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P133
RIG-I mediates recognition of Salmonella RNA in non-phagocytic cells and contributes to early bacterial replication in vivo
M. Schönölke1, J. Patel1, J. Miller2, M. Sanchez Aparicio1, B. Manicassamy1, M. Merad3, A. Garcia Sastre1, A. Marlu1,2, V. Leroy1,2,3, N. Sturm1,2, J.-P. Zarski1,2,3, P.N. Marche1,2,3,
Hoang 1,2, A. Merad2, A. Garcia Sastre1, 1
(1,2). However, the association of rs12979860 polymorphism with immune function activity. Expression of CD8
CD45, CD3, CD4, CD8, CD56 and FoxP3 as markers and CD107a for degranulation (T, Treg, NK and NKT) were identified by flow cytometry for the expression of
were collected from the needle biopsy achieved for the diagnosis prior any treatment.
Salmonella strains in RIG-I+/+ and RIG-I
production was measured by 293T IFNbeta- reporter cells or qPCR. Intracellular bac-
bacterium Salmonella typhimurium is a ligand for RIG-I, triggers production of
type I–/– interferons and contributes to pathogenicity in vivo.
Methods. We use RIG-I−/–/MEFs [2] or human epithelial cells depleted of RIG-I by lentiviral shRNA transfection for transfection and infection experiments. IFNβeta
expression was measured by 293T IFNbeta-reporter cells or qPCR. Intracellular bac-
terial titers were determined by gentamycin protection assay. In vivo infection exper-
iments were performed as described [3] using SL1344 or SL1344 aroA deficient Salmonella strains in RIG-I+/+ and RIG-I−/– mice [4].
Results. Infection of fibroblasts and epithelial cells with S. typhimurium, but not
with non-invasive E. coli, triggers IFNβeta transcription, suggesting that intracellular replication is required. RIG-I dependent recognition of bacterial RNA is dominant in
non-phagocytic cells, as shown in RIG-I knockout and knockdown model cells. Despite
presence of other pathogen-associated patterns (PAMP), like LPS or flagellin, no IFNbeta was produced in fibroblasts and epithelial cells lacking RIG-I upon S.
typhimurium infection. In contrast, in macrophages TLR dependent recognition of
bacterial PAMPs through TRIF/Md88 overcomes RIG-I deficiency and leads to robust
induction of type I interferon. We observed higher bacterial titers at early time points
after infection in the cumc of S. typhimurium infected RIG-I−/– mice, when compared
to wild type animals. However, at later stages of infection, S. typhimurium overcomes
the innate immune response leading to similar weight loss and mortality in wild-
type and RIG-I−/– mice.
Conclusion. In summary, our data implicate a role of RIG-I mediated innate immune
recognition of bacterial RNA in early control of bacterial replication, most likely medi-
ated by non-phagocytic intestinal epithelial cells targeted by S. typhimurium.
Disclosure of interest: None declared.
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http://dx.doi.org/10.1016/j.cyto.2012.06.225

P134
IL28B associated polymorphism, rs12979860, controls the activity of liver
lymphocytes
E. Jovuin-Marche1,2, E. Fugier1,2, M.A. Theluy1,2,3, N. Van Campenhout1,2,3, X.S. Hoang1,2, A. Marlu3, V. Leroy1,2,3, N. Sturm1,2, J.-P. Zarski1,2,3, P.N. Marche1,2,3,
U823, INSERM, France, 2 Institut Albert Bomette, Université J Fourier Grenoble1,
Grenoble, France, 3 Pole Digi-Dune, Centre Hospitalier Universitaire de Grenoble, La
Tronche, France

Introduction. The single nucleotide polymorphisms (rs12979860), near the IL28B
gene, is correlated with a sustained virological response (SVR) in Hepatitis C Virus
(HCV) infected patients treated with Pegylated Interferon-α combined with Ribavirin
(1,2). However, the association of rs12979860 polymorphism with immune function of the
liver, the site of HCV production, and the mechanism of SVR remain still undefined.
Methods. Patients chronically HCV-infected patients were genotyped for
rs12979860 defining C/T polymorphism. C allele is associated to SVR. Liver samples
were collected from the needle biopsy achieved for the diagnosis prior any treatment.
Single cell suspensions were prepared by mechanical disruption. Liver lymphocytes
(T, Treg, NK and NKT) were identified by flow cytometry for the expression of
CD45, CD3, CD4, CD8, CD56 and FoxP3 as markers and CD107a for degranulation
activity. Expression of CD8β, FoxP3, IL10 and HPRF genes was measured by PCR.
Immunohistochernistry were performed on in paraffin sections for the detection of
CD8 and FoxP3. Statistical analysis was done with Mann–Whitney U test and Wil-
coxon matched-t test.
Results. Lymphocytes from 52 fresh liver biopsies displayed similar distributions of
T (CD3), NKT (CD3, CD56) and NK (CD56) among CD45 cells by flow cytometry multi
parametric analysis, whatever the IL28B genotype of the patients. Strikingly, higher
degranulation activity, revealed by CD107a surface expression, was observed in T
(p < 0.0001), NKT (p = 0.002) and NK cells (p = 0.015) of patients with CC genotype
(n = 17) compared to patients with CT or TT genotypes (n = 35); patients with CC
 genotype displayed two fold higher degranulation activity than patients with CT
 genotype in T (p = 0.001), NKT (p = 0.002) and NK cells (p = 0.011); no significant dif-
fERENCE was observed between patients with CT and TT genotypes. Sections of liver
from 19 patients showed the frequency of CD4-FoxP3 lymphocytes two fold higher
(p = 0.004) in patients with CC genotype (n = 11) as compared to patients with CT
 genotype (n = 8) supporting the presence of Treg. Previous study demonstrated that the
ratio between the number of CD68 cells and FoxP3 cells in parenchymatous
necro-inflammatory areas is maintained in the early stage of the chronic hepatitis
(3). This is found only in patients with CC genotype, whereas this ratio is reduced
in patients with CT genotype due to lower number of FoxP3 cells. Transcriptional
analyses confirmed these data and further showed two strong correlations between:
one between FoxP3 and CD8β, another between FoxP3 and IL-10 expressions in
patients with CC genotype.
Conclusion. Collectively these data provide new insights into the role of IL28B poly-
morphism related to SVR in treatment of HCV infected patients. CC genotype, which is
linked to good response, is associated to higher efficiency of effector lymphocytes (T,
N and NKT) of the liver. The liver immune response appears tightly regulated as sug-
gested by the links between CD8 cells and CD4-FoxP3 cells, and between IL10 and
FoxP3 gene expression.
Disclosure of interest: None declared.

http://dx.doi.org/10.1016/j.cyto.2012.06.226

P135
Defining the intracellular innate immune synapse
S.M. Horner 1, A. Krasnoselsky 2, C. Willians 2, M.G. Katze 2, M. Gale Jr. 1, 1 Immunology,
University of Washington, Seattle, United States, 2 Microbiology, University of Washington, Seattle, United States

Introduction. - Innate immunity to RNA virus infection is triggered when the cyto-
solic pathogen recognition receptor RIG-I engages viral RNA in infected cells. RIG-I
pathway signaling is transmitted by the RIG-I adaptor protein MAVS, which resides
on mitochondria, peroxisomes, and the mitochondrial-associated membrane (MAM), a
distinct membrane that links ER to mitochondria. During RNA virus infec-
tion, MAVS is recruited into the MAM where it binds MAVS and drives the actions
of a signalosome that mediates downstream induction of antiviral, proinflammatory,
and immunomodulatory genes that impart control of infection and immunity. MAVS-
tethering to mitochondria and peroxisomes coordinates MAVS localization to form a
signaling synapse between membranes. The importance of the MAM within this
“innate immune synapse” is highlighted by the fact that the hepatitis C virus (HCV)
NS3/4A protease cleaves MAVS on the MAM, but not the mitochondria, to evade
immunity.
Methods. To identify the components that regulate formation and function of the
innate immune synapse and the MAVS signalosome, we characterized the proteome
of MAM, ER and cytosol subcellular fractions from uninfected cells and from cells
with either chronic (HCV) or acute (Sendai) RNA virus infections.
Results. Comparative analysis of protein trafficking dynamics during both chronic
and acute infection reveals differential protein profiles in the MAM compartment
under RIG-I pathway activation. We also identified molecules recruited to the MAM
in both chronic and acute RNA viral infections representing proteins that drive immu-
nity and/or regulate viral replication.
Conclusion. Our proteomic analysis reveals dynamic cross-talk between subcellular
components during both acute and chronic RNA virus infection, and demonstrates the
importance of the MAM as a central platform that coordinates innate immune sig-
naling to initiate immunity against RNA virus infection.
Disclosure of interest: None declared.

http://dx.doi.org/10.1016/j.cyto.2012.06.227

P136
Combined action of type I and type III IFN restricts initial replication of SARS-
coronavirus in the lung but fails to inhibit systemic virus spread
T. Mahlahoko1, D. Ritz2, L. Enjuanes1, M.A. Müller2, C. Drosten2, S. Staeheh3
1 Department of Virology, University of Freiburg, Freiburg, Germany, 2 Institute of Virology,
University of Bonn Medical Center, Bonn, Germany, 3 Department of Molecular and Cell
Biology, Campus Universidad Autonoma de Madrid, Madrid, Spain, 4 Institute of Virology,
University of Freiburg, Freiburg, Germany

Introduction. STAT1-deficient mice are more susceptible to infection with SARS-
Coronavirus (SARS-CoV) than type I IFN receptor-deficient mice. The increased sus-
cceptibility of STAT1-deficient mice is potentially due to the lack of functional type
III IFN (IFN-λ) signaling.

Disclosure of interest: None declared.

http://dx.doi.org/10.1016/j.cyto.2012.06.227
Methods. We used mice lacking functional receptors for both type I and type III IFN (dKO) to evaluate the possibility that type III IFN plays a decisive role in SARS-CoV protection.

Results. We found that viral peak titres in lungs of dKO and STAT1-deficient mice were similar, although significantly higher than in wild-type mice. The kinetics of viral clearance from the lung was also comparable in dKO and STAT1-deficient mice. Surprisingly, however, infected dKO mice remained healthy, whereas infected STAT1-deficient mice developed liver pathology and eventually succumbed to neurological disease.

Conclusion. Our data suggest that the failure of STAT1-deficient mice to efficiently control initial SARS-CoV replication in the lung is due to impaired type I and type III IFN signaling, whereas the failure to control subsequent systemic viral spread is due to unrelated defects in STAT1-deficient mice.

Disclosure of interest: None declared.

http://dx.doi.org/10.1016/j.jcyto.2012.06.228

P137

Type III interferon impairs bacterial clearance through PDCD4 regulated inflammatory cytokine production

T.S. Cohen, A. Prince, Columbia University, New York, United States

Introduction. The balance between pro and anti-inflammatory signaling in innate immune responses to bacterial infection is especially critical in the lung. Airway epithelial cells, in addition to resident and recruited cells of immune origin, participate in what must be coordinated proinflammatory signaling in response to inhaled pathogens. The type III interferons are especially important in pulmonary infection, produced in response to viral infection and bacterial PAMPs. IFN-λ is activated by and induces NF-kB signaling and promotes expression of Th1 cytokines. We postulated that common respiratory pathogens, Staphylococcus aureus and Pseudomonas aeruginosa, would stimulate an IFN-λ response and that this would have an important effect on bacterial clearance from the airway.

Methods. To establish that bacterial components can stimulate IFN-λ, we measured the induction of IFN-λ by RT-PCR in murine BMDCs. Biological significance of IFN-λ induction was determined by comparing the ability of wt C57BL/6 and IL-28R−/− mice (lacking the IFN-λ receptor) to handle an intranasal inoculation of 10^5 cfu of either PAK or USA300.

Results. In response to either P. aeruginosa PAK or USA300 MRSB on BMDCs there was a 10-fold increase in IFN-λ transcript by 4 h post infection which persisted for up to 8 h; in contrast to the 1000-fold induction of IFN-β by both organisms. The IL-28R−/− mice had significantly improved clearance of either pathogen from the airway and lung tissue (P < 0.05 for each). Consistent with the expected participation of IFN-λ in NF-kB signaling, we measured decreased expression of KC, TNF and GM-CSF and increased IL-10 in the IL-28R−/− BAL (P < 0.05), although there were no significant differences in the populations of immune cells (neutrophils, DC, macrophages) recruited to the airways of the wild type or IL-28R−/− mice. Deleterious effects of IFN-λ on bacterial clearance were confirmed by exogenous treatment of wt mice with IFN-λ that significantly diminished clearance of both organisms. To address how IFN-λ signaling interferes with bacterial clearance we examined the role of PDCD4 (programmed cell death protein 4) in this model system. PDCD4, which is negatively regulated by IFN-λ signaling, is an important regulator of the Jak-STAT pathway. Upregulation of USP18 results in a long-lasting suppression of IFN-stimulated genes. We further analyzed the subcellular localization of USP18, and its potential substrates, including β-catenin. Microarray experiment was performed to determine the target genes of β-catenin.

Results. We found that IFN-γ markedly increased IFN-γ-regulated USP18 expression and nuclear accumulation. Inhibition of USP18 using pharmacological inhibitors or RNA interference reversed IFN-γ suppression of HES1 and XIAP, suggesting that USP18 mediates the downregulation of inhibitory gene expression by IFN-γ, β-catenin, a major substrate of USP18, is a transcription factor recently implicated in induction of anti-inflammatory mediator IL-10 in murine dendritic cells [3]. However, effective knockdown of β-catenin by siRNAs had little effect on IFN-γ-mediated gene expression, as assessed by genome-wide microarray analysis. In contrast, we found that the expression of AP-1, another target of USP18, as well as its nuclear accumulation was significantly suppressed by IFN-γ.

Conclusion. We provided several lines of evidence that USP18 was the major mediator in the crosstalk between IFN-γ and IFN-λ signaling. AP-1 transcription factor, but not β-catenin, was the major target of USP18 in this scenario. These findings yield insight into mechanisms of crosstalk between IFN-γ and IFN-λ that are important for the orchestration of cytokine production and inflammation.

Disclosure of interest: None declared.

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http://dx.doi.org/10.1016/j.jcyto.2012.06.230

P139

Continuous exposure to PEG-IFN-Alpha only transiently activates JAK-STAT signalling in human liver

Z. Makowska ,1 M.T. Dill,1 J.E. Vogt 2 M. Filipowicz 1, L. Terracciano 1, V. Roth 2 ,M.H. Hein 2 1 Biomedicine, University of Basel, Switzerland, 2 Computer Science, University of Basel, Switzerland, 3 Pathology, University Hospital Basel, Basel, Switzerland

Introduction. IFN-α signals through the JAK-STAT pathway to induce expression of IFN-stimulated genes (ISGs) with antiviral functions. Pegylated forms of IFN-α are currently used in clinical use for treatment of chronic hepatitis C virus infection. PegIFN-α2 is shown increased anti-hepatitis C virus efficacy compared to nonpegylated IFN-α. This has been attributed to the significantly longer plasma half-life of the pegylated form. However, the underlying assumption that persistently high plasma levels obtained with pegIFN-α therapy result in ongoing stimulation of ISGs in the liver has never been tested. In the present study we there-

Disclosure of interest: None declared.

http://dx.doi.org/10.1016/j.jcyto.2012.06.229