Analysis of the intestinal mucosal immune response to an experimental infection with *Salmonella typhimurium* in pigs

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Introduction

Salmonellosis caused by the non-host–adapted bacteria *Salmonella typhimurium* is an important disease in animal safety and human health [1]. The early immune response will be crucial for successful host defence or pathogen colonization. In this work we have characterized the immune response to *S. typhimurium* infection *in vivo* along the porcine intestinal tract (jejunum, ileum and colon) by quantifying the gene expression of 28 immuno-related molecules.

Materials and Methods

Sixteen piglets were orally infected with *S. typhimurium* and sequentially sacrificed at 0 dpi (controls), 1, 2, and 6 dpi. Intestinal mucosa was isolated and RNA purified using the RNeasy Mini Kit (QIAGEN). Quantitative real time PCR assays were carried out using RNA pools and Sybr-Green in a iQ5cycler (BioRad). Relative gene expression was addressed using two constitutive genes. Expression changes were quantified both within a section along the infection and among different sections at the steady state. Statistical analysis was carried out by t-Student. Genes assayed:

Cytokines: TNFα, IL1β, IFNγ, IL6, IL12p40, IL4, IL10.

Chemokines: IL8, MCP1, MIP1α, MIP1β.

PRRs:TLRs 1-10, NOD1, NOD2.

Signal transducer: MyD88, Caspase1, NFκB1.

Antimicrobial and others: β-Defensin 1 and 2.

Table 2. TLRs and β-defensins where differences among sections were found at steady state (p<0.05). na, no amplification. Same letters means no differences.

	JEJUNUM	*ILEUM*	*COLON*
β-defensin 1	1[a]	9,92[b]	na
β-defensin 2	1[a]	-3,65[b]	3,50[c]
TLR-7	1[a]	3,83[b]	5,30[b]
TLR-9	1[a]	17,39[b]	1,75[c]
TLR-10	1[a]	11,80[b]	1,69[a]

Results

Table 1. Fold-change at different days post infection respect to non-inoculated pigs (0 dpi). [a]Only molecules where changes in gene expression were quantified at p<0.05 (*) are shown. (**, p<0.1)

	Fold-change[a]				*Fold-change*[a]		
	JEJUNUM	*ILEUM*	*COLON*		*JEJUNUM*	*ILEUM*	*COLON*
IL-8				***TNF-α***			
1 dpi	**3.54***	**5.29***	1.23	1 dpi	2.59	**2.86****	1.02
2 dpi	**8.43***	**5.59***	**3.53***	2 dpi	**3.59***	1.95	**2.22****
6 dpi	3.31	3.61	**3.30***	6 dpi	1.65	1.48	1.48
MCP-1				***IL-1β***			
1 dpi	2.27	**4.80***	-1.37	1 dpi	1.72	1.46	-1.22
2 dpi	**2.77****	**3.59****	1.25	2 dpi	**15.38***	1.02	**3.09****
6 dpi	-1.06	1.62	1.11	6 dpi	**-3.48***	**-5.02***	**3.33***
MIP-1α				***IFN-γ***			
1 dpi	2.19	1.6	-1.01	1 dpi	1.36	1.9	-1.21
2 dpi	**3.86****	**3.84***	1.64	2 dpi	**2.87***	-1.35	1.31
6 dpi	1.76	**3.58****	**4.52***	6 dpi	-2.14	1.46	**3.61***
TLR-2				***IL-6***			
1 dpi	-1.44	**3.59***	-1.59	1 dpi	2.09	2.1	1.07
2 dpi	1.34	**2.56****	1.08	2 dpi	**5.43***	-1.32	**2.89****
6 dpi	**-3.31****	**5.58***	-1.2	6 dpi	-1.98	**-4.00***	2.23
NOD1				***IL-12p40***			
1 dpi	2.34	**2.85****	1.15	1 dpi	1.32	-1.49	-1.69
2 dpi	**5.86***	**2.97****	1.57	2 dpi	1.05	**-4.36***	1.58
6 dpi	1.77	1.75	1.43	6 dpi	**-3.03****	**-4.61***	**2.44****
β-defensin 2							
1 dpi	**-19.56***	**2.95****	1.02				
2 dpi	**-17.21***	**6.97***	2.34				
6 dpi	**-18.30***	1.1	-1.22				

Discussion and conclusions

Our results demonstrate that each intestinal section responds differently to the infection. According to the pattern of induction of pro-inflammatory cytokines, ileum mucosa seems unable to mount such an appropriate response to *S. typhimurium* as did jejunum (Table 1). Nevertheless, expression level at steady state of TLRs or β-defensins did not show ileum as a less protected section to the infection (Table 2). On the other hand, colon mucosa, although able to mount the response, it did later. Finally, none TLR but TLR2 showed an increase in gene expression upon *S. typhimurium* infection. Similarly, the expression of the cytosolic pattern recognition receptor (PRR) NOD1 was increased after the infection.

References

[1]Boyen F. et al., Vet. Microbiol. (2008) 130:1-19.

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