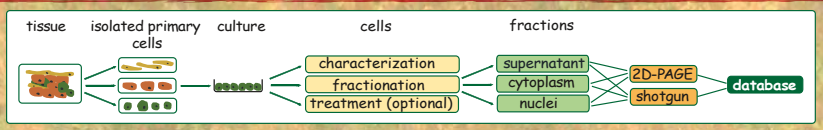
Inflammation-induced secretion of cytokines, chemokines and growth factors in human primary white blood cells, endothelial cells and fibroblasts: cell type-specificity and response to Dexamethason

Agnes Schwarzmeier, Astrid Slany, Verena Haudek-Prinz, Anastasia Meshcheryakova, Philip Klepeisz, Thomas Mohr, Christopher Gerner

Department of Medicine I, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria



Experiments:

- Human lung fibroblasts (HLF), human umbilical vein endothelial cells (HUVEC) and freshly isolated white blood mononuclear cells (WBC) were inflammatory activated, followed by treatment with 100ng/ml Dexamethason. Cells were alternatively left untreated for controls, or treated with Dexamethason alone. After 20 hours, cells were metabolically labelled for 4 hours with 35S methionine/cysteine. Cytoplasmic proteins were then separated by **2D-PAGE** and detected by **autoradiography** (**Figure 1**).
- The different treatments were accompanied by morphologic alterations of the cells which could be observed by **phase contrast microscopy** (**Figure 2**).
- In addition, unlabelled cell aliquots were fractionated in cytoplasmic, nuclear and secreted protein fractions and analysed by **shotgun proteomics** (LC-MS/MS). The relative protein abundance index (**emPAI**) was calculated from the MS data for each subcellular fraction and compared for the different treatments as exemplified in **Figure 3** and **Figure 4**.

Figure1:
Autoradiography images after 2D-PAGE of differently treated WBCs, HUVECs and HLFs: controls (con); treatment with Dexamethason alone (dex); inflammatory activation (act); inflammatory activation followed by Dexamethason treatment (act+dex). Increased spot intensities relate to higher protein synthesis rates.

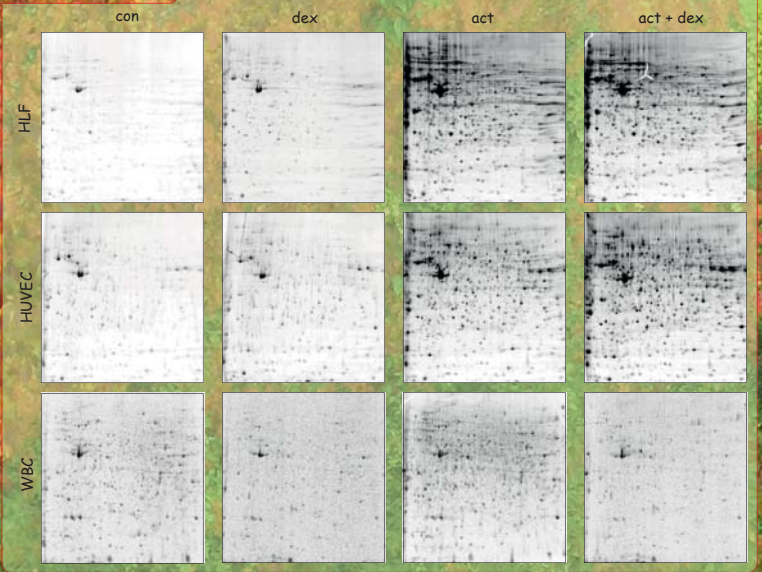


Figure 3:
The average emPAI of at least three independent experiments was calculated from the MS data for selected proteins and is represented by corresponding colour intensities for each cell fraction: nuclear fraction: inner circle; cytoplasm: outer circle; secretome: extracellular space.

Conclusions:

Inflammation is triggered and further promoted by different cell types in different ways. Proteomics is a powerful method to investigate inflammation-associated cell functions in detail. Anti-inflammatory drugs such as Dexamethason may differently interfere with the inflammation-associated functions exerted by different cell types.

Results 1: Cells respond differently to inflammatory activation.

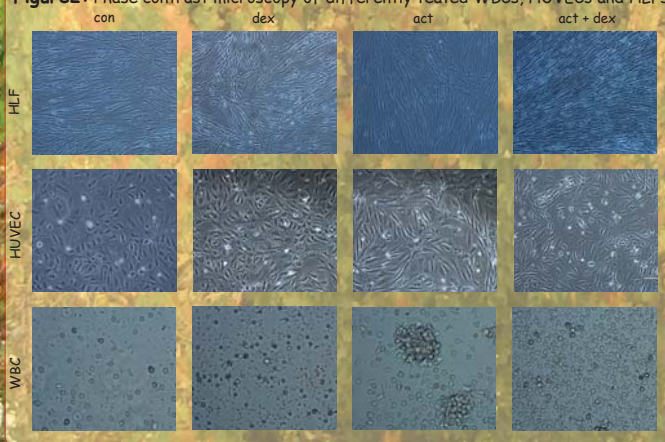
- Figure 1 shows that inflammatory activation of cells goes along with an increased protein synthesis.
- These effects are accompanied by morphologic alterations as seen in Figure 2 especially for WBCs, which show high aggregation.
- Shotgun analysis demonstrated that several proteins were found specifically and reproducibly (n>=3) induced upon inflammatory activation: Interleukin-8 for example in all analysed cells, Stromelysin-1 in HLF only, VCAM in HUVECs only and Granzyme B in WBCs only (Figure 3).

Results 2: Cells respond differently to dexamethason.

- Figure 1 shows that while Dexamethason alone reduces protein synthesis in WBCs, the opposite effect is observed in endothelial cells and fibroblasts.
- Intriguingly, Dexamethason profoundly affected HLF morphology as well as that of HUVECs, as seen in Figure2.
- Dexamethason affected the protein expression profiles only in a subtle fashion (Figure3)

Figure 4:
EmPAI values for specific proteins are represented as in Figure 3.

Figure2: Phase contrast microscopy of differently teated WBCs, HUVECs and HLFs.



Result 3: Dexamethason is exerting anti-inflammatory activity in a cell type-specific fashion.

- Figure 1 shows that increased protein synthesis upon inflammatory activation is disrupted in WBCs but not in HUVECs and HLFs.
- Morphologic alterations after inflammatory activation were reversed by Dexamethason treatment. This can be well seen in the case of WBCs in Figure 2, where aggregation of the cells was largely reduced.
- Analysis of shotgun experiments revealed that the induction of most inflammation-related proteins was successfully inhibited or at least down-regulated by Dexamethason (demonstrated for selected proteins in Figure 3).

Result 4: Inflammation-associated cell responses to Dexamethason treatment were in part deviating from expectations.

- The chemokine CXCL10, for example, specifically induced in inflammatory activated WBCs, was not suppressed by Dexamethason. Similarly, the growth factor CSF-1, specifically induced in inflammatory activated HUVECs, and the growth regulator Inhibin beta A, were not suppressed by Dexamethason. In contrast, the hepatoma derived growth factor was successfully suppressed in HUVECs, but not in the HLF cells (Figure 4).

Contact:
Agnes.Schwarzmeier@meduniwien.ac.at
Christopher.Gerner@meduniwien.ac.at
poster design: Editha.Bayer@meduniwien.ac.at

Supported by
Christian Doppler Forschungsgesellschaft
FWF Der Wissenschaftsfonds
CLINICAL PROTEOMICS LABORATORIES
MEDICAL UNIVERSITY OF VIENNA