

Effect of humanin on inflammation in transgenic mice

Polish-Norwegian Research Fund (OPI/EEA)

Rune Blomhoff group

Torunn E Tjelle

Task 5

Measurements of NF-kB expression response to HNs and/or L-arginine and/or FFA in luciferase transgenic mice in proinflammatory conditions

Model system

NFkB transgenic mice

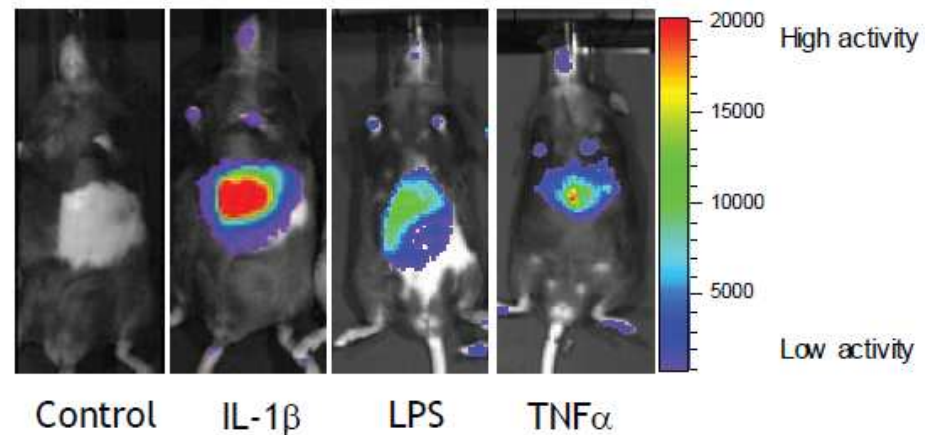
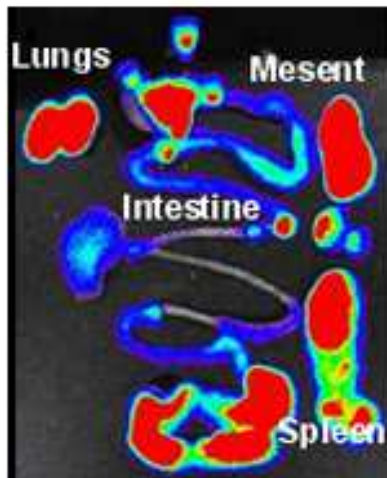
- Blomhoff group (Harald Carlsen) has developed a series of mice expressing luciferase under the control of different gene regulatory elements
- The first made, was the mice expressing luciferase under the control of the transcription factor NFkB



Model system

NFkB transgenic mice

- Injecting luciferin (substrate of luciferase), mice can be imaged *in vivo* (CCD camera) and luciferase activity quantitatively determined as a measure of the activity of NFkB
- NFkB plays a crucial role in regulating the expression of genes involved in inflammatory responses

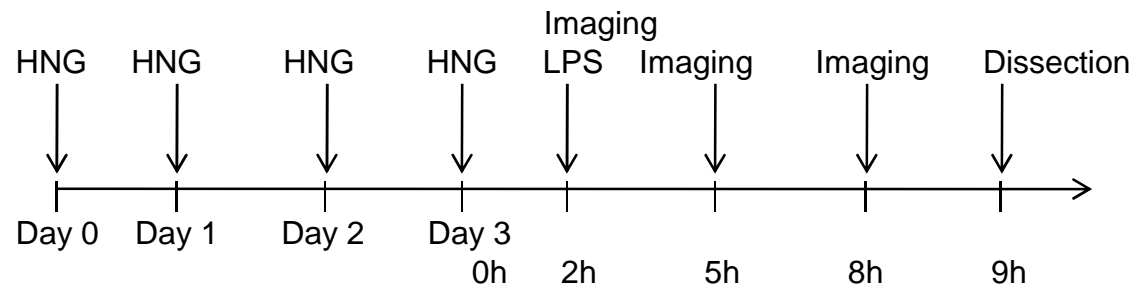


NF- κ B stimulation by classical stressors

IL-1 β (4 ug/kg), lipopolysaccharide (LPS-3 mg/kg) or TNF α (4 ug/kg) were injected i.v. (IL-1 β) or i.p. (LPS and TNF α). Picture is taken 4-5 hours after injections.

Protocol

- Animals
 - 8 week old NFkB and wild type (C57Bl6/CBA) mice
- Treatment
 - Injection of HNG (250 ng/mouse) once a day for 4 days
 - Challenge with LPS
 - *In vivo* imaging after 3h and 6h
 - Dissection after 7h



Protocol

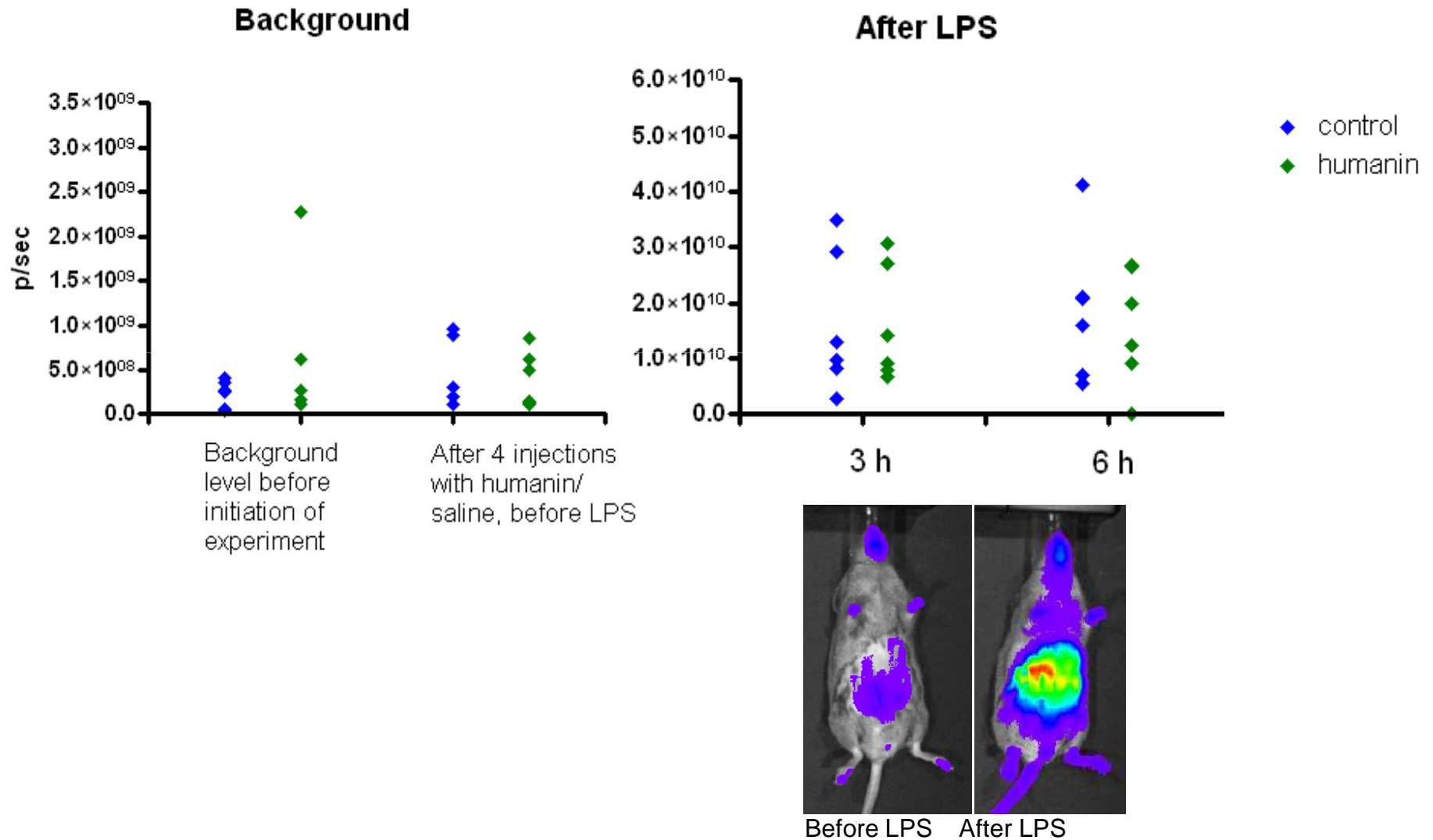
- Groups

	Group	Treatment	N
1	TG mice: Control	4 x saline, LPS	6
2	TG mice: Humanin	4 x humanin, LPS	6
3	WT mice: Control	4 x saline	6
4	WT mice: Humanin	4 x humanin	6
5	WT mice: Control + LPS	4 x saline, LPS	6
6	WT mice: Humanin + LPS	4 x humanin, LPS	6

- Organs for analyses

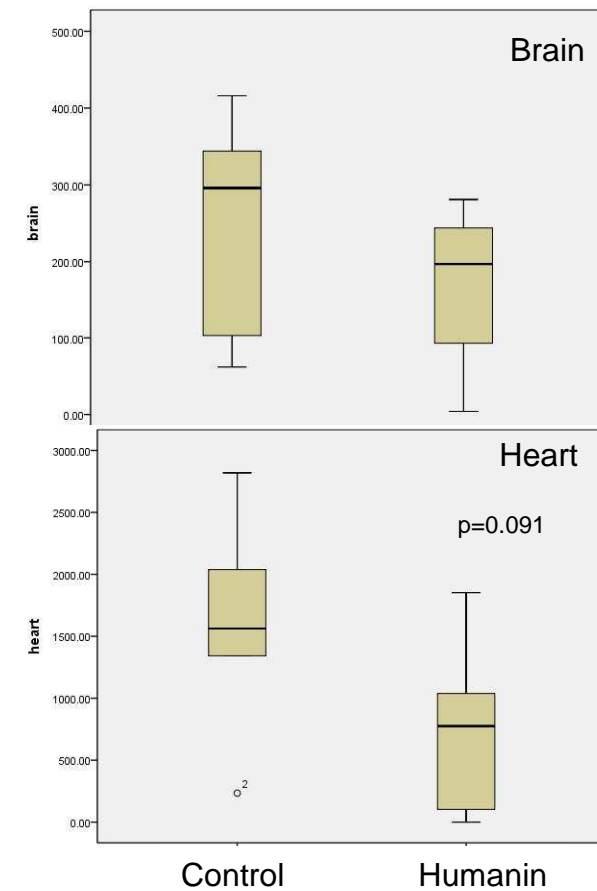
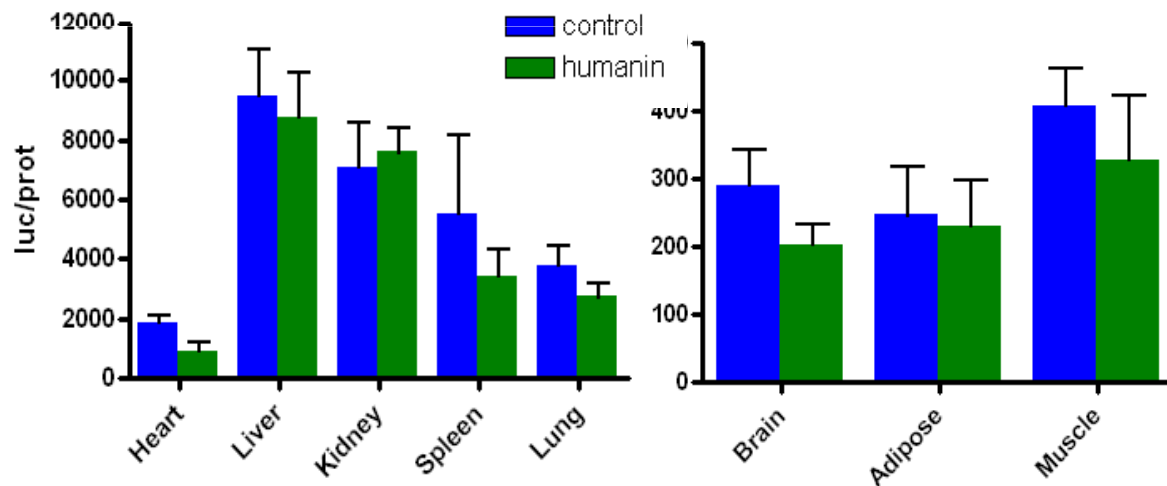
- snap freeze for luciferase activity
- transfer to RNAlater for mRNA analyses
- transfer to formalin for immuno histochemistry
- brain, heart, liver, adipose, muscle, lung, spleen, kidney, blood

In vivo imaging



Luciferase activity in organs

- Measured in homogenates of organs



Remaining analyses

- Serum from the wt mice (4 groups) will be analyzed for levels of cytokines
 - IL-6, IL-1b, TNF-a, IL-10, GMCSF, KC
- Assay: "Milliplex" from Millipore

Summary

- Mice were successfully injected with humanin, challenged with LPS and NFkB activity was measured as luciferase activity
- The *in vivo* imaging could not reveal any suppression of luciferase (NFkB) activity in mice treated with humanin
- The *in vitro* measurements of luciferase in different organs did not show any differences between the humanin and control group
- Cytokine levels in serum is yet to be analyzed

Remaining experiment

Hypothesis

Humanin will inhibit stressor induced NFkB activity in cells

- NFkB-luciferase stably transfected U937 cells will be used as there are no available brain cell lines with NFkB-luciferase expression
 - HNM, HNG, 13ThrHN10b will be incubated with cells for 24 h
 - Next day, add fresh peptides
 - 1 h later, add LPS for immune challenge
 - Measure luciferase activity after 3-6 h

Publication

- If positive results from cells, an outline of a paper could be:
 - Humanin inhibit inflammation, measured as inhibition of NFkB activity in cells
 - Different humanins
 - Different stressors
 - The principle was tested in an NFkB-transgene mouse model but the same effect was not reproduced *in vivo*
 - Results from cytokine levels in plasma
 - (Results from gene expression in brain? – or separate pub?)
 - (Results from gene expression in cells? – or separate pub?)

