EADGENE

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

Genomics for Animal Health: Outlook for the Future

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Chicken Macrophage Transcriptional Response to TLR Pathway Stimulants

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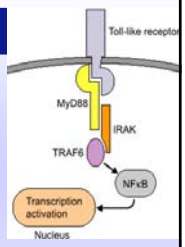
Background

Chicken Macrophages
- Utilize many receptors for microbial recognition and internalization
- Major roles in innate immune response
- HD11 cell line: virus-transformed, bone-marrow derived

Toll-like Receptors
- Recognize many specific PAMPs
- Initiate innate immune response by activating pro-inflammatory cytokine genes



OBJECTIVE 1: Characterize the stimulants and functions of TLR15 in early immune response in chicken macrophages.

OBJECTIVE 2: Determine the kinetic profile of *Salmonella* endotoxin-induced transcriptional response of chicken macrophages.

Beug *et al.*, 1979
Bliss *et al.*, 2005
http://genomebiology.com/content/figures/gb-2000-1-6-reports0079-1.jpg

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Summary and Discussion I

CpG ODN → mammalian TLR9 chicken – no TLR9???

TLR2, TLR15, TLR21 (not TLR4) → IFNA, IL10

- What functions as the "missing" TLR9?
- Brownlie et al. (2009) propose chicken TLR21 as one functional homolog of mammalian TLR9 in recognition of CpG ODN.
- Our data on HD11 cell response supports their hypothesis, as well as suggesting that chicken TLR2 and TLR15 may also respond to CpG.
- Future studies will further characterize the roles of each of these TLRs.

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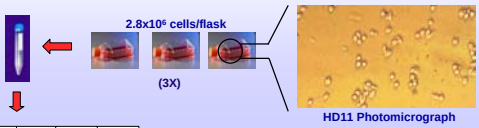
Materials and Methods II



Time (hr)	1	2	4	8
Endotoxin ST-798 Dose (μg/ml)	0.0	0.0	0.0	0.0
	0.1	0.1	0.1	0.1
	1.0	1.0	1.0	1.0
	10	10	10	10

qPCR → IL1β, IL6, IL8, IL10, IFN-γ, TLR15

Time (hr)	1	2	4	8
Dose (μg/ml)	0.0 versus 1.0			

Affymetrix GeneChip chicken genome array N = 20 → Ingenuity Pathway Analysis Software

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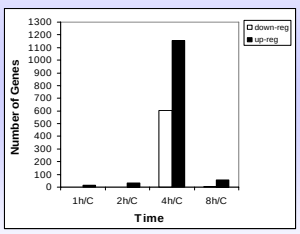
Differentially expressed genes, by time

Results II-A Number of genes differentially expressed (q < 0.05) in chicken HD11 cells, ST-798 stimulated versus non-stimulated



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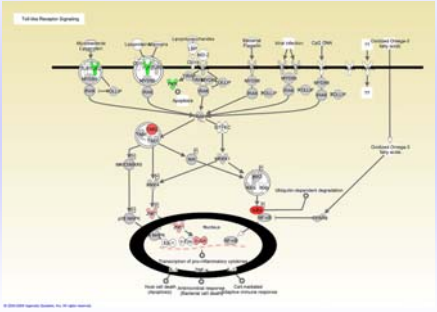
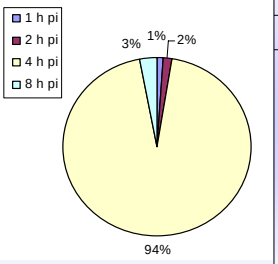
Differentially expressed genes, by time

Results II-B Toll-like receptor canonical signalling pathways



Differentially expressed chicken inflammatory response genes, by time

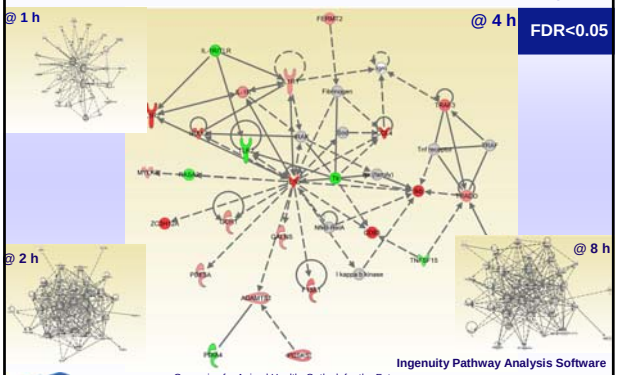
Results II-C



NFKBIA, IL1B, IL8 signature

Time	Inflammatory response genes
1	NFKBIA, IL1B, IL8
2	CCL4, PROK2, NFKBIA, LIPG, CD83, PIK3R5, BLNK, IL1B, IL8
4	PARP4, SBNO2, PIK3CG, NFKBIZ, ADIPOR1, F2RL1, EIF2AK2, CYBB (includes EG:1536), CD93, PIK3CD, IL28RA, LSP1, PRNP, BPI, ANKRD1, UACA, NFKBIA, IRAK2, DPP8, TRAF3, FANCC, CD274, RALB, GNA13, GNAI3, IL1R2, FAS, NFE2L1, NT5E, IL1R1, FYN, CD8A, JAM3, NCF4, F13A1, CSMD1, ADA, IFNGR2, SLC7A2, ETS2, DLL1, C19ORF2, XPO1, ZC3HAV1, PLA2G6, KSR1, AKIRIN2, IL1B, ELF1, CCL4, RPS15A, YARS, B2M, CCDC88A, PTPRC, ADORA2B, ID2, ABCC1, IL8, PIK3R5, C5AR1, PLSCR1, EDNRB, NFRKB, MYLK, ITCH, NCF2, CD44, SLC11A1, PODXL, MYLK3, TLR2, IRF2, HPS1, CCR9, ITGB3, MAP3K5 (includes EG:4217), HRH3, MAPK8, AHSG, CCND1, DIAPH1, CD83, RASA1, PIK3CB, NCOR2, LAT2, SYK, KIT, PIK3AP
8	CCL4, PROK2, NFKBIA, FAS, LIPG, ADORA2B, CD83, PRNP, IL8, PIK3R5, IL1B

Results II-D — Gene Network: Immune Cell Trafficking



Ingenuity Pathway Analysis Software

Red: up-regulation
Green: down-regulation

Summary and Discussion II

- Maximal numbers of differentially expressed genes occurred at 4 hours after in vitro stimulation with bacterial endotoxin.
- Higher number of differentially expressed genes after 4 hr endotoxin treatment gave rise to a more refined gene interaction map.
- 10% of the quantified genes were involved in the inflammatory response.
- NFKBIA + IL1B + IL8 = persistent signature of induction of inflammatory response by bacterial endotoxin.