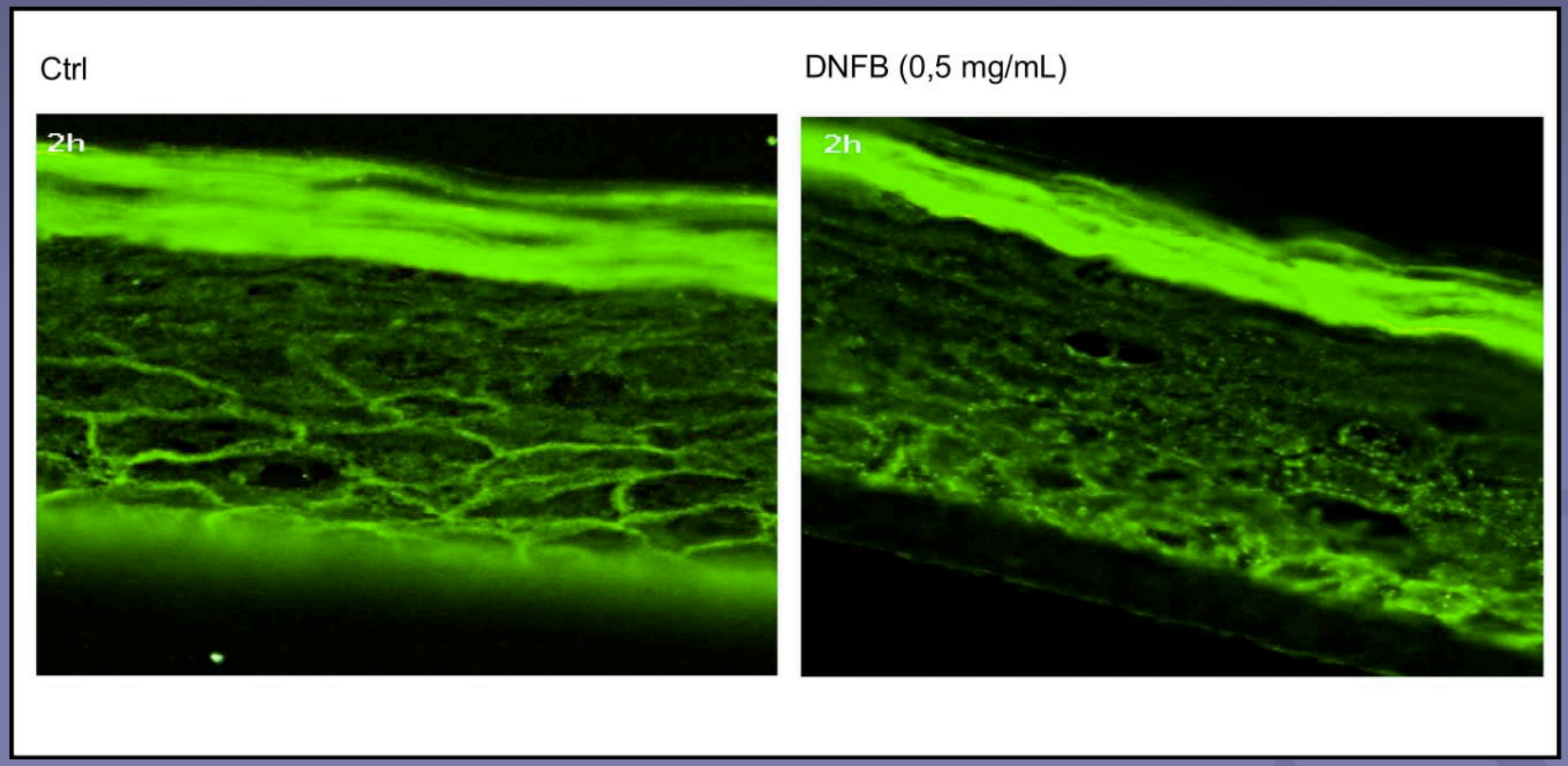
⇨ On RHE treated with DNFB we observed a phosphorylation of the EGFR

⇨ Internalization of the EGFR on RHE?



EGFR localized on membrane

Internalization of the EGFR

Conclusions

- This results are preliminary results
- Repeat experiments → statistical results
- Investigation with more sensitizers
- Investigation with irritants → compare with sensitizers
- Systematic study of the 3 MAPK activation: ERK1/2, p38 and JNK

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Analysis of MAP-kinase activation provides a promising tool for the identification of sensitizing compounds *in vitro*

Thank you for your attention