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Comparing the stability and activity of recombinant IFNλ3 and IFNλ4

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Hepatitis C virus (HCV) infection is one of the major causes of liver cancer worldwide and every year 3–4 million people become infected. Whereas some infected people eradicate the virus spontaneously, almost 85% do not and instead develop a chronic infection. Recently, it was shown that people with a functional *IFNL4* gene have a lower chance of clearing the virus spontaneously or in response to treatment than people with a non-functional *IFNL4* gene. Why it is a disadvantage to have a functional *IFNL4* gene during HCV infection is currently not known although a causal relationship between the activity of the IFNλ4 protein and poor HCV clearance has been demonstrated.

IFNλ4 belongs to the type III IFNs together with IFNλ1, -2, and -3. However, it differs from the others not only by its low sequence similarity but also by its impaired secretion. This impairment is not due to a weak signal peptide, as swapping the signal peptides between IFNλ3 and -4 had no effect on secretion. When we purified IFNλ3 and -4, we found that IFNλ4 is far more difficult to refold *in vitro* than IFNλ3 suggesting that the poor secretion of IFNλ4 could be due to an inherent problem of folding the protein. Because such a problem could also mean that IFNλ4 could be far unstable than the other type III IFNs, we decided to compare the stability of recombinant IFNλ3 and IFNλ4. Our results demonstrate that IFNλ4 like IFNλ3 is surprisingly stable once it has folded properly.

http://dx.doi.org/10.1016/j.cyto.2015.08.195

ID: 192
Role of AhR in IL-22 production by four types of immune cells, including CD4-CD8- TCRß T cells, present in psoriasis-like skin after topical imiquimod

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IL-22 has a detrimental role in skin inflammatory processes, particularly in the imiquimod-induced psoriasis model.

As transcription factor AhR controls IL-22 production by several cell types, we analyzed its role in IL-22 production by immune cells in the inflamed skin. We used a model in which imiquimod is applied on the ears.

Our results indicate that IL-22 is still expressed in the skin of imiquimod-treated AhR−/− mice but in lesser amounts than in wild-type mice. We then studied the role of AhR on each of the three cell populations known to produce IL-22 in the skin: γδ T cells, Th17 cells and ILC3. We studied also a new IL-22 producing cell type that we identified in this setting: CD4-/CD8- TCRβ+ T cells.

In the imiquimod-treated ears, AhR was required for IL-22 production by Th17 but not by the three other cell types. For the latter but not for Th17 cells, AhR had a role in their recruitment into the inflamed skin or in their local proliferation, as their numbers were reduced in AhR−/− vs wild-type imiquimod-treated ears.

http://dx.doi.org/10.1016/j.cyto.2015.08.196

ID: 193
ADAM10 controls a novel IL-11 trans-signalling pathway

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Interleukin (IL)-11 is a member of the IL-6 family which has been initially described to have anti-inflammatory properties. However, recent studies revealed that overshooting IL-11 activity is involved in inflammation and the progression of epithelial cancers. IL-11 binds to the membrane-bound IL-11 receptor (IL-11R), and this complex can then initiate signal transduction by recruiting two molecules of the β-receptor glycoprotein (gp)130, which predominantly leads to activation of the JAK/STAT pathways. Gp130 is ubiquitously expressed, and IL-11R expression was found on several cell types including osteoblasts, osteoclasts, T cells and macrophages.

Proteolytic processing of cytokine receptors is an important regulatory element for their signalling capacity, which not only regulates the amount of the receptors on the cell surface, but also creates soluble receptors with distinct biological functions. Here, we show for the first time that the metalloprotease ADAM10, but not ADAM17, is able to cleave the IL-11R and release its ectodomain. Chimeric receptors of the IL-11R and the IL-6R revealed structural traits required for proteolytic processing and showed that a small part of the stalk region is responsible for ADAM17's ability to discriminate between substrate and non-substrate. Furthermore, we show that a single amino acid mutation within the stalk is sufficient to generate a proteolysis-resistant IL-11R variant.

The generated soluble IL-11R binds IL-11, and the resulting complex can then bind to gp130 and thus activate cells even though they do not express the membrane-bound IL-11R. This novel IL-11 trans-signalling pathway can be specifically inhibited by the anti-inflammatory designer protein sgp130Fc.

http://dx.doi.org/10.1016/j.cyto.2015.08.197

ID: 194
Role of microRNAs in signal transduction pathways of the inflammatory cytokine Interleukin-6: Relevance for liver diseases

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IL-6 plays important roles in regulation of liver functions and promotes hepatocarcinogenesis. As the contribution of IL-6-induced miRNAs to these effects is largely unknown, we investigate the effects of IL-6 on the miRNome of hepatoma cells and non-transformed hepatocytes. Moreover, little is known about miRNAs regulating key players of IL-6 signal transduction. Therefore, our aims were to identify such miRNAs in a miRNA mimics screen and to elucidate their effects on this pathway. Integration of the results of both approaches is expected to lead to the identification of regulatory circuits.

While IL-6 (and hyperIL-6) induce the differential regulation of thousands of mRNAs in HepG2 and HuH-7 hepatoma cell lines, only selected miRNAs change their expression level significantly, including miR-21, miR-100, miR-145, miR-146b-5p. In contrast, we have observed previously that IFN-g has a profound effect on the miRNome of melanoma cells (Schmitt, Cell Comm. Sign. 2012). Therefore we investigate whether IL-6-type cytokines may have generally weaker effects on the miRNome compared to interferons and/or whether our observation is due to cell type-specific differences.

To extend our *in vitro* findings, we analyse and correlate the expression levels of miRNAs and inflammatory cytokines in patients with HCC and advanced nonalcoholic steatohepatitis (NASH), the latter being at high risk to develop HCC. Bio-Plex cytokine immunoassays revealed that the serum level of IL-6 and HGF is higher in HCC patients than in healthy controls and NASH patients. Our work may contribute to the identification of novel biomarkers for the progression of chronic liver diseases.

http://dx.doi.org/10.1016/j.cyto.2015.08.198

ID: 195
Identification of cytokine cell sources in a new mouse model of systemic juvenile idiopathic arthritis highlights innate immunity of this disorder

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Objective: Systemic juvenile idiopathic arthritis (sJIA) is a rheumatic childhood disease, with distinctive systemic inflammatory features and a typical cytokine profile. The etiology of sJIA is largely unknown. Here, we aimed to elucidate the role of specific cell populations and cytokines in the pathogenesis, by means of a new mouse model of sJIA relying on the injection of complete Freund's adjuvant (CFA) in interferon-gamma deficient (IFN-g KO) mice. Wild type (WT) mice developed a minor inflammation, indicating that IFN-g provides protection in the disease process.

Methods: Cytokine mRNA levels were analyzed in organs and purified immune cell populations of CFA-challenged IFN-g KO and WT mice. Cytokine antagonists and cell depleting antibodies were administered to mice. Clinical, laboratory and immune features of sJIA were evaluated.

Results: In the diseased IFN-g KO mice, elevated expression of IL 1β, IL-6, IL-17 and IL-22 were found in lymph nodes and – remarkably – also in lung tissue. Gamma-delta T cells were a major cell source for IL-17, and anti-IL-17 antibodies abrogated the disease. In the non-diseased WT mice, expression of IFN-g was almost exclusively found in NK, NKT and gamma-delta T cells. Transient neutralization of IFN-g as well as depletion of NK cells in WT mice both resulted in a fulminant sJIA-like disease.