porcine primary cells. Inoculation of NPTR cells with adenovirus vectors expressing all three types of IFN inhibited CSFV infection, with the AdIFNα/β and λ constructs inducing a greater anti-CSFV effect than the AdIFNγ constructs.

Conclusion: All three types of porcine IFN have some antiviral activity against CSFV and delivery via adenovirus vectors may offer a promising targeted intervention strategy against CSF. Future studies will assess the kinetics of protection of single and combined vectored PoIFN treatments in vitro and in vivo.

http://dx.doi.org/10.1016/j.cyto.2015.08.236

ID: 234
Dynamics of antiviral defense

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Early recognition of viral pathogen-associated molecular patterns (PAMPs) by RIG-I-like receptors (RLRs) and endosomal Toll-like receptors (TLRs) is crucial to protect the host from extensive viral propagation. These sensors activate intracellular signaling cascades leading to the induction of type I and type III Interferons (IFNs). During the acute phase of infection the efficiency of the IFN system in time, space and strength determine the outcome of antiviral protection. To understand the dynamics of antiviral activity within a cell community and to define the limiting steps during signal propagation we studied temporal and spatial dynamics of IFN induction and IFN-stimulated gene expression on single-cell level. Time-resolved imaging uncovers a large extent of heterogeneity for several key steps of antiviral activity that is based on stochastic decisions. This heterogeneity applies with respect to the ability of the individual cells to respond as well as with respect to the onset times of the responding population. Especially at lower IFN concentrations the temporal variability of ISG induction increases explicitly. To determine the molecular origin of the heterogeneity upon PAMP recognition we combined single cell reporter systems for K63-polyubiquitination and NF-κB activation. We find a temporal variability of polyUb aggregation events that is paralleled by subsequent NF-κB activation. However, the temporal variability between both events was low compared to the overall variability of NF-κB activation onset. We conclude that heterogeneity of the IFN induction results mainly from the dominant stochastic nature of high-order complex formation of RIG-I upon ligand recognition.

http://dx.doi.org/10.1016/j.cyto.2015.08.237

ID: 235
Sensing accuracy of interferons' concentrations

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Interferons exhibit their key role of immune modulators through activation of the Jak-Stat signalling pathway. We know substantial amount of molecular details regarding functioning of the pathway. However, to what extend the action of the pathway is dose dependent at the single cell level remains largely unclear. Specifically it is not know if single cells respond in a digital fashion or their output is continuously dependent on the stimulant's concentration. We have combined an information-theoretic framework with high-throughput confocal imaging of mouse embryonic fibroblasts to provide a thorough, single-cell analysis of the Jak-Stat signalling in response to interferon beta and interferon gamma. We showed that in a baseline state single cells have information hardly sufficient to distinguish between presence or absence of interferons. However they can be put in an alert state by an action of interferons, which allows them to respond more in an analogous fashion. Our results show that the accuracy with which signalling pathways transmit information is not fixed but can be modulated on the contextual basis.

http://dx.doi.org/10.1016/j.cyto.2015.08.238

ID: 236
IL-37 inhibits HIV-1 replication in human T-cells

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The inflammasome cytokines IL-1β and IL-18 have well described pro-inflammatory effects. IL-37 is a recently characterized member of the IL-1 family of cytokines and has been identified as a regulator of innate immune responses. This study aims to determine the effects of IL-1 family cytokines on HIV-1 replication in human primary CD4+ T-cells. Activated primary human T-cells were spinoculated with HIV-1 and infection and release were measured by flow-cytometry using a GFP expressing HIV-1 virions and p24 ELISA. Expression of the cytokine receptors was confirmed via flow-cytometry. We find that activated T-cells express receptors for all IL-1 family cytokines. IL-37 uniquely suppresses HIV-1 entry and replication in activated CD4+ T-cells infected with laboratory-adapted strains of HIV-1 as well as virus like particles. Conversely the pro-inflammatory cytokine IL-1β promoted HIV-1 replication in activated T-cells. Our studies suggest that inflammasome cytokines are not inherently antiviral and demonstrates suppression of HIV-1 replication by IL-37.

http://dx.doi.org/10.1016/j.cyto.2015.08.239

ID: 237
In virus-induced hepatitis local IFN-I responses modulate myeloid suppressor cell infiltration

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Most viruses developed mechanisms that inhibit the induction or the function of type I interferon (IFN-I). Vaccinia virus (VACV), a large DNA-encoded poxvirus, encodes several IFN-I evasins, including the viral IFN-I receptor B18. Nevertheless, here we found that VACV infection still induced local IFN-I responses in secondary lymphoid organs, which are mainly sensed within the liver. VACV infected IFN-I receptor deficient (IFNAR−/−) mice developed fulminant disease, associated with high virus loads in organs such as liver and spleen, imbalanced cytokine responses within the blood, and eventually succumbed to infection. Elevated ALT/AST levels in the serum of VACV infected IFNAR−/− mice supported the hypothesis that IFNAR triggering of the liver played a major role in the antiviral response. Histological analysis of livers of VACV infected IFNAR−/− mice revealed a severe acute hepatitis, massive infiltration of immune cells, and acute tissue apoptosis in the absence of IFNAR-signaling. Interestingly, VACV infected mice with a hepatocyte selective IFNAR ablation (Alb-CreIFNARflox/flox) showed moderate hepatitis as observed in wild-type mice, whereas mice with a cell type-specific IFNAR deletion only in myeloid cells (LysM-CreIFNARflox/flox) showed enhanced hepatitis. Thus, local IFN-I responses sensed by myeloid cells prevented immunopathology within the liver, presumably due to the induction of myeloid derived suppressor cells (MDSC).

Collectively these results indicate that although the induction of serum IFN-I is sequestered, VACV infection induces local IFN-I responses that play a central role in modulating immune responses and thus in reducing immunopathology and hepatitis.

http://dx.doi.org/10.1016/j.cyto.2015.08.240

ID: 238
IL-15 markedly augmented the number of circulating NK cells and enhanced ADCC mediated by tumor-directed monoclonal antibodies

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Interleukin-15, that activates antitumor T and natural killer cells, has potential applications in cancer immunotherapy. IL-15 administered at 20 μg/kg/day by continuous intravenous (CIV) infusion for 10 days to nonhuman primates yielded an 80–100-fold expansion of circulating CD8+ effector memory T-cells, a 15-fold increase in the number of monocytes and an 8-fold increase in NK cells. Based on these observations we performed a first-in-human phase 1 clinical trial of rhIL-15 administered by bolus infusions and a trial of IL-15 by CIV in patients with metastatic malignancy. When administered by CIV at 2 μg/kg per day IL-15 led to a 30-fold increase in the number of NK cells and over a 200-fold increase in the number of CD56+ CD16− NK cells. It was clear from these clinical trials that to be effective IL-15 would have to be administered in combination with agents with specificity directed toward the tumor. Given the capacity of IL-15 to increase NK cells an attractive strategy was to use it in conjunction with an antitumor antibody to augment ADCC. When IL-15 was administered with rituximab it markedly increased the ADCC effectiveness of this antibody in a syngeneic mouse model of human B-cell lymphoma involving EL4