

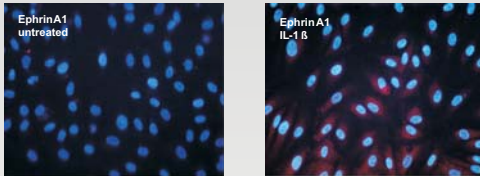
Cell type specificity of inflammatory secretome profiles of primary human endothelial and dendritic cells

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Methods

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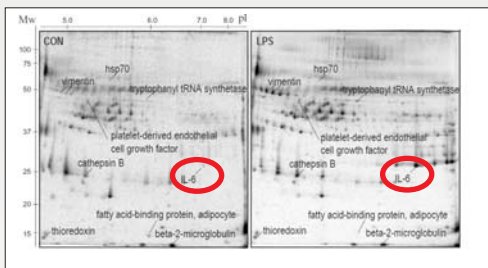
Immunofluorescence is a technique allowing the visualization of a specific protein or antigen within cells or tissue sections by binding of a specific antibody which is chemically conjugated with a fluorescent dye.



The induction of Ephrin A1 expression upon IL-1 β treatment observed by shotgun proteomics was verified by immunofluorescence

2D PAGE

Two-dimensional polyacrylamide gel electrophoresis is an advanced form of gel electrophoresis commonly used to analyze complex protein mixtures. Protein extracts are separated based on their specific charge and molecular weight into individual spots. For secretome analysis, cells have been metabolically labeled with 35S- methionine/cysteine and proteins detected by autoradiography.



Inflammation induced upregulation of protein secretion is clearly detectable in the following 2D-gels of immature (CON) and LPS-treated DCs. Protein spots were identified by LC-MS/MS analysis of tryptic digests. Note: IL-6 is strongly expressed in LPS treated DCs, but still detectable at very low levels in immature DCs.

Shotgun proteomics

Untreated and treated cells were fractionated into secreted proteins, cytoplasm and nuclear proteins. These protein fractions were further separated by SDS PAGE and cut into 6-12 gel slices. Each slice was digested with trypsin and the eluted peptides were analysed by LC-MS/MS (Agilent 1100 system, chip HPLC, Agilent XCT ultra ion trap MS).

Conclusion

Secretome profiling of inflammatory activated dendritic cells in comparison to endothelial cells revealed high levels of IL-6 and IL-8 secretion by ECs; levels considerably above those of DCs. Furthermore the cell number of ECs in any tissue by far exceeds those of DCs. Based on this consideration, IL-6 and IL-8 quantified in blood serum may be derived from ECs rather than DCs. Concomitant determination of cell type specific inflammatory cytokines such as MCP 1, or MIP 4 may support the identification of the cell types contributing to the inflammation state. This information may help to optimize the choice of anti-inflammatory drugs.

Background

Dendritic cells belong to the main regulators of inflammation. In addition to these well known players other cell types were found to contribute significantly to the progression of inflammatory responses. Different cell types may produce specific sets of inflammatory cytokines. Furthermore different cell types may respond in a cell type specific manner to anti-inflammatory drugs. In contrast to dendritic cells, the inflammatory contribution of endothelial cells is hardly considered, although these cells secrete considerably amounts of inflammatory cytokines with a similar cytokine pattern. Systematic secretome profiling of inflammatory cells have not yet been established. Knowledge of such cell type specific cytokine expression pattern may allow to determine the contribution of individual cell types to inflammatory diseases and thus aid individual choice of therapy.

Results

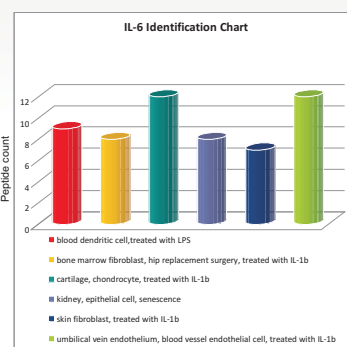
Secretome profiling was performed by nano LC-MS/MS. We identified 1407 proteins in immature DCs. 129 of them were genuinely secreted. In inflammatory activated DCs we identified 1436 proteins. 150 proteins of those were genuinely secreted, whereas 39 proteins were found to be newly induced upon inflammatory activation.

In untreated ECs we identified 192 genuinely secreted proteins out of a total of 2091. From ECs treated with IL-1 β we were able to identify 2098 proteins. 200 of those were genuinely secreted and 38 proteins were newly induced by the inflammatory stimulus.

Intriguingly 9 of the inflammation induced cytokines, including IL-6 and IL-8, were identified both in DCs and ECs, while 30 (29) secreted proteins were found specifically induced in DC (EC).



The CPL/MUW Database contains protein expression data of more than a 100 different human cell types/states. The query for IL-6 reveals that beside inflammatory activated DCs and ECs four other cell types secreted this cytokine.



Strategy

- 1 Endothelial cells were released from umbilical vein by collagenase IV treatment



- 2 CD34 positive cells isolated by Dynabeads



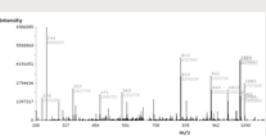
- 3 *in vitro* reference experiments with primary cells derived from healthy donors: treatment with IL-1 β in order to investigate alterations characteristic for inflammatory activation.

- 4 Isolation of cell supernatant, cytoplasm and nuclei by hypotonic cell lysis and differential centrifugation. Extraction of nuclear proteins by high salt and detergent (NP-40). Protein precipitation to remove non protein impurities and solubilisation in sample buffer.

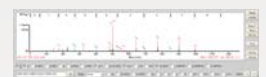
- 5 tryptic digests of gel slices



- 6 nano-LC-MS/MS



- 7 Determination of amino acid sequence out of peptide fragmentation pattern



- 8 Assembly of protein lists

Q9UKD2	miRNA turnover protein 4 homolog - Homo sapiens (Human)
Q15233	Non-POU domain-containing octamer-binding protein (NcoD) p
Q9WV22	Non-structural maintenance of chromosomes element 3 homolog
Q95662	Nuclear cap-binding protein subunit 1 OS=Homo sapiens GN=V
Q15298	Nuclear cap-binding protein subunit 2 - Homo sapiens (Human)
Q9H945	Nuclear envelope pore membrane protein POM 25 OS=Homo
Q14980	Nuclear mitotic apparatus protein 1 [NUAAP protein] (SP-H) and
P17740	Nuclear pore complex protein Nuq107 OS=Homo sapiens GN=V
Q9WAA0	Nuclear pore complex protein Nuq133 (Nucleoporin Nuq133) 1
Q15294	Nuclear pore complex protein Nuq155 - Homo sapiens (Human)
Q12795	Nuclear pore complex protein Nuq160 OS=Homo sapiens GN=V
Q92621	Nuclear pore complex protein Nuq205 OS=Homo sapiens GN=V
P15568	Nuclear pore complex protein Nuq214 (Nucleoporin Nuq214) 1
Q95667	Nuclear pore complex protein Nuq88 (Nucleoporin Nuq88) (S)
Q9H177	Nuclear pore complex protein Nuq95 OS=Homo sapiens GN=V
Q15248	Nuclear pore complex protein Nuq98 Nuq98 OS=Homo sapiens
P17198	Nuclear pore glycoprotein p62 (25 kDa nucleoporin) - Homo sap
Q9H279	Nuclear pore protein Nuq11 (Nucleoporin-associated nucleoporin)
Q9H425	Nuclear receptor coactivator 5 OS=Homo sapiens GN=HCCAS 1
Q9H805	Nuclear RNA export factor 1 (Export-associated protein) (Exp-as-1)
P19269	Nuclease sensitive element-binding protein 1 (Y-box-binding) p
Q9B214	Nuclear GTP-binding protein 1 OS=Homo sapiens GN=CTP1914
Q14978	Nuclear phosphoprotein p180 (Nucleoporin 180 kDa protein) 1
Q06936	Nucleolar protein 3 - Homo sapiens (Human)