



Blood transcriptome kinetic response to LPS in pigs

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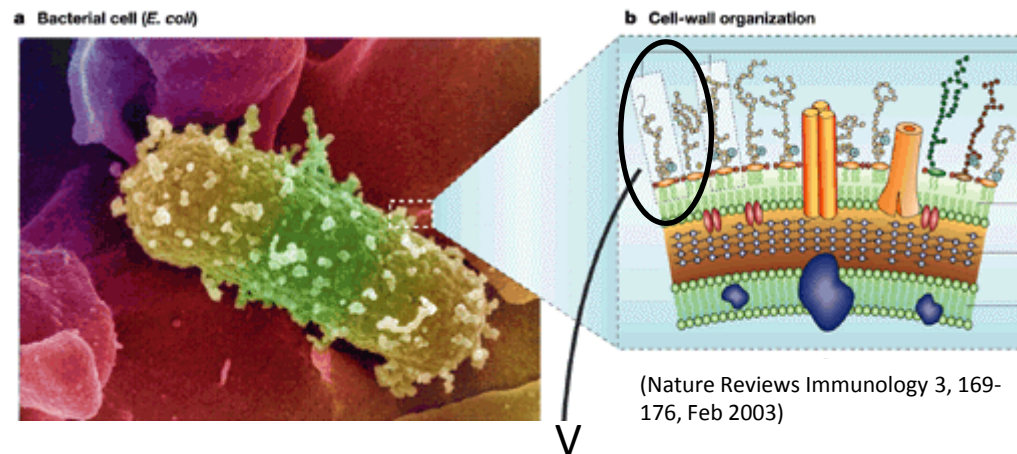
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Response to inflammation in pigs

Inflammatory model using *E.coli* lipopolysaccharide (LPS):



(Nature Reviews Immunology 3, 169-176, Feb 2003)

LPS

- **Immune response**
(inflammatory cytokines, acute phase proteins, fever)
- **Metabolic response**
(hypoglycaemia, hyperlipidemia)
- **Endocrine response**
(stress hormones: catecholamines, cortisol)

Aims



Can we have a global view of the response to inflammation using **blood transcriptome**?

- Can we observe the time-response to LPS?
- Can we observe not only the immune but also the endocrine and metabolic responses ?

Blood mRNA => **immune cells**: lymphocytes (T, B, NK), polynuclear cells, monocytes

Material and methods



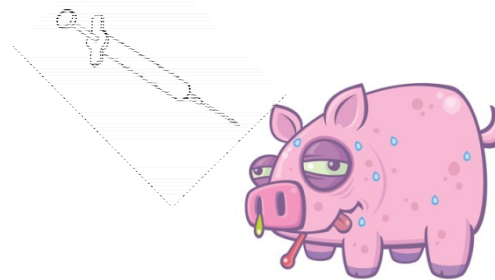
Jugular catheters by surgery



i.v. LPS
(O55:B5, 15 μ g/kg)
in pigs fasted for 6 h



Blood sampling

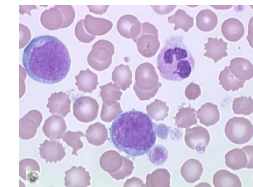


Measurements

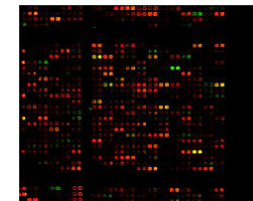


- Blood sampling at 4 time points (-30min, 1h, 4h and 24h post LPS)

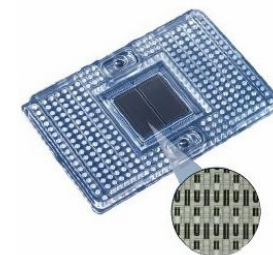
- Differential blood cell count



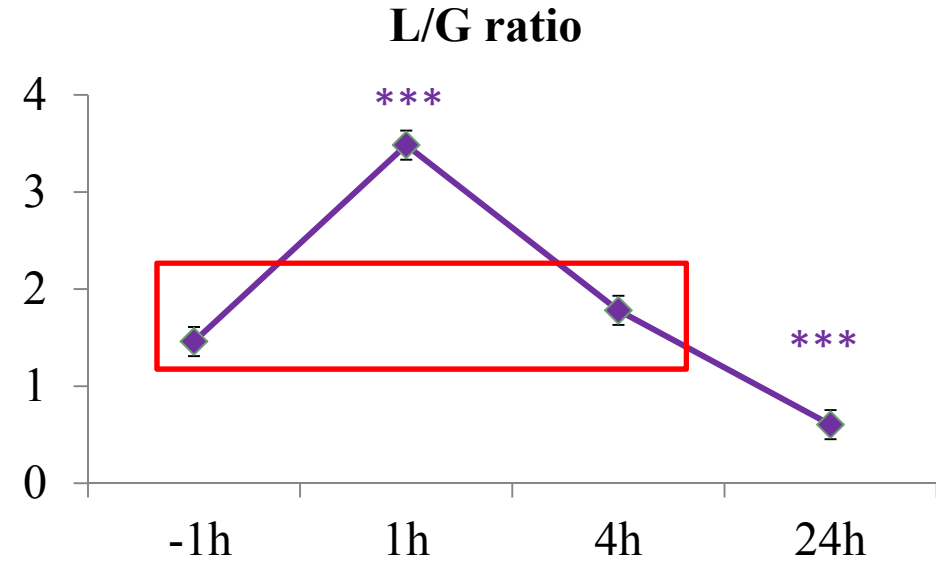
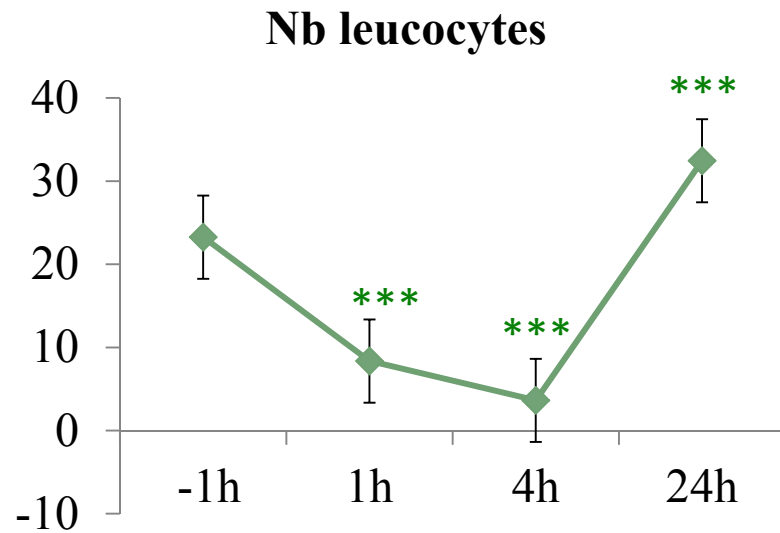
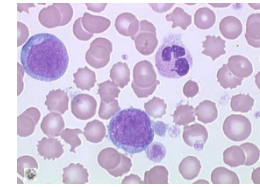
- Transcriptome analysis (on 7 pigs only)
using the 60K agilent microarray



- Validation on a set of differentially expressed genes (on 32 pigs)
PCR of 52 transcripts using the fluidigm method



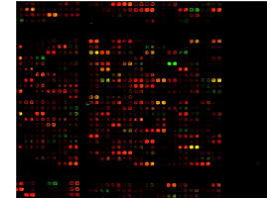
Blood cell numbers



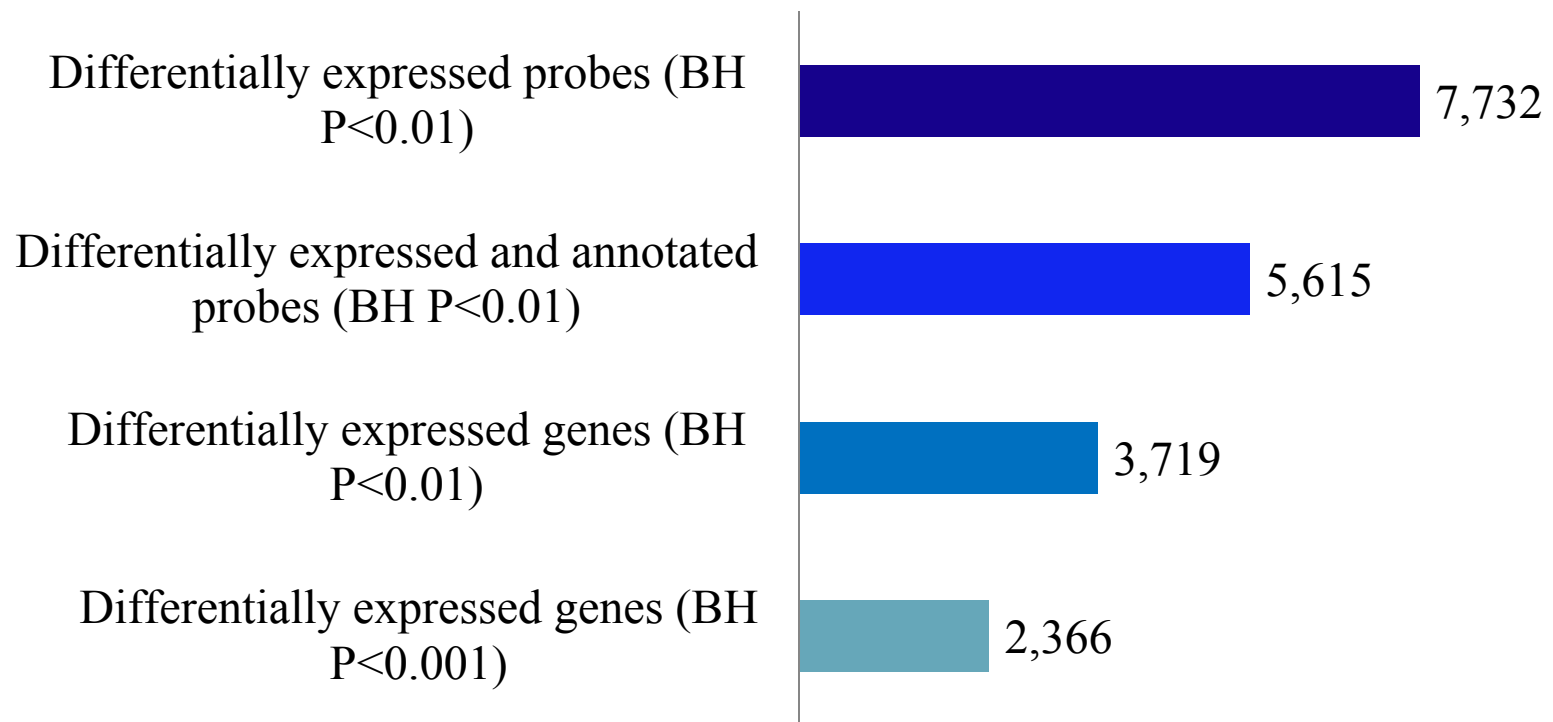
⇒ LPS induces dramatic variations in blood cell number and lymphocyte / granulocyte ratio

⇒ Confusion between the time and L/G effects: a major problem for the interpretation of transcriptomic data

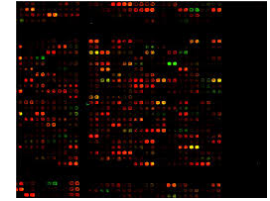
Microarray results



⇒ 3 719 annotated genes vary along time (Benjamini-Hochberg adjusted P values < 0.01)



Gene ontology enrichment analysis



- David software, “Biological processes” gene ontology => 46 clusters significant at $P < 0.05$
- Exclusion of too generic clusters (morphogenesis, transcription, locomotion...)
- Aggregation of the 25 first clusters (638 genes) into 8 “superclusters”:

	Super cluster	Nb of clusters	Nb of genes
1	Immunity and inflammation	5	209
2	Chemotaxis	5	196
3	Apoptosis	3	185
4	Ionic transport and Ca metabolism	2	127
5	Metabolism	5	121
6	Hormonal responses	1	111
7	Cell growth	2	71
8	Hemostasis	2	66

« Immunity and inflammation » cluster

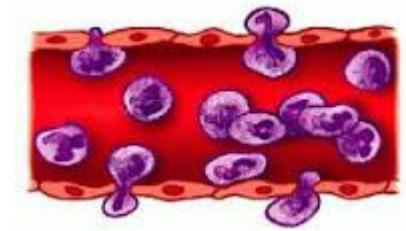
⇒ 209 genes

⇒ Inflammatory cascade after activation of leukocytes by LPS via TLR4 receptor (DF genes for TLR4, CD14, IRAK1, NFκB pathway)

⇒ Selection of 23 genes for fluidigm PCR:

	Super cluster	Nb of genes	Ex of functions	Ex of genes
1	Immunity and inflammation	23	<ul style="list-style-type: none">•Cytokines and receptors•Transcription factors•Immune cell products	<ul style="list-style-type: none">•TNFSF13B, IFNGR1, TGFBR1•NFKB1A, STAT2•SAA1, AZU1

“Chemotaxis” cluster



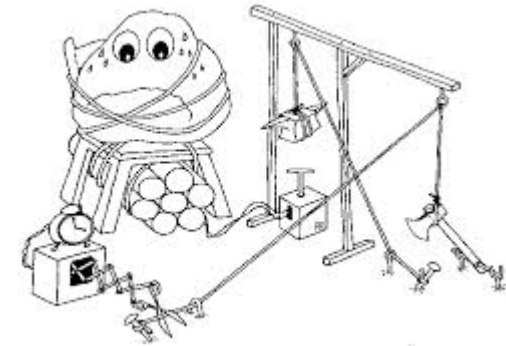
⇒ 196 genes

⇒ immune cell trafficking: in agreement with the observed blood cell redistribution

⇒ Selection of 14 genes for fluidigm PCR:

	Super cluster	Nb of genes	Ex of functions	Ex of genes
2	Chemotaxis	14	<ul style="list-style-type: none">•Chemokines and receptors•Matrix organization and cell adhesion	<ul style="list-style-type: none">•IL8, CCR5•ADAM10,TGFBI

“Apoptosis” cluster



⇒ 185 genes

⇒ apoptosis: down-regulation of the immune response

⇒ Selection of 19 genes for fluidigm PCR:

	Super cluster	Nb of genes	Ex of functions	Ex of genes
3	Apoptosis	19	<ul style="list-style-type: none">•Receptors•Intracellular control and execution of apoptosis	<ul style="list-style-type: none">•LTBR, TNFRSF1A•BCL2, CASP6

“Metabolism” cluster

⇒ 121 genes

⇒ lipid and protein metabolism

⇒ Selection of 9 genes for fluidigm PCR:

	Super cluster	Nb of genes	Ex of functions	Ex of genes
5	Metabolism	9	<ul style="list-style-type: none">•Antioxydant metabolism•Lysosom and proteasome enzymes•AA catabolism	<ul style="list-style-type: none">•GSR•GUSB•PSMC5, SDS

“Hormonal responses” cluster

⇒ 113 genes

⇒ immune paracrine / endocrine control of the immune response

Cytokines with pseudo-endocrine actions (TNF, IL-1)

Prostaglandins and leukotriens

⇒ neuro-endocrine control of the immune and systemic responses

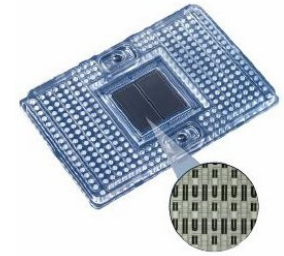
Sympathic (catecholamines, dopamine) and parasympathic (cholinergic) systems

Oxytocin

Adiponectin receptor, cholesterol and insulin pathways

	Super cluster	Nb of genes	Ex of functions	Ex of genes
6	Hormonal responses	16	<ul style="list-style-type: none"> •Synthesis of prostanglandins and leukotriens, androgens •Catecholamine receptor and degradation 	<ul style="list-style-type: none"> •PLA2G4A, ALOX12 •HSD17B11 •ADRB2, MAOA

PCR results using fluidigm method



Analysis of the time...

using times points -1 and +4h (where L/G ratios are ~)

using the L/G ratio as a covariate

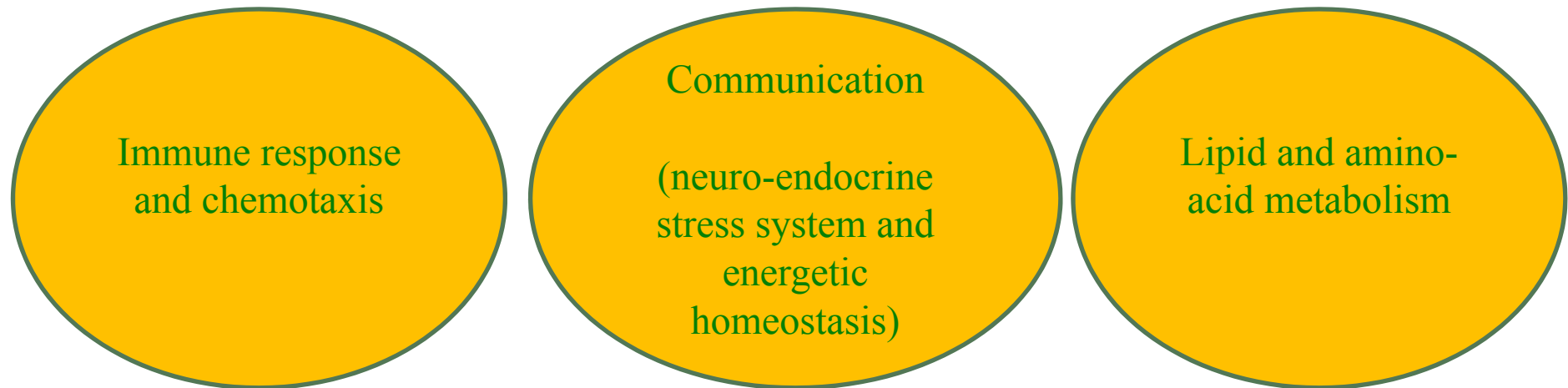
⇒ 30 genes influenced by the L/G ratio

⇒ 40 genes among 52 confirmed to vary between -1h and +4h

	Super cluster	Nb of genes	Down-regulated at 4h / -1h	Up regulated at 4h / -1h
1	Immunity and inflammation	23	11	10
2	Chemotaxis	14	7	7
3	Apoptosis	19	11	5
5	Metabolism	9	7	1
6	Hormonal responses	16	9	5

Conclusion

- Blood transcriptome allows the investigation of blood leucocytes...



- However...
Be careful with **blood cell redistribution!!**
=> only samples with close **Lymphocyte / Granulocyte ratio** ratio can be compared
=> include it as a covariate in the analysis
=> even the L/G ratio might be too rough



Thank you for your attention!

