EADGENE
European Animal Disease Genomics Network of Excellence for Animal Health and Food Safety

Mastitis Workshop: Biology and Meta-analysis
15th October 2009, Muséum National d'Histoire Naturelle, Paris, France

Dissection of pathogen-specific signal transduction in Mammary Epithelial Cells

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Central problem: chronic mastitis

Why can some pathogens manage to persist in the udder as latent infection, but others not?

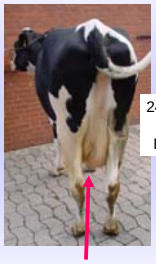
Some Questions:

Do they elicit an immune alert?

Are they recognized by the adequate receptors?

Which component of the host's immune defense do they inactivate?

Mastitis induces changes



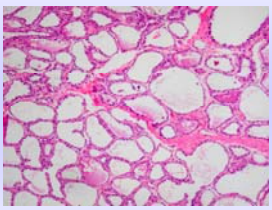
24 h p.i. with 500 CFU E. coli1303

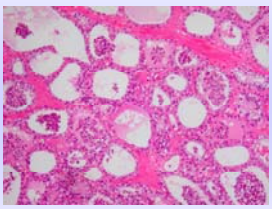
Healthy: 50 l milk / d

Acute Mastitis: <30% of secretion (~no milk!)

Infection recruits immune cells

MEC | **MEC, Granulocytes…**





healthy

24h p.i.
500 CFU E. coli 1303

MEC outnumber by far any other cell in the healthy milk parenchym!

MEC is a sentinel and effector cell of Innate Immunity

TLR2:

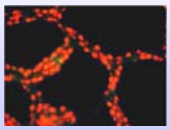
Toll-like-Receptor 2

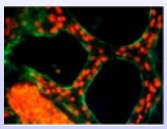
Polyclonal Antiserum against Bovine TLR2

Wei Yang, FBN Dummerstorf

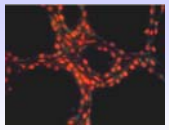
Perceives pathogen

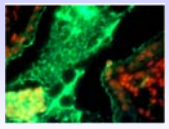
control | 24 h infected





α TLR2





α LAP

LAP:

Lingual Antibacterial Peptide

Polyclonal Antiserum against Bovine LAP,

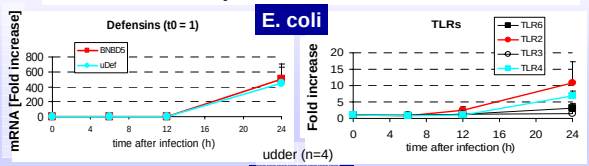
Tom Wheeler, Adrian Molenaar AgResearch

Kills pathogen

The phenomenon:

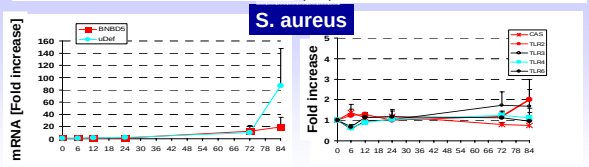
Pathogen-specific modulation of the immune response in the udder

E. coli



udder (n=4)

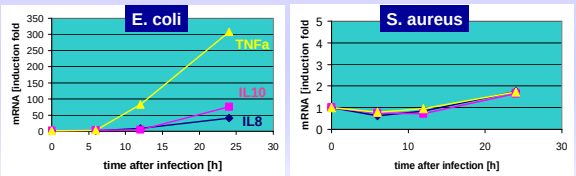
S. aureus



E. coli* alerts faster and stronger than *S. aureus

Cytokine-Induction *in vivo in the udder*: *E. coli* induces, *S. aureus* ~not!

4 cows, infected with 500 CFU E. coli or 10 000 CFU S. aureus
qRT-PCR



Inadequate recognition of *S. aureus* pathogens?

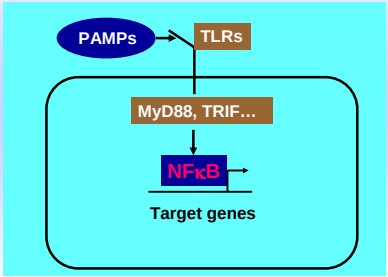
Analyse TLR2 activation by *S. aureus*!

Mounting the Immune Defence, our concept: Pathogen-dependent signal transduction in MEC

TLRs
Pathogen-specific receptors

PAMPS
Pathogen Associated Molecular Patterns

TF
Transcription-factor



our Paradigms:

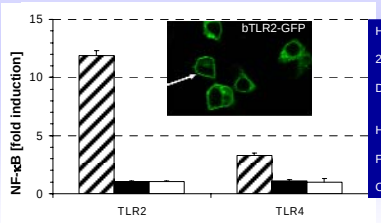
Defense: β-Defensins (LAP)

Sentinel: IL8, TNFα

Which TFs activate sentinel- and effector genes of the immune defence in MEC?

Reconstitution of TLR-mediated signal transduction in HEK293

- **Co-transfect** into HEK293 a NF-κB reporter together with expression constructs of bovine TLRs
- **stimulate** (pathogen or PAMP),
- **Verify** TLR signalling by blocking with DN-MyD88 or dual DN-MyD88-DN-TRIF



HEK, Human Embryonic Kidney 293 cells: no own TLRs!

DN, transdominant negative

Hatched: positive control

Filled: 10x DN-MyD88

Open: 10x DN-dual

***E. coli* activates both TLRs, DN-factors block signal: system functions**

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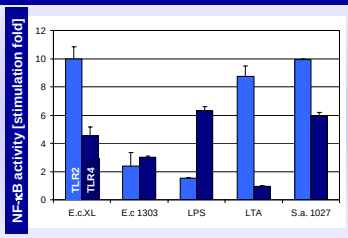
E. coli and *S. aureus* activate TLRs

TLR expression in HEK293, stimulation with heat killed bacteria or PAMPs



E. c.:
XL, XL-1 blue
1303, mastitis pathogen

S.a. 1027,
mastitis pathogen

LTA, LTA from 1027

LPS, LPS from 1303

Bovine-pathogenic *S. aureus* is recognized

Will both pathogens activate the immune response in MEC?

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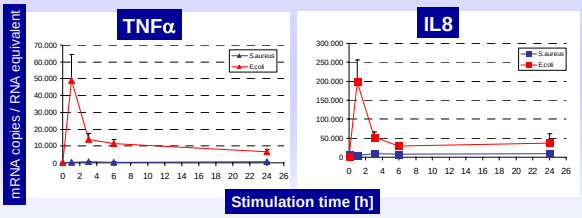
E. coli elicits a much stronger cytokine response in MEC than *S. aureus*

Challenge of pbMEC, 10^7/ml heat killed *E. coli*$_{1303}$ or *S. aureus*$_{1027}$
qRT-PCR, three biological replica



Does the MEC perceive the *S. aureus* presence at all?

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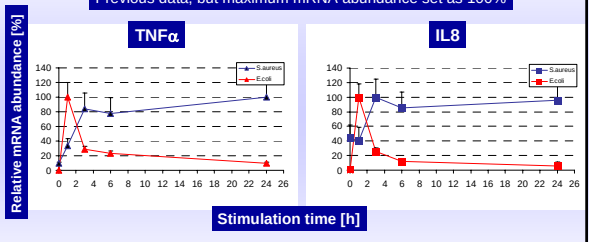
MEC recognize the presence of *S. aureus*, as quickly as *E. coli*

Previous data, but maximum mRNA abundance set as 100%



***S. aureus* elicits a significant response, but in magnitude only 2% of the E. coli output.**

Why?

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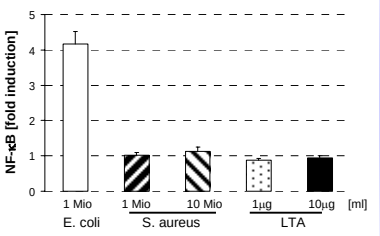
S. aureus fails to activate NF-κB in MEC

Transfect NF-κB reporter into pbMEC, stimulate o/n

E. coli: >20 Isolates checked, including ~10 strains from persistent mastitis



S. aureus 6 Isolates checked

ATTENTION: ***S. aureus*** **activates** NF-κB in ΦMo, PBMC!

Failure to activate NF-κB in MEC is a severe impairment to mounting an immune response in the udder.

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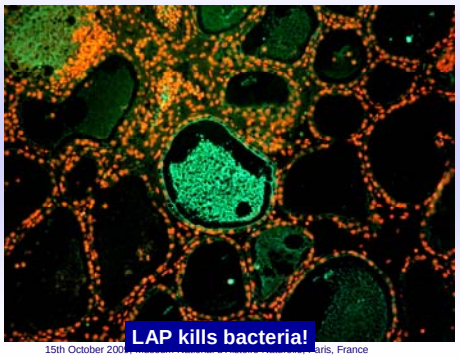
LAP, Lingual Antibacterial Peptide:
Effector gene of immune defence



LAP kills bacteria!

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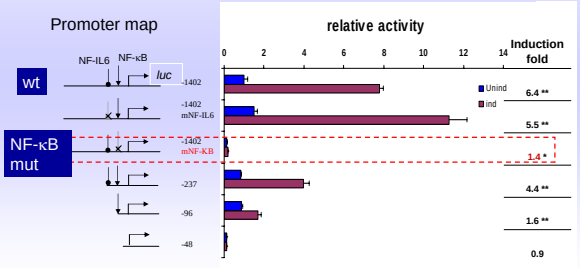
Activating the defence: NF-κB is the key-factor

Method: Analyse LAP promoter function in reporter gene assays; Delete or mutate 1 (!) base from a relevant site



Which NF-κB factor matters?

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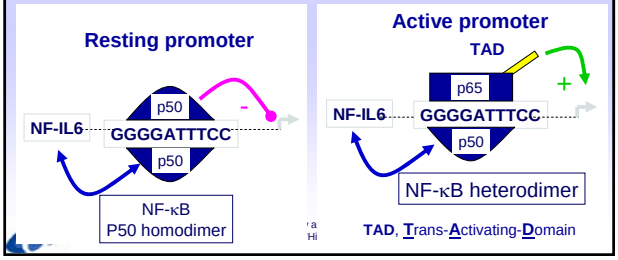
NF-κB: Ubiquitous regulator of immune gene expression

- NF-κB activates >>600 immune relevant genes
- Consists of five different factors,
- Inactive in the resting cell, blocked by IκB

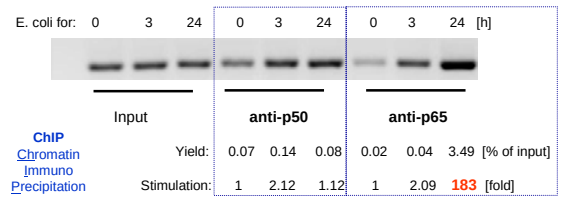
Alternate composition of NF-κB:



TAD, **T**rans-**A**ctivating-**D**omain

NF-κBp65, but not NF-κBp50 is selectively recruited to stimulate LAP-expression

ChIP, q-PCR, pbMEC, heat killed E. $coli_{1303}$



ChIP Chromatin Immuno Precipitation

Yield:	0.07	0.14	0.08	0.02	0.04	3.49	[% of input]
Stimulation:	1	2.12	1.12	1	2.09	183	[fold]

Is this true?

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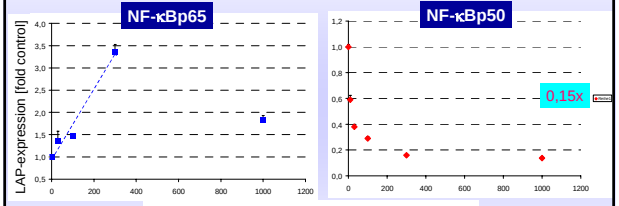
Differential effects of NF-κB factors on LAP-promoter activity

CMV-expression constructs of bovine p50, p65; HC-11 cells; LAP-reporter



NF-κBp65 triggers LAP expression!

NF-κBp50 represses LAP!

What controls NF-κBp65 recruitment onto the LAP-promoter? This principal pulls the trigger!

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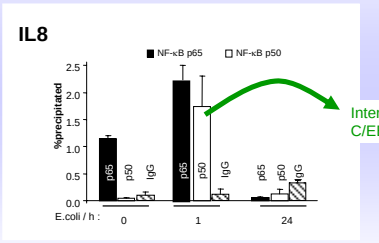
Will NF-κB p65 also drive the expression of "Immediate-Early" sentinel genes?

pbMEC, Stimulation 10^7/ml E.coli$_{\text{i303}}$(inaktiviert), CHIP

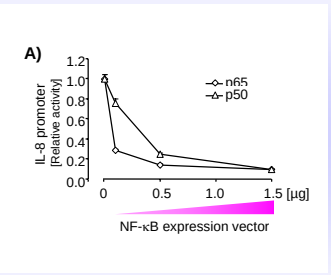


Interacts with C/EBP-factors

Result: C/EBP Factors activate IL8 promoter

Does NF-κB p65 block IL-8-expression in quiescent MEC?

NF-κB blocks expression of IL8 in MEC, but not in HEK293

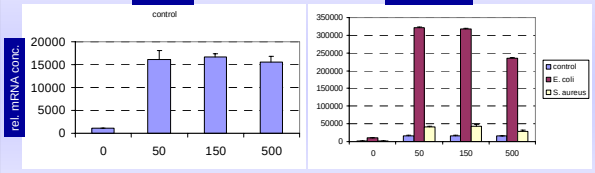


Also true in vivo?

NF-κB inhibitor(s) enhance IL8 expression

pbMEC, PDTC: from -1 h to 6h stimulation with 10^7E. coli/ ml

Control | Stimulation



µ Mol PDTC

NF-κB blockage causes overshooting IL-8 induction!

Essence 1:

All these defense mechanisms are regulated in a cell-type specific fashion.

Hence, immune defense is always also

Patho- & Gene-specific!

Essence 2: Activation of the Immune Defense in the Udder

NF-κBp65

L A P

Also : iNOS SAA Haptoglobin…

Chromatin remodeling at the LAP-promoter!?!

Essence 3: Pathogen-specific modulation of the response

E. coli **[Acute mastitis]**

Instantaneous **Blitz!**

TNFα IL8 CCL20 CCL5

S. aureus **[chronic mastitis]**

Blocks or fails to activate **Blitz!**

No early Cytokine respose **No** **NF-κB** in MEC **Weak** **TNFα**

Thanks!

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Thanks for your attention

Basis for systematic analyses: Experimental Mastitis

Example: **Infect healthy heifers** (4th month into lactation) with pathogens,
E. coli$_{1303}$ or **S. aureus**$_{1027}$
keep 1 quarter for control, infect the others consecutively

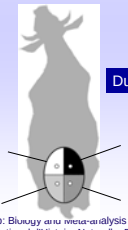
Micro array

18000 genes

Take aslo samples from:

Lymphknodes,
Blood
Milk cells…

N = 4



Duration of infection

Control

24 h

6 h

12 h

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