BELVIR Workshop on IFN

PARTICIPANTS

Seminar

> 20 members of BELVIR labs attended the seminar on Thursday morning
+ extra persons

Workshop

Tim Dierckx	KUL Debyser
Olivier Vandersleyen	Coda-Cerva
Fiona Ingrao	Coda-Cerva
Thu Giang Nguyen	Coda-Cerva
Jochen Lamote	U-Ghent
Niraj Mishra	KUL Neyts
Joanna Zmurko	KUL Neyts
Yuan Gao	ULG Vanderplasschen
Lea Morvan	ULG Vanderplasschen
(Nicolas Gillet) Excused	ULG Willems

Contributors from host lab UCL Michiels:

Michael Peeters

Melissa Drappier

Aurélie De Cock

Muriel Minet

Stephane Messe

IFN workshop

February 12-13th, 2014

All welcome

Auditorium de Visscher,
de Duve Institute,
74 avenue Hippocrate, B-1200 Brussels

9h00-9h30: Registration

9h30-9h55: Welcome session and Introduction on IFN – Thomas Michiels

9h55-10h20: RNase L activity and its inhibition by Theiler's virus - Melissa Drappier

10h20-10h40: Activation and inhibition of PKR – Fabian Borghese

10h40-11h00: Coffee Break

11h00-11h25: HIV triggers a cGAS-dependent type 1 IFN response in primary CD4+ T cells that is regulated by Vpu and Vpr - Jolien Vermeire* (U Ghent)
* kindly replaced by B. Verhasselt for medical reasons.

11h20-11h45: Methods to measure IFN – Thomas Michiels

12h00-13h00: **Seminar**

Fine control of type I interferons signaling : relevance in health and disease

Sandra Pellegrini, Pasteur Institute, Paris

13h00-14h30: Lunch

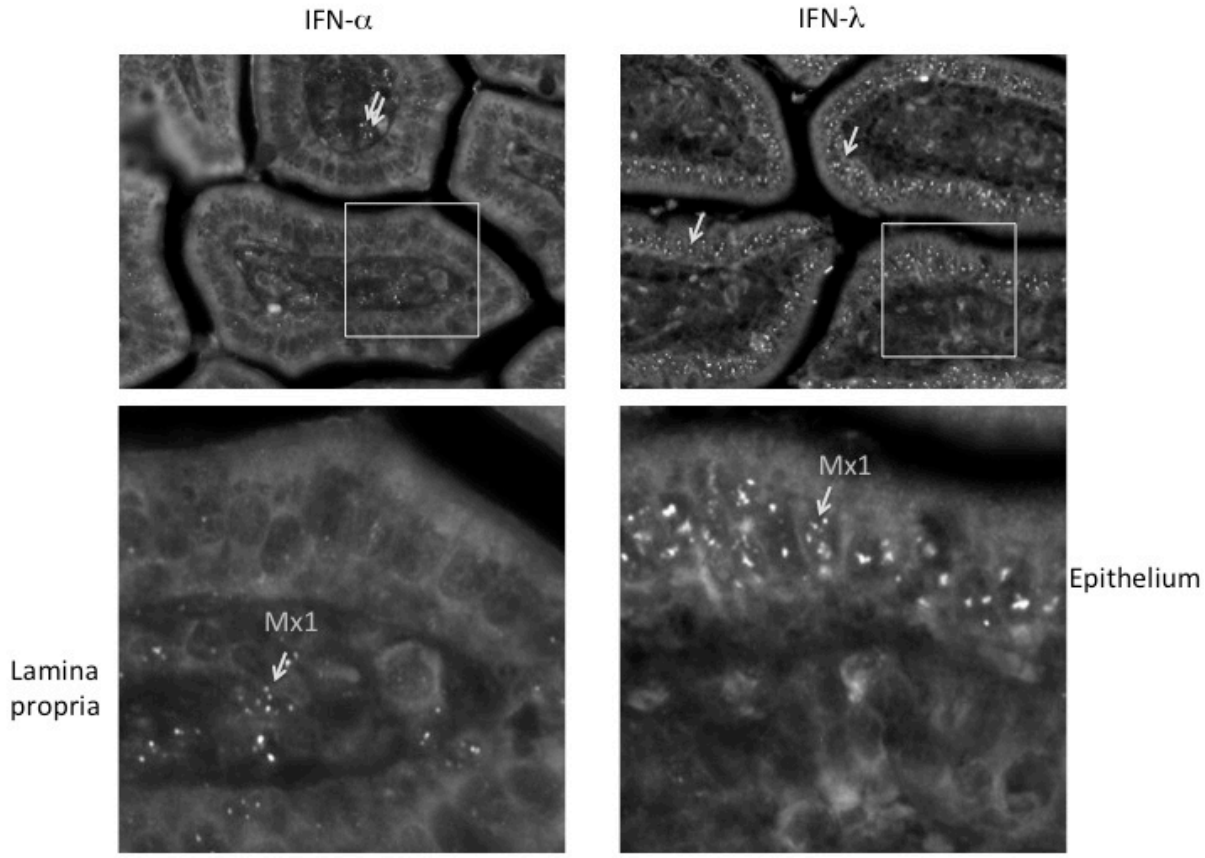
AFTERNOON and FRIDAY: PRACTICAL TRAINING

Mx immunolabeling

Thursday :
- deparaffination : successive bath of Xylene, Ethanol -> H_2O to rehydrate the sections
(5 min each in : Xylene, Xylene again, Ethanol 100, Ethanol 95, Ethanol 70, Ethanol 50, H_2O)
- epitope retrieval : 30 min. 95°C in citrate buffer pH 4.5
- permeabilisation : 5 min in 0,2 % Triton X-100
- blocking : incubation in 100 ul of blocking buffer (goat serum 1/50)
- Primary antibody : diluted 1/150 in blocking solution (overnight 4°C) – wet room(box)

Friday :
- wash : 3 x 5 min in PBS – 0,1% Tween-20
- incubate 1h with secondary antibody : Alexa488-conjugated anti-rabbit antibody (1/400).
- (optional) add Hoechst nuclear stain 1 ug/ml
- wash : 3 x 5 min in PBS – 0,1% Tween-20

Mount with a hydrophilic mounting medium (Mowiol).
Observe under the fluorescent microscope.



IFN-α induces Mx1 expression in the lamina propria
IFN-λ induces Mx1 expression in the epithelium.

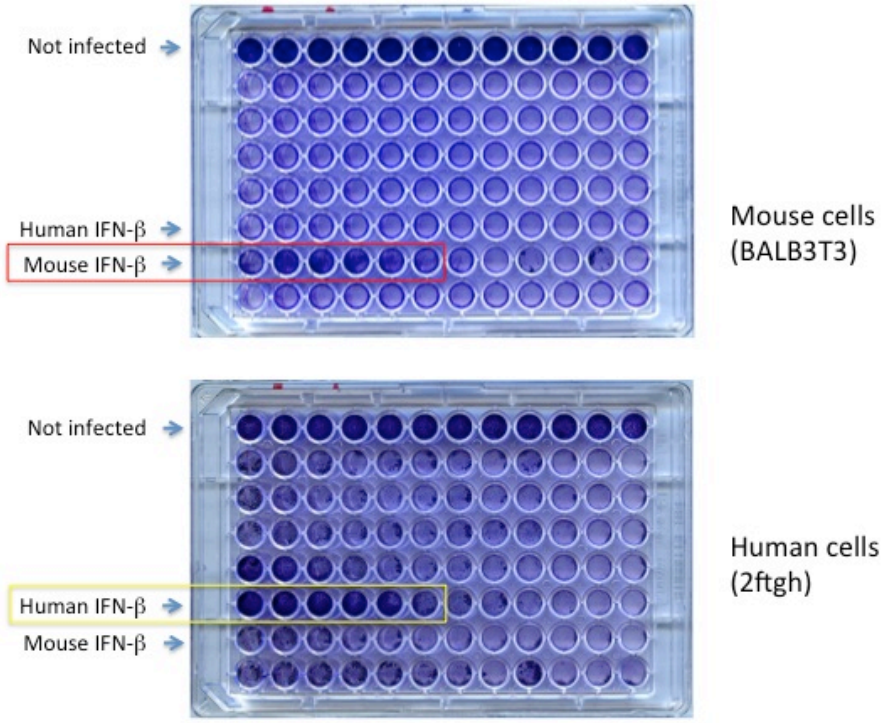
IFN assay

Tuesday
- Cells : BALB/3T3 (mouse fibroblasts) and 2ftgh (human fibrosarcoma) cells
- seed 8000 cells per well in 150 ul of medium (DMEM-10% FCS) of in 96-well plates

Wednesday
- Add 150 ul of medium containing IFN (or control) in the first raw of wells.
- make 2-fold dilution series by pipetting 150 ul from well to well (change tips)
- control cells treated with culture medium
- positive control with reference IFN

Thursday
- infect wells with 0,5 PFU per cell of Mengovirus (EMCV) or VSV-GFP
- control non-infected wells

Friday
- observe cytopathic effect under the light microscope
- observe VSV-GFP-infected cells under the fluorescence microscope.
- fix the cells by adding 40 ul formaldehyde 37% per well
- after > 2h, discard the supernatant and color the plates with violet cristal.



RNase L activity

Wednesday
- co-transfection 6-cm dishes (27 cm^2) of 293T cells (60-70% confluence) with plasmids expressing
    muRNase L
    muRNase L and L*

Thursday
- wash cells with ice-cold PBS
- lyse cells
- scrape cells and centrifuge the lysate
- divide the lysate supernatant into aliquots
- add 2-5A
- Prepare RNA after 5 or 20 min incubation

RNA prep : (according to Chomczynski and Sacchi, 1987)
- add 400 ul of D solution (Guanidine Thiocyanate 4M, Na citrate 25mM, pH7 ; N-lauroylsarcosine 0.5% ; β-mercaptoethanol 0.1M)
- mix vigorously
- add 500 ul of phenol+Na acetate and mix vigorously
- add 125 ul of $CHCl_3$ and mix vigorously
- centrifuge for 20 min at 4°C
- collect 400 ul supernatant and add 400 ul isopropanol -> Mix -> incubate ON at -20°C

Friday
- centrifuge RNAs for 20 min at 4°C
- wash pellets with icecold Ethanol
- resuspend RNAs in RNase-free water
- run on standard 1 % agarose gels

Observation : unfortunately, degradation was hardly visible in this experiment.

RT-qPCR for IFN and ISG mRNA expression

Miqe guidelines about design and report of qPCR reactions
http://miqe.gene-quantification.info/

Web site for primer design
http://primer3plus.com/cgi-bin/dev/primer3plus.cgi
Several other web sites exist to define primers and probes.
Note that Taqman probes may be chosen with a higher melting temp (5 to 10°C higher than primers Tm)

Thursday
- mix the qPCR master mix (Sybr Green) with primers

    - Primers for IFN-beta (mouse)
        `Fwd : ATG AAC AAC AGG TGG ATC CTC C`
        `Rev : AGG AGC TCC TGA CAT TTC CGA A`

    - Primers for Oasl2 (mouse)
        `Fwd : GGA TGC CTG GGA GAG AAT CG`
        `Rev : TCG CCT GCT CTT CGA AAC TG`

- prepare the plasmid dilutions for the standard curve

- prepare the RT-qPCR plate :
    - 5 ul of diluted cDNA
    - 20 ul of qPCR Mix
- controls include :
    - non-infected samples
    - cDNA samples generated without RT
    - H_2O

- Run (note that more recent enzymes allow to do much faster runs)

    95°C – 10 min (to activate « hot-start » Taq polymerase)

    -> 40 cycles :
        95°C – 15 sec : denaturation
        60°C – 1 min : primer annealing – elongation – fluorescence measurements

    -> 30 x 10 sec steps of + 0,5°C : melting curve
        (melting curve allows to assess the specificity of the products
        when Sybr green is used instead of Taqman probes)

Friday
- analysis of the data