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**Vet. Res. (2009) 40:05** DOI: 10.1051/vetres:2008043

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## **Original article**

## Early immune response following *Salmonella enterica* subspecies *enterica* serovar Typhimurium infection in porcine jejunal gut loops

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(Received 25 July 2008; accepted 13 October 2008)

**Abstract** – Salmonella enterica subspecies enterica serovar Typhimurium, commonly called S. Typhimurium, can cause intestinal infections in humans and various animal species such as swine. To analyze the host response to Salmonella infection in the pig we used an in vivo gut loop model, which allows the analysis of multiple immune responses within the same animal. Four jejunal gut-loops were each inoculated with  $3 \times 10^8$  cfu of S. Typhimurium in 3 one-month-old piglets and mRNA expressions of various cytokines, chemokines, transcription factors, antimicrobial peptides, toll like and chemokine receptors were assessed by quantitative real-time PCR in the Peyer's patch and the gut wall after 24 h. Several genes such as the newly cloned CCRL1/CCX-CKR were assessed for the first time in the pig at the mRNA level. Pro-inflammatory and T-helper type-1 (Th1) cytokine mRNA were expressed at higher levels in infected compared to non-infected control loops. Similarly, some B cell activation genes, NOD2 and toll like receptor 2 and 4 transcripts were more expressed in both tissues while TLR5 mRNA was down-regulated. Interestingly, CCL25 mRNA expression as well as the mRNA expressions of its receptors CCR9 and CCRL1 were decreased both in the Peyer's patch and gut wall suggesting a potential Salmonella strategy to reduce lymphocyte homing to the intestine. In conclusion, these results provide insight into the porcine innate mucosal immune response to infection with entero-invasive microorganisms such as S. Typhimurium. In the future, this knowledge should help in the development of improved prophylactic and therapeutic approaches against porcine intestinal S. Typhimurium infections.

Salmonella / Th1 cytokines / pig / CCRL1 / pattern recognition receptor

#### 1. INTRODUCTION

Enteropathogenic Salmonellae such as Salmonella enterica subspecies enterica serovar Typhimurium (commonly called S. Typhimurium) and Salmonella Typhisuis cause inflammation and necrosis of the small and large intestines of cold and warm blooded animals, resulting in diarrhea that may be accompanied by generalized sepsis. In pigs, all ages are susceptible; however, the disease is most common in weaned and growingfinishing animals. Even though *Salmonella* has been well characterized in terms of genetics, physiology and virulence factors, the understanding of the molecular mechanisms of host pathogen interaction is quite limited. In the pig, few studies have been carried out in the last few years [32, 39, 41–44]. Among

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these studies, some [39, 42] used the original approach of the small intestinal segment perfusion (SISP) [30]. This model was originally described to study the metabolite effects on intestine water absorption capacity [30], but has also proven to be a valuable technique to carry out genomic studies [39]. Markedly different host transcriptional profiles between Salmonella serovars Cholerasuis (narrow host range) and Typhimurium (broad host range) have been revealed [39]. Serovar Typhimurium-infected swine presented a transient induction of genes involved in innate and T-helper type-1 (Th1) response early in the infection (24-48 h) followed by a significant repression of Interleukin 12 p35 (IL12 p35), IL12 p40, IL4, IL8 and Granulocyte/Macrophage Colony-Stimulating Factor (GM-CSF) [39]. The observed upregulation of serum IFN gamma and TNF alpha supported the involvement of Th1mediating cytokines in the porcine response to Salmonella infection. The clearance of intracellular pathogens such as Salmonella by the host is primarily accomplished by the activation of Th1-mediated immune responses [7, 11, 21, 45]. More recently, a few studies [41-43] have been interested in porcine antimicrobial peptides in the context of Salmonella infection. An antimicrobial activity has been demonstrated for porcine beta-defensin 2 (PBD-2) against various bacteria such as S. Typhimurium, Listeria monocytogenes and Erysipelothrix rhusiopathiae and using porcine intestinal cell culture infected with different bacteria, PBD-2 gene expression was shown to increase 10fold upon infection with S. Typhimurium [41]. By contrast, Arcobacter cryaerophilus and Salmonella Enteritidis, pathogenic bacteria with comparable adhesion and invasion characteristics, failed to increase PBD-2 mRNA expression. Gene expression of PBD-1 was regulated differently since an increase in mRNA expression was only observed upon Salmonella Enteritidis infection.

In the current study, we describe the mRNA expression of multiple chemokines, cytokines, pattern recognition receptors, transcription factors and antimicrobial peptides within the

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Peyer's patch (PP) and the gut wall (GW) of porcine gut loops 24 h after the inoculation of S. Typhimurium. This model originally described in sheep [15] is an interesting methodology which allows, similarly to the SISP procedure, the in vivo infection of isolated jejunal segments with a dose of pathogens without removing the blood supply and innervation. Like the SISP procedure, this technique minimizes the effect of individual variation between animals since the control and the infected loops are within the same part of the intestine in the same animal. Moreover, it has been demonstrated that this procedure does not induce any macroscopic or histological alterations in lymph or blood supply with normal cell population and functional mucosal associated lymphoid tissues. This study constitutes a broad and original assessment of the early immune response in the pig gut loop model. In addition, we describe the cloning of ChemoCentryX Chemokine Receptor (CCX-CKR), commonly named CCRL1, the second receptor of CCL25 and we analyzed for the first time its mRNA expression during an infection. As CCRL1, many mRNA expressions were measured to our knowledge for the first time in the pig, particularly the gene coding for recently discovered cytokines such as IL17a, IL22, IL27 p28 and IL33 contributing to a better understanding of porcine immunology.

#### 2. MATERIALS AND METHODS

#### 2.1. Animals

Landrace piglets between 28 and 32 days of age were used for the experiments. The pigs were healthy and raised in commercial swine herds. Prior to the experiment, the pigs were determined to be free of culturable *Salmonellae* organisms. Twentyfour hours post-surgery, the pigs were euthanized by barbiturate overdose. All experiments were conducted in accordance with the ethical guidelines of the University of Saskatchewan and the Canadian Council for Animal Care.

# 2.2. Bacterial strain, experimental inoculation of intestinal loops and tissue collection

The Salmonella enterica subspecies enterica serovar Typhimurium strain SL1344 [23] was used



Figure 1. Schematic presentation of the jejunal segments used in the experiment (N = 3 piglets). Tissues (Peyer's patch and Gut wall) were collected after 24 h. Two loops were used as control (Growth Media) and four loops were infected with *Salmonella enterica* subspecies *enterica* serovar Typhimurium SL1344 (*Salmonella* Typhimurium SL1344). IS: Interspace.

in the current study. Bacteria were prepared freshly for the experiment by cultivation from a frozen stock at 37 °C in Luria Bertani broth (LB: Tryptone 10 g, Yeast extract 5 g, NaCl 10 g/L). Before the experiment, the overnight culture was subcultured 1:100 and incubated for 2 h at 37 °C. Bacteria were then collected in the exponential phase, spun down and resuspended in LB broth. Four jejunal gutloops were inoculated with  $3 \times 10^8$  colony forming units (cfu) of S. Typhimurium. As controls, clean bacterial growth media was injected into the two loops. To constitute the loops, a sterile 2-4 m long segment of intestine was surgically prepared in the jejunum, where PP can be individualized, of 3 one-month-old piglets (for a detailed description of the surgical procedure see [15]). This "intestinalsegment" was then subdivided into consecutive segments, designated as "loops" (10-20 cm long, 6 loops), that included a PP, or "interspaces" (20-100 cm long, 7 interspaces), that lacked a visible PP (Fig. 1). All 'loops' were collected 24 h post-surgery before bacteria enumeration. Tissues were cut open in five  $3 \times 3$  mm pieces, laid flat, washed with ice cold phosphate-buffered saline, snap-frozen in liquid nitrogen and stored at -80 °C. Additionally few other tissues (duodenum, jejunum, ileum, colon, caecum, mesenteric lymph node, liver, thymus and heart) were collected to check the mRNA expression of CCRL1.

Invasion of *S*. Typhimurium was established, after plating of serial dilution of lumen content and homogenized tissues on SS Agar (Salmonella-Shigella) (Oxoid limited, Basingstoke, UK), by the enumeration in infected and control loops of all the bacteria. The enumeration was performed after

an overnight culture at 37 °C. The results showed a massive invasion of the subjacent tissues by the bacteria in infected loops while in control loops only few bacteria, most probably few *Proteus* spp., were detected mainly in the lumen. Moreover a quantitative real-time PCR (qPCR) directed against *sipA* [14] which is involved in the invasion of the bacteria showed a clear up-regulation of *sipA* mRNA expression in all the infected loops versus the control loops confirming the infection in *Salmonella* loops and the absence of bacteria in control loops. Moreover, the level of *sipA* mRNA expression was similar in the different infected loops.

#### 2.3. Cloning of the porcine CCRL1 gene

Total RNA was extracted from the pig mesenteric lymph node samples using Trizol reagent (Invitrogen, Cergy-Pontoise, France). The fulllength Open Reading Frame (ORF) of porcine CCRL1 was cloned using sequence information of the 3' end of CCRL1 from a porcine expressed sequence tag (EST) (NCBI accession no. BW955277) and the 5' end of the porcine CCRL1, which was obtained using a 5' RACE template switching method [27] with the primer sets CCRL1GSP and CCRL1nGSP (Tab. I). PCR products were cloned using Zero Blunt<sup>®</sup> TOPO<sup>®</sup> PCR Cloning Kit for Sequencing from Invitrogen. Inserts were sequenced and their homology to human CCRL1 was determined with Clone Manager 9 (Scientific & Educational Software, Cary, North Carolina, USA). The nucleotide sequence for CCRL1, based on results from five clones,

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Table I. Primer sequences,	annealing	temperatures	of	primer	sets,	expected	PCR	fragment	sizes	and
accession numbers.										

$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Primer name	Primer sequence	Annealing	PCR	Accession
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APRIL/TNFSF13       S: TGCTCACCCGTAAACAAAG       60       172       EST BP170456         AS: TAAACTCCAGCACHTCCAAAG       60       103       NM_001097498         AS: GCATGCCACTGTTCGAATC       60       103       NM_001097498         CCL20MIP3 alpha       S: GCTTCCCGGCCACCACTTTGATGTC       60       146       NM_001025214         CCL25TECK       S: GCTCCCGCCACCACTTAAG       64       136       NM_001024695         AS: TAGGGGCTGACACGATTC       62       144       NM_001024695         CCL2WMEC       S: GTGGCGCAGCACCACC       69       143       NM_001024695         AS: ACGGCACCACGATGAACAGATTC       66       136       NM_00104563         AS: CAGGGCAGCACCGGGTTGGAA       AS       CCR10       A: GCCCCGCAGACCGGGTTGGAA         CCR10       A: GCCCCCGCAGACCGGGTTGGAA       72       na       EST BW955277         AS: CCACCCATGAACTGCTTAACTCCAGGG       72       na       EST BW955277         CCRLIACSK       S: CCCCCCTTGATGCTGCTATACTC       72       na       EST BW955277         CS: CCCCGCCTTCTTCTACCCCAGGAG       61       147       NM_001097430         AS: CACACTTCGTTGTGTGATTC       72       na       EST BW955277         CCLLIACSK       A: CACCTTCTGTGTGGATTGC       72       na       EST B			(°C)	(bp)	
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AS: GCTTCCGGACACCATCTT CCL28/MEC S: GCTGCGCACTGAGGTTTC 62 144 NM_001024695 AS: TGAGGGCTGACACACAC 69 143 NM_001001624 AS: ACGGCACCCAGTGACACCACC 69 143 NM_001001624 AS: ACGGCACCCAGTGGACACCACC 72 na EST BW955277 AS: CCGCCCCAGGACACTGGGTTGGAA CCRLIGSP S: CTGCCTGCCTTCTCACAGTAGCTTTCATC 72 na EST BW955277 AS: CCACCCATGAACTGGCATTAACTGCCCAGAG CCRLIGSP S: CTGCCTGCCTTCTCACAGTAGCTTTCATC 72 na EST BW955277 CCRLIGSP A: CCTTCTATCACTCTGGGCCGTAACTG CCRLIGSP S: CCGCCCCTGTGTGTAACTGCCCAGAG CCRLIGSP A: CCTTCTTTCACCAGTGACTGCCATTAACTGCCCAGAG CCRLIGSP A: CCTTCTTTCACCTCTGGG 72 na EST BW95277 CCRLIGSP A: CCTTCTTTCACCTCAGGAG CCRLIGSP A: CCTTCTTGTCTACATCCAGGAG CCRLIGSP A: CCTTCTTGTGTTTACTCCAGGAG CCRLIGSP A: CCGCCCCTGGTCTTGTGATTG CCACCTCTGGTCCATGCCATTAC AS: CAGACTCCCCCCCAGTGAATG CX3CLIFractalkine S: GCGACTCCTGGCCATTAC AS: CACCATTCTGGACCCAGAG AS: CACCATCTGGCCTTCTGG AS: CGGCCCGGTTGTTCTGGACTC AS: CGGCCCGGTGTTCTCGGACACAC CXCL2/GRO beta S: TGCGTCCTGTGGACTC AS: CGGTCCCGGTTGTTCGAGGC CXCL10/IP-10 S: CCCACATGTGAGTCCTTTGG CXCL10/IP-10 S: CCCACATGTGAGTCCTTGG AS: CGGTCCAGTGCCTTGGAACAC AS: GGGTCGAGTCCTGGGACACAC AS: GGGTCGAGTGCCTGGACACAC AS: GGGTCGAGTGCCTGGGCG AS: CGGTCCAGTGGCGCG AS: GGGTCGAGTGCCTGGGACATAC CXCL10/IP-10 S: CCCACTGTGTGGAACAATC AS: GGGTCGAGTGCTGGGCG AS: GGGTCGAGTGCGTGCG AS: GGGTCGAGTGCGGGCGCG AS: GGGTCGAGTGCTGGGACGCG CXCL10/IP-10 S: CCCACTGTGTGGAGCGTTG AS: CGGTCGAGTGGCTGGGCG AS: GGGTCGAGTGGCTGGCG AS: GGGTCGAGTGGCTGGGCG CXCL10/IP-10 S: GCCACTGGCTGGG AS: GGGTCGGAGTGGCTGG AS: GGGTGGAGGCGTCGGGCGGCG AS: GGGTGGAGGCGCGCGCGGGGGGGGGGGGGGGGGGGGGGG	CCL25/TECK	S: GCCTACCACAGCCACATTAAG	64	136	NM_001025214
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AS: TGAGGGCTGACACAGATTC CCR9 A: TACGGCTATGACGCCACACC 69 143 NM_00101624 AS: ACGGGCACCCACGATGAACAC CCR10 A: GCCGCCAGAGCAGCTTTCCA AS: CCAAGGAACACTGGGTTGGAA CCRL1GSP S: CTGCCTGCTTCTTCACAGTAGCTTCATC 72 na EST BW955277 AS: CCACCCATGAACTGCCATTAACTGCCCAGAAG CCRL1AGSP A: CCTTCTATTCACTCTGCCCAGAAG CCRL1/CCX-CKR A: ACAGATACTGCGCATAACTGCCCAGAAG AS: CCACCCGCTTGTGTTACATCCAGGAG CCRL1/CCX-CKR A: ACAGATACTGGCCAGTAAG AS: CACCCTGCTTTGTGTACATCCAGGAG AS: CACCCCGCTTTGTGTACATCCAGGAG CCRL1/CCX-CKR A: ACAGATACTGGCCAGTAAG AS: CACCACTCGCTTTGTGATTG CD40L A: TACGCCCAAGTCACTTGC AS: CACCCTCGCTTTGTGATTG CCACL1/Fractalkine S: GCAGCTCCTAGTCCATTAC AS: CACCACTCCGCAGTGAATG CCXCL2/GRO beta S: GCGGCTCCTAGTCCATTAC AS: CACCATTCTGGACCTC AS: CCGCCCCAGTGACTGC AS: CCGCCCCTGTGTTGGG CCXCL2/GRO beta S: TGCTGCCCTTCTGGG CCXCL2/GRO beta S: GGGCGCCGTGGTTGTTGG CCXCL2/GRO beta S: GGGCGCCGTGGTTGTGG AS: CGCGCCCTCTGGGACAATG CCXCL2/GRO beta S: GGGCGCCGTGGTTGTGG CCXCL2/GRO beta S: GGGCGCCGTGGTTGTGG CCXCL2/GRO beta A: GGGTGCAGTGCAGTAG AS: CGGCCCCTAGTGGAACAC AS: GGGGCAGTCTCTGGAACAC AS: GGGCCAGTGGGAACAAC AS: GGGCCGCGTGGTTGTGG CCXCL2/GRO beta A: GGTGCCGTCGTGGGAACAAC AS: GGGCCAGTGGCAGAAC AS: GGGCCAGTCGTCGGGAACAAC AS: GGGCCAGTCGTCGGGAACAAC AS: GGGCCCAGTGGCACAATAC AS: GGGCCGCTCTGGAACAAC AS: GGGCCGTCGTGGGAACAAC AS: GGGCCGTCGATGGCGG CGACSF/CSF-2 S: GAAACCGTAGGGCGGCTTG AS: GGGCCGTCGGGAACAAC AS: GGGCCTCTCAGGGAACAC AS: GGGCCTCTAGGGCACAATAC AS: GGGCCTCTCAGGGAACAC AS: GGGCCTCTAGGGCACAATAC AS: GGGCCTCTCAGGGAACAC AS: GGGCCTCTAGGGCACAATAC AS: GGGCCTCTCAGGGCACAATAC AS: GGGCCTCTCAGGGCACAATAC AS: GGGCCTCTCAGGGCACATAC AS: GGGCCTCTAGGCCGCTGG AS: GGGCCTCTCAGGGCACATAC AS: GGGCCTCTCAGGCGCTTG AS: CCCCCCAGGGCTTTGG AS: GGGCGCCATCGGCTTGG AS: GGGCCTCCGGCATTGGCTCG AS: GGGCGCCTCCGGCTTGG AS: GGGCGCCTCCGGCTTGG AS: GGGCGCCTCGGTAGGTCG AS: CCCCCCAGGGCTTGGGCTTG AS: CCCCCCAGGGCTTGGGTAGG AS: CCTCCCCAGGGCTTAGGCCG AS: CCTCCCGCGCTGGGTAGG AS: CCTCCCGCGCAGGCTTGG AS: CCTCCCGCGCAGGCGCTGGGTAGG AS: CCTCCCGCGCGCGCGTGGGAAGG AS: CCTCCCGCGCGCGCGTGGGAAGG AS: CCTCCGCGCCAGGGCGCGCGTGGGAAGG AS: CCTCCGCGCCTGGGCACGGCGCGTGGGAAGG	CCL28/MEC	S: GCTGCTGCACTGAGGTTTC	62	144	NM_001024695
CCR9A: TACGGCTATGACGCACACC69143NM_001001624AS: ACGGCACCCACGATGAACACAS: ACGGCACCCACGATGAACAC66136NM_001044563CCR10A: CCCCGCCGAGAGCAGGTTTCC72naEST BW955277AS: CCACCCCATGACTGCCCTTCTGG72naEST BW955277AS: CCACCCCGCCTTGTCTTACCCAGGAG72naEST BW955277SS: CCCGCCCTTGTCTTACCCAGGAG61147NM_001097430AS: CCACCCCGCCTTGTGGTAACG62172AF248545CCRLI/CCX-CKRA: ACAGATACTGGGCAGTAACG62172AF248545CCACCCTCGCCCTTGTGGCATTAC58167EST CK464144A: ACGGCTCCTGGTCCATTAC58167EST EK464144A: ACGGTCCCCTGGTCCTTGGGACTC60182EST BX919199AS: ACGGTCCCTGGCTCTGGGACTC60182EST BX919199AS: ACGGTCCCTCTGGACTC60188NM_001008611AS: ACGGTCCCTCTGGGACTC60188NM_001008612AS: ACGGTCCCTCTCTGGACACATACC60193EST BW971285AS: GGTGCAGTCTCTGGGACACATAC61148AY669812AS: GGTGCCAGTGGCACACATAC61150DQ108393AS: GGTGCCATCTAGGGAAC61150DQ108393AS: GGTGGCAGTGGTGGTTGG62150DQ108393AS: GGGGGCAGAGCCTCTGGGTTAGTG63159NM_213861AS: GCCCCCGGGTTAGGGCTACGTTGG63159NM_21423AS: CCCCCGGGTTGGGATAGGTC64105NM_21423AS: CGCCCGCGTTGGGTAGGTAGGTC65173NM_214399 <td></td> <td>AS: TGAGGGCTGACACAGATTC</td> <td></td> <td></td> <td></td>		AS: TGAGGGCTGACACAGATTC			
AS: ACGGCACCACGATGAACAC CCR10 A: GCCCGCAGAGGCAGGTTCC 66 136 NM_001044563 AS: CAAAGAGACACTGGGTTGGAA CCRLIGSP S: CTGCCTGCTTCTTCACAGTAGCTTTCATC 72 na EST BW955277 AS: CCACCCATGGACTGCATTAACTGCCCAGAAG CCRLIAGSP A: CCTTCTATTCACTCTGGCCATTAACTGCCCAGAAG CCRLIAGSP A: CCTTCTATTCACTCTGGCCATTAACTGCCAGGAG CCRLIACX-CKR A: ACAGATACTGGGCAGTAACG 61 147 NM_001097430 AS: CACACTCGGCCATGACGA AS: CACACTCGGCCATGACG CD40L A: TACGGCCCAGAGGACT CCACLI/Fractalkine S: GCAGCTCTAGTCCATTAC AS: CACCATCTGGGCAGTAGAG CX3CL1/Fractalkine S: GCAGCTCTCTGGACCAGAAG CX3CR1 A: ACCTTGCCCTTCTGGACCA AS: CACCATCTGGACCCAGAAG CXCL2/GR0 beta S: TGCGCCCTTGTGTCATTGC CXCL2/GR0 beta S: TGCGCCCTCTGGGCAGTAGC CXCL2/GR0 beta S: GCGGCCAGTGGATGG AS: CGCCACTCTTGGAACAAC GATA3 A: CCCTGCCTCTAGTCCGTTGG A: CCCGTCCTAGTCCAGTGC GM-CSF/CSF-2 S: GGAACGCTCTCTGGAACAAC GATA3 A: CCCGTCGTCTAGTGGCAGAAC CXCL10/IP-10 S: CCCACATGTGGGCAGAAC GM-CSF/CSF-2 S: GGAACGCGTCGTTGG GM-CSF/CSF-2 S: GGAACGCGTCGTTGG GM-CSF/CSF-2 S: GGAACGCGTCGTGG AS: GTGGCGGGGAGAGCGCTCTG GM-CSF/CSF-2 S: GGAACGGCGCTCTG AS: GGGGCGCAGGGCGAGGGCTCGG GM-CSF/CSF-2 S: GGAACGCGCCGTGGTGG L1/BCGF S: CGCAGGCGCCTCTGG CCCL10GACGCCGTCGTGGGCAACACC CCCGTCCTACTACGGGCGTCGTGG CCCGTCGTGGAGAGGCCCTCGG CCCGTCGTGGGAGGCGCCTGG CCCGTCGTGGGAGGCGCCTGG CCCGTCGTGGGGAGGCGCCTGG CCCGTCGTGGGGGAGGCGCCTGG CCCGTCGTGGGGGAGGCGCCTGG CCCGTCGTGGGGGGAGGCGCCTGG CCCGTCGTGGGGGGAGGCGCGCGGG L1/BCGF S: CGCAGGCGCGCGTGGTGG CCCGTGCGTGGGGGAGGCGCGCGGG L1/BCGF S: CGCATGGCGGGGAGGCTGG CCCGTGGGGGGGGGGGGGGGGGGGGGGGGGGG	CCR9	A: TACGGCTATGACGCCACACC	69	143	NM_001001624
CCR10A: GCCCGCAGAGCAGGTTTCC66136NM_001044563 AS: CAAAGGAACTGGGTTGGAACCRL1GSPS: CTGCCTGCCTTCTTCACAGTAGCTTTCATC72naEST BW955277 AS: CCACCCTGCCTTCTGGCCCTTCTGGCCRL1nGSPA: CCTTCTATTCACTCACGTGCAGTAACG72naEST BW955277 SS: CCGCCCCTTGTGTCACACGTGAGGCCRL1nCX-CKRA: ACAGATACTGGGCAGTAACG61147NM_001097430 AS: CACACCTCGCTTGTGGATTGCD40LA: TACGCCCAAGTCACTTCTG62172AF248545AS: CACACCTCGCCTCAGTGCATGG58167EST CK464144 AS: CACCATTCTGACCCAGAGGCX3CL1/FractalkineS: CGCGCCCTAGTCATTGC60182EST BX919199 AS: CACCATTCTGGCCTTCTGGGACTGAS: CACCATTCTGCCCTTCTGGACTC60168NM_001008691 AS: CACCCTCTGTGGACTGC60168CXL1gRO betaS: TGCTGCTCCTGGATTGG60193EST BW971285 AS: GGTCCCACATGCTGGGAAAC60193FOXP3A: GCGTGCCAGGACACATCG60193EST BW971285 AS: GTGGCGGAGAACC611001008691 AS: CAGTCCTATACGGCAAACGATA3A: CCCGTCCTACTACGGAAAC6091DQ815175 AS: GTGGTGGAGGACGTCTTG62150DQ108393 AS: GTGGTGGAGGACGTCTGGM-CSF/CSF-2S: GACATGCGTGGTGGTGTGGACGT63159NM_21423 AS: CGGCTCCTCAGTGCTTG63159NM_21423 AS: CGCTCCCCAGGGTTTGL1/BCGFS: CACCCCTGCTGGGGATTGC63159NM_21423 AS: CGCTCCCCAGGTTTGTGGGATTG110/8-TCGC110/8-TCGL4/BCGFS: ACACCCGGTGGTGTGGATTG61105NM_214239 AS: TGGTGGCTTGGCTTGGGGGGGTTGG		AS: ACGGCACCCACGATGAACAC			
AS: CAAGAGACACTGGGTTGGAA CCRLIGSP S: CTGGCTGCTTCTTCACAGTAGCTTCATC 72 na EST BW955277 AS: CCACCCATGAACTGCATTAACTGCCCAGAAG CCRLInGSP A: CCTTCTATCACTCGCCCTTCTGG 72 na EST BW955277 SS: CCACCCATGATCACTGGCCATTACATGCCCAGAAG CCRLIACX-CKR A: ACAGATACTGGGCAGTAACG 61 147 NM_001097430 AS: CACACCTGCTTTGTGATTG 62 172 AF248545 AS: GCACCTCCGCTCAGTCCATTAC CD40L A: TACGCCCAAGTGCATCG 58 167 EST CK464144 AS: CACCATCTGGACCTAGTC CX3CL1/Fractalkine S: GCAGCTCCTAGTCCATTAC AS: CACCATTCTGACCCGAAG CX3CL1/Fractalkine S: GCAGCTCCTGGCATTG AS: ACGTTCCCGCCTAGTCCATTAC 60 182 EST BX919199 AS: ACGTTCCCTCTGGACTCA AS: CGCGTCCTTGTGAGTCG CXCL2/GRO beta S: TGGTGCTCTGGGATCA AS: GGTGCCAGTGCTGTGG CXCL10/IP-10 S: CCCACATGTTGAGTCGG FOXP3 A: GGTGCCAGTGCTGCGG FOXP3 A: GGTGCCAGTGCTAGTGC GATA3 A: CCGTGCCTAGTGCGACAAC AS: GGTGCCAGTGGCTACAATAC GATA3 A: CCGTGCCTAGTGGCACCAATAC GATA3 A: CCGTGCCTAGTGGCACCAATAC GATA3 A: CCGTGCCTCTGGGAACAAC AS: GGTGCCAGTGGCTACAATAC GATA3 A: CCGTGCCTCTGGGACCACC HPRT-1 S: GGACTGCGAGCGTCTTG GM-CSF/CSF-2 S: GAAACCGTAGGCTCTTG GM-CSF/CSF-2 S: GAAACCGTAGACTCTGG HPRT-1 S: GGACTGCGAGACGTCTTGG HPRT-1 S: GGACTGCGAACAAC AS: GTGGCGGCACTGCTGTG HPRT-1 S: GGACTGGCAGCGTCTTG GAACGGT AGACGTACATGCC HPRT-1 S: GGACTGGCAGCGTCTTG GAGAGGCCCATGGTCCTGG HPRT-1 S: GGACTGGCAGCGTCTTG GM-CSF/CSF-2 S: GAAACCGTAGGCTCTTG GM-CSF/CSF-2 S: GAAACCGTGGCTCTG AS: GTGGTGCCATGGGCTGCTTG HPRT-1 S: GGACTGGAGCGTCTTG GAAGGGCCCATGGTCGTCGGC HI beta/LAF S: AGAGGCCCATCGTCGTG HPRT-1 S: GGACTGGCGGCTTGG HPRT-1 S: GGACTGGCTGCTGGGAGTCC HI beta/LAF S: AGAGGCCCTACGTGGTCG HI beta/LAF S: AGAGGCCCTACGTGGCTTG HI beta/LAF S: AGAGGCCCACGTGGTCGC HI beta/LAF S: AGAGGCGCACTGGTGGCTTG HI beta/LAF S: AGAGGCGCACTGGTTGG HI beta/LAF S: AGAGGGCGCACTGGTGGCT HI beta/LAF S: AGAGGGGCGCGCTTGGGATTG HI beta/LAF S: CGGGGGCTACGTGGGATGG HI beta/LAF S: CGGGGGGCACTGGTGGATGG HI beta/LAF S: ACCGGGGCGACTGGTGGATGG HI BACGF S: ACCCGGCGGCGGCTGGGATGGGATGG HI BACGFAC S: CCTGCGGCGACGGCGCGCGCGCGCGCGCGCGCGCGCGCGC	CCR10	A: GCCCGCAGAGCAGGTTTCC	66	136	NM_001044563
CCRL1GSPS: CTGCCTGCCTTCTTCACAGTAGCTTCATC72naEST BW955277 AS: CCACCCATGAACTGCCCTTGCGCCAGAAGCCRL1nGSPA: CCTTCATTCACTCTGCCCTTCTGG72naEST BW955277 AS: CCCGCCTGTCTTGAATGCCRL1/CCX-CKRA: CCTTCCATTCACTCTGCCCTTCTGG61147NM_001097430 AS: CACACTCGCCTTGTGATTGCD40LA: CACGATACTGGGCAGTAACG62172AF248545 AS: AGACTCCGCCCAAGTGAATGCX3CL1/FractalkineS: GCAGCTCCTAGTCCATTAC58167EST CK464144 AS: CACCATTCTGGACTCAS: CACCATTCTGGACTC60182EST BX919199 AS: CACCATCTTCTGGGACTC60171NM_001001861 AS: CACCATGTGAGATCATTGCAS: CACCATGTTGGAGTCCTCTGTGGAGTC60168NM_001008691 AS: CATCCTTCTGAGATCATTGC60168NM_001008691 AS: CATCCTTCTGAGTGCGGCXCL10/IP-10S: CCCACATGTTGAGATCATTGC60193EST BW971285 AS: GGTGCCAGTGGCTACAATACGATA3A: CCCGTCTCATGACGGCAGCACAC61148AY669812 AS: GGTGCCAGTGGCTACAATACGM-CSF/CSF-2S: GAAACCGTAGGCGTCTG AS: CAGCGTGGAACACACC61193EST BW971285 AS: GGGCTGCAAGCGCGTCTGHPRT-1S: GGACTTGAAGACCAGCGTCTG AS: CGGCTCTCAAGTCATGTTTCTGG61105NM_214123 AS: CCCTCCCAGGCTTGGCCTGHS/EDFS: GCCATTGGCTGCTGGAGAGTTGC63159NM_214123 AS: CCTCCCCTAGCTGGTTGGATTACL1/beta/LAFS: AGCAGCGCCATGGTTGGTGGATG AS: CGTGCCGCTAGGTTAGCG62177NM_214239 AS: CGCTCCCAGGTAGGTGGATGGL1/beta/LAFS: ACACCGGTGGCTAGGTTAGC AS: CGGTGCCAAGGTTGGTGGATTG61105NM_		AS: CAAAGAGACACTGGGTTGGAA			
AS: CCACCCATGAACTGCATTAACTGCCCAGAAG         CCRLIAGSP       AS: CCACCTCGACTGACTGCGCCATTACGGAG         AS: CCACCTCGGCTTGTGTTACATCCAGGAG       147         NM_001097430         AS: CACACCTCGCTTGTGTTACATCCAGGAG       147         NM_001097430         AS: CACACCTCGCTTGTGTTACATCCAGGAG       61         AS: CACACCTCGCTTGTGATTG       62         CD40L       A: TACGCCCCAAGTCACTTCTG         AS: CACCATTGCCCATAC       62         AS: CACCATTGCCCATAC       60         AS: CACCATTGCCCTTCTGGACTC       60         AS: CACCATTGCCCTTCTGGACTC       60         AS: CACCATGTGCCTGTGTCTAGTG       71         CXL2/GRO beta       S: GCGTGCCGTGTGTCAGTGC         AS: CACCATTGCCCTTCTGGAACAAC       60         AS: CACCATGTGCCGTGTGGCAAAAC       60         AS: CACCATGCCGGCTACAATAC       61         CXL10/IP-10       S: CCCACAGTGGCACCAATAC         AS: GGTGCCAGTGGCACGTAACC       62       148         AY669812       AS: GTGGCAGTGCACTGG         AS: GGTGCCAGTGGCACGTTG       61       193         GATA3       A: GCGTGCCAGTGCTGG       62       150         AS: GGGGTGAAGACCTCAGGTCGTG       63       159       NM_201005149         AS: GGACTTGAATCATGGCTAGGTGCTG       63	CCRL1GSP	S: CTGCCTGCCTTCTTCACAGTAGCTTTCATC	72	na	EST BW955277
CCRL1nGSPA: CCTTCTATTCACTCTGCCCTTCTGG72naEST BW955277 SS: CCCGCCCTTGTCTACATCCAGGAGCCRL1/CX-CKRA: CACGATACTGGGCAGTAACG61147NM_001097430 AS: CACACCTCGCTTGTGGATTGCD40LA: ACAGATACTGGCACCTTCG61147NM_001097430 AS: CACACCCCCCAAGTCACCTTCGCX3CL1/FractalkineS: GCAGCTCCTAGTCCATTAC AS: CACCTCCGGTTGTTCATGG58167EST CK464144 AS: CACCATCCGGCCAAGTGCX3CR1A: ACCTTGCCCTTCTGGACTC AS: CACGTCCCGGTTGTTCATGG60182EST BX919199 AS: CAGTCCGGTTGTCATGGCXCL10/IP-10S: CCCACATGTGAGATCACTTGC60171NM_001001861 AS: CAGTCCCAGTAGACCATTGCCSCL30S: CCCGCCATGTGGAGACACTTGG60168NM_001008691 AS: CATCCTTATCAGTAGTGCCGGFOXP3A: GGTGCCAGTGGCTACAATAC AS: GGTGCCAGTGGCTCACAATAC60193EST BW971285 AS: GTGCTGCAGCAGTCTTGGATA3A: CCCGTCCTACTACGGAAAC AS: GTGCTGCTACTAGCGACTGTGG62150DQ108393 AS: GTGCTGCTACTAGGACTGTGGGM-CSF/CSF-2S: GAAACCGTGCTTGAAACTCATGT AS: GAGGCTTCAAGTGCTTGG62139NM_201005149 AS: GAGAGCCTTCAGGCTGTGL1 beta/LAFS: GGCATTGCTGGCTTACTGG62173NM_213861 AS: CCTCCCAGAGCTTCGGTTGTGL2/TCGFS: GCAGTGGCTGCTGGGTTAGTG62173NM_214205 AS: CCTCCCAGAGGTTGCTL4/BCGFS: CCAGCAGTGGTTAGTGG62173NM_214205 AS: CGGGCGCACTGGTTGGTGGATTGCL5/EDFS: TCGGGCTGCTGCTGGGGTTGC62100NM_214205 AS: TGGGGCTGCTTGCTGGGATTAGTL6/IFN beta 2S: ATCCAGGAGGCTGCTTGCTGGGATTGC<		AS: CCACCCATGAACTGCATTAACTGCCCAGAAG			
SS: CCCGCCCTTGTCTTACATCCAGGAG CCRL1/CCX-CKR A: ACAGATACTGGGCAGTAACG 61 147 NM_001097430 AS: CACACCTGCGCTTGTGTGATTG 62 172 AF248545 AS: AGAGTCCGCCCAAGTGAATG 62 172 AF248545 AS: CAGACTCCGCCCAAGTGAATG 72 CX3CL1/Fractalkine S: CGCAGTCGCTTACC 58 167 EST CK464144 AS: CACCCATTCTGACCCAGAAG CX3CR1 A: ACCTTGCCCTTCTGGACTC 60 171 NM_001001861 AS: CGGGTCGCGGTTGTTCATGG 60 171 NM_001001861 AS: CGGGTCGTCGTTCTGGACCG 60 168 NM_001008691 AS: CGGGTCGTGTTCAGTGG 60 168 NM_001008691 AS: CGGGTCGTGGTTGGTCAGTGG 60 168 NM_001008691 AS: CGGTGCCAGTGGTCAGTGCG 60 168 NM_001008691 AS: CGGTGCCAGTGGTCAGTGCG 60 168 NM_001008691 AS: GGGTGCAGTGTTGGGACAAAC 60 193 EST BW971285 AS: GGGTGCCAGTGGCTGCT GATA3 A: CCCGTCCTAGTCGGAAAC 60 193 EST BW971285 AS: GTGGTGGATGGACGTCTTG GM-CSF/CSF-2 S: GGAACCGTAGGCTGG AS: CGGGCCAGTGGCTGG GM-CSF/CSF-2 S: GGAACCGTAGGCTGG HPRT-1 S: GGACTTGAGACGACG L1 beta/LAF S: GGACTGGCCACTGTG AS: CGTGCGCCAGTGGCTGG L2/TCGF S: CACACTGGGCTCATGG L1/BCGF S: CACCCTGGCTTGGAGTGCTG L1/BCGF S: CACCCTGGCTTGGAGTGGC L1/BCGF S: CACCCTGGCTTGGATTAC L1/BCGF S: CACCCTGGCTTGGAGTGGTGG L5/EDF S: CGACCTGGGTGGGTTAGTG L5/EDF S: GGAGCCGCGCTGTGG L6/IFN beta 2 S: ATCGGGCGAGTGTGG L6/IFN beta 2 S: ATCGGGCGCTGCTGG L6/IFN beta 2 S: ATCGGGCGCTGCTGG L6/IFN beta 2 S: ATCGGGCGTGGTGGAGGCTGCTG L6/IFN beta 2 S: ATCGGGCGCTGCTGG L6/IFN beta 2 S: ATCGGGCTGGTGGAGGGCGTC L6/IFN beta 2 S: ATCGGGCTGGCTTAGTG L6/IFN beta 2 S: ATCGGGCTGGCTGGTGG L6/IFN beta 2 S: ATCGGGCTGGCTGGCTTAGTG L6/IFN beta 2 S: ATCGGGCTGGCTGGTTGG L6/IFN beta 2 S: ATCGGGGCGACTGCTG L6/IFN beta 2 S: ATCGGGCTGGCTTGGTTGG L6/IFN beta 2 S: ATCGGGCTGGCTGGTTGG L6/IFN beta 2 S: ATCGGGCTGGCTGGTTGGT L6/IFN beta 2 S: ATCGGGCGCTGCTTGGTGGATGG L6/IFN beta 2 S: ATCGGGGCGACTGCTGGGATGG L6/IFN beta 2 S: ATCGGGCTGGCTTGGTGGATGG L6/IFN beta 2 S: ATCGGGCGGCTGCTGCTGGGATGG L6/IFN beta 2 S: ATCGGGGCGACTGCTGGGATGG L6/IFN beta 3 S: TGCTGCTTTGTGGGAGGGGGGAGTGC L6/IFN beta 4 S: TGCTGGCTTGGTGGGAATG L6/IFN beta 4 S: TGCTGGCTTGGTGGGAATG AS: GGTGGAAAGGGTGGGAATG AS: TGCTGGCTTGGTGGGAATG AS: TGC	CCRL1nGSP	A: CCTTCTATTCACTCTGCCCTTCTGG	72	na	EST BW955277
CCRLI/CCX-CKR       A: ACAGATACTGGGCAGTAAGG       61       147       NM_001097430         AS: CACACTCGCTTGTGATTG       62       172       AF248545         CD40L       A: TACGCCCAAGTCACCTTCTG       62       172       AF248545         CX3CL1/Fractalkine       S: GCAGCTCCTAGTCCATTAC       58       167       EST CK464144         AS: CACCATTCGGCCTTCTGGACTC       60       182       EST BX919199         AS: ACCTTGCCGTTGTTCATGG       60       171       NM_001001861         AS: TGGCTAGCTTCTAGGG       60       168       NM_001008691         AS: CACCATCTTGGAACTACCGG       60       168       NM_001008691         AS: CATCCTTATCAGTAGTGCCGG       60       168       NM_001008691         AS: CATCCTTATCAGTAGTGCCG       60       168       NM_001008691         AS: CATCCTTATACAGTAATACC       65       148       AY669812         GATA3       A: CCCGTCCTACTACGACACC       60       193       EST BW971285         AS: GTGGTGGCTCATAGTGGTCTG       62       150       DQ108333         GM-CSF/CSF-2       S: GAAACCGTAGTCGTCTGTG       62       150       DQ108393         AS: GTGCTGCTCATAGTGCTCTGG       62       139       NM_001005149         AS: CACCATGGCTCAGTCGTCTG       63		SS: CCCGCCCTTGTCTTACATCCAGGAG			
AS: CACACCTCGCTTTGTGATTG CD40L A: TACGCCCAAGTGAACTGTCTG AS: AGACTCCGCCCAAGTGAATG CX3CL1/Fractalkine S: GCAGCTCCTAGTCCATTAC AS: CACCCATCTGGACCAGAAG CX3CL1/Fractalkine A: CCCGTCGTGGCATTAC AS: CACCCTTCTGGACCAGAAG CX3CL1/Fractalkine S: GCAGCTCCTGGTTCATGG CX3CL1/GP.10 AS: ACGTTGCCTTCTGGACTC AS: TGGTGCAGTTGTCATGG CXCL10/IP-10 S: CCCACAGTTGAGATCATTGC AS: GGGTGCAGTTGTGAGATCATTGC FOXP3 A: GGTGCAGTCTCGGGAACAC AS: GGGCCAGTGGCTAGATAC GATA3 A: GGTGCAGTCTCGGGACAAC AS: GGGCGCAGGGCGCTGG GM-CSF/CSF-2 S: GAAACCGTGGGCTG AS: GGAGCTCTGAGTGGTGGA AS: GGGTGCAGTGTCGTGTGG HPRT-1 S: GGACTGCCATGTTGGG HPRT-1 S: GGACTGCAAGTGTCTGG AS: GGAGCTTCGAAACC AS: GGAGCGTGCAAGTGTCTGG HPRT-1 S: GGACTGCCAGGGGCTCAAGTGG HPRT-1 S: GGACTGCCAGGGGTGCAACAC AS: GGAGCGTGGATGGACGTCTG AS: GGAGCTTGAATCAGTGTGTGG HPRT-1 S: GGACTGCCATGGTGTGG HPRT-1 S: GGACTGCCATGGTCTGG AS: GGAGCTTGGATGACGTCGTGG HPRT-1 S: GGACTGCCATGGTCTGG AS: GGAGCTTCGGATGACGCCATGG HPRT-1 S: GGACTGCCATGGTGTGG HPRT-1 S: GGACTGCCTGGCTGGGTGG HPRT-1 S: GGACTGCCTGGCTGGG HPRT-1 S: GGACTGGCTGCTGG HI beta/LAF S: AGAAGGCCCATCGTCTGG HI beta/LAF S: GGACTGCCTGGCTTGG HI beta/LAF S: GGACTGCGCTGGGTTGG HI beta/LAF S: GGACTGCGCTGGGTTGG HI beta/LAF S: GGACTGCGCTGGGGTGGATTAC AS: CCCTCCCAGGGCTTGGGTTGG HI beta/LAF S: GGAGCCGCTTGGGTTGG HI beta/LAF S: GGAGCTGCCTGGCTTGG HI beta/LAF S: GGAGCCGCATCGTCGTGG HI beta/LAF S: GGAGCCGCTTGGGTTGG HI beta/LAF S: GGAGCCGCTTGGGTTGG HI beta/LAF S: GGAGCCGCTTGGGTTGGG HI beta/LAF S: GGAGCCGCTTGGGTTGGG HI beta/LAF S: GGAGCCGCTGGCTGGGTAGG HI beta/LAF S: GGAGCCGCCTGCGTGGGGGGGGGG HI BI BEA/LAF S: TGGGGGCGCCTGCGTGGGGGGGGGGGGGGGGGGGGGGG	CCRL1/CCX-CKR	A: ACAGATACTGGGCAGTAACG	61	147	NM_001097430
CD40LA: TACGCCCAAGTCACCTTCTG62172AF248545AS: AGACTCCGCCCAAGTGAATGS: GCAGCTCCTAGTCCATTACS8167EST CK464144AS: CACCATTCTGACCCAGAAGAS: CACCATTCTGACCCAGAAG182EST BX919199AS: ACGGTCCTGGCTTCTGGACTC60182EST BX919199AS: ACGGTCCTGGCTTCTGGACTC60171NM_001001861CXCL2/GRO betaS: TGCTGCTCTGCTGTTTGG60168NM_001008691AS: CACCATTGTGAGATCATTGC60168NM_001008691AS: CATCCTTATCAGTAGTGGCCGAS: CATCCTTATCAGTAGTGGCCG182EST BW971285FOXP3A: GGTGCAGTCTCTGGAACAAC65148AY669812AS: GTGGTGGATGACAATAC60193EST BW971285GM-CSF/CSF-2S: GAAACCGTAGACGTCGTCTG62150DQ108393AS: GTGCTGCTCATAGGACGTCGTTG61190NM_001005149AS: GGAGCTTCAGGCTCATGG62139NM_001005149AS: GGAGGCTTCAGGTCTTG62139NM_213861L1/BCGFS: CACAGTGGCTACAGTTGG64105NM_214205L5/EDFS: TGGAGCTGCTACGTTAGTG64105NM_214205L5/EDFS: TGGAGCTGCCTACGTTAGTG62100NM_214399AS: TGGTGGCTTACGTAGGTGGATTG62100NM_214396L10/B-TCGFS: ACCAGATGGCGCACTGTGTGGAATG63123NM_214041AS: CCCTGCTTCTGGGATTGC62100NM_214041AS: CCCTGGCTACGTTGTGGAATGG64105NM_214041AS: TCCTGCTTCTGGGAATTGC62100N		AS: CACACCTCGCTTTGTGATTG			
AS: AGACTCCGCCCAAGTGAATG CX3CL1/Fractalkina AS: CACCATTCTGACCCAGAAG AS: CACCATTCTGACCCAGAAG CX3CR1 A: ACCTTGCCCTTCTGGACTC A: ACCTTGCCCTTCTGGACTC A: ACCTTGCCCTGCTGGAGTG AS: TGCGCTGCTCCTGGTGGTGG AS: TGCTGCTCCTGCTTCAGTG AS: TGCCTGCTCTCGGTTGG AS: TGCCTGCTCTCGGTTGG AS: CCCACATGTTGAGATCATTGC AS: CCCACATGTTGAGATCATTGC AS: CCCACATGTTGAGATCATTGC AS: GGTGCAGTCTCTGGAACAAC AS: GGTGCCAGTCTCTGGAACAAC AS: GGTGCCAGTGCTCACAGGAAAC AS: GGTGCGAGTGCACGGCAAAAC AS: GTGCTGGCAATGGCGCG AS: GGTGGGAGGAGGCTTTG GM-CSF/CSF-2 S: GGAACCGTGAACAGCGTCGTG AS: GGACTTGAATCATGTTGTG AS: GGACTTGAACCATGGTCTGG AS: GGACGTGCCAATGGCTCGG HPRT-1 S: GGACTTGAATCATGTTTGTG AS: GAACGTGCACATCGTCTTG AS: GAACGTGCACTCGTCTG AS: GAACGTGCACTCGTCTG AS: GGACTTGCAACTCGTCTG AS: GCCATTGCTGCTCTGG HPRT-1 S: GGACTTGAACCATGGTTGG HJ beta/LAF S: GGCCATGCTCTGCTGGATTAC AS: CCCTCCCAGAGCTTCGGCTTG HJ/BCGF S: GCCATTGCTGCTGCTGGTGGTG HJ/BCGF S: GCCATGCTGCTGCTGGTGGTG HJ/BCGF S: CCACCGGCCTACGTCGTGG HJ/BCGF AS: TGGTGGGCGCACGTTGG HJ/BCGF AS: TGGGGGGAACGCGTTGG HJ/BCGF AS: TGGGGGGCGCCACGTTGGGCTTG HJ/BCGF S: CCACCGGCTGCTGGTGGTTG HJ/BCGF AS: TGGGGGCGCCACGTTGGGCTTG HJ/BCGF AS: TGCGCCTACGCCACGTTGGT HJ/BCGF AS: TCCTGCCTTGCGGCTTGGGCTTG AS: TCCTGCCTTGCGGCTTGGG AS: TCCTGCCTTGCGGCTTGGGCTTG HJ/BCGF AS: TCCTGCCTTCGGCATTGTG AS: TCCTGCCTTGGGATTGC AS: TCCTGCCTTGGGAATGG AS: TCCTGCCTTGGGAATGGGAATGG AS: TCCTGCCTTGGGAATGGC AS: TCCTGCCTTGGGAATGGAATGG AS: TCCTGCCTTGGGAATGGGAATGGAATGGACGACTGGCTGG	CD40L	A: TACGCCCAAGTCACCTTCTG	62	172	AF248545
CX3CL1/FractalkineS: GCAGCTCCTAGTCCATTAC58167EST CK464144 AS: CACCATTCTGGACCCAGAAGCX3CR1A: CCCTTCGCCTCTGGACTC60182EST BX919199 AS: ACGTCCCGGTTGTTCAGTGAS: ACGTCCCGCTCTGCTCTAGTG60171NM_001001861 AS: TGGCTAGTGAGATCATTGCAS: CCCACATGTTGAGAGTCATTGC60168NM_001008691 AS: CCCACATGTTGAGAGTCATTGCFOXP3A: GGTGCAGTCTCTGGAACAAC60193EST BW971285 AS: GGTGCGAGTGGCTACAATACGATA3A: CCCGTCCTACTACGGAAAC60193EST BW971285 AS: GTGGTGGATGGACGTCTTGGM-CSF/CSF-2S: GAAACCCTAGGTGCTGTG6091DQ108393HPRT-1S: GGACTGCATAGTGTTGTG6091DQ815175AS: GTGCTGCTATAGTGGTTGG62139NM_001005149 AS: GAGCCTTCAGCTCATGTGHPRT-1S: GGACTTGAGCTGATGAGTC63159NM_214123AS: CCTCCAGAGGCTGTGGGAGGTCTTG63159NM_214123L1 beta/LAFS: GGAGCCGCACGTCATGGTG64105NM_214205 AS: CTCCCCAGAGGTTTGAGTTCIL2/TCGFS: TGGAGCTGCCTACGTAGTGG64105NM_214205AS: CTCTCCGGGGTGCTTACTG62177NM_214399AS: TGGTGGCTTCTGGGTTTCCTG177NM_214399AS: TGGTGGCATGCTGCGAGTTGG62100NM_214399AS: TGGTGGCTTGCTGGATTGC62100NM_214399AS: TGGTGGCATGCTGCGGAGGAGTTCG62100NM_2143867L6/IFN beta 2S: ATCAGGAGCCGCACTGCTGGAGTTC62100NM_2143867AS: TGGTGGCATGCTTCGGAGTTCGTGGATTG<		AS: AGACTCCGCCCAAGTGAATG			
AS: CACCATTCTGACCCAGAAG CX3CR1 A: ACCTTGCCCTTCTGGACTC 60 182 EST BX919199 AS: ACGGTCCGGTTGTTCATGG CXCL2/GRO beta S: TGCTGCTCCTGCTTCAGTG 60 171 NM_001001861 AS: TGGCTATGACTTCCGTTTGG CXCL10/IP-10 S: CCCACATGTTGAGATCATTGC 60 168 NM_001008691 AS: CATCCTTATCAGTAGTGGCCG FOXP3 A: GGTGCCAGTCTCTGGAACAAC 65 148 AY669812 AS: GGTGCCAGTGGCTACAATAC GATA3 A: CCCGTCCTACTAGGAACAAC 60 193 EST BW971285 AS: GTGGTGGATGGACGTCTTG GM-CSF/CSF-2 S: GAAACCGTAGGCGGCTGC 62 150 DQ108393 AS: GTGCTGCTCATAGTGCTTGG HPRT-1 S: GGACTTGAATCAGTTTGTG 60 91 DQ815175 AS: CAGATGTTTCCAAACTCAAC IL 1 beta/LAF S: GAGAGCCATCGTCTTG 62 139 NM_001005149 AS: GAGAGCCTTCAGGCTCATGTG IL2/TCGF S: GCCATGGTGGTTATCC 65 173 NM_214123 AS: CTTCTCCGAGGCTTAGTGG L5/EDF S: GGAGGCGCTCTGGTTTGC AS: CCTCCAGAGCTTGGTTGC GM-CSF/CSF-2 S: GGAGGCGTCTGGCTTG 62 177 NM_214399 AS: CTGCTCATACGAGTTC AS: TGGTGGAACGGACTTGTGG L6/IFN beta 2 S: TCCTGCTTGTGGATTG L10/B-TCGF S: ACCAGAGGCCTTGGCATTG L10/B-TCGF S: ACCAGAGGCCTTGGAATG	CX3CL1/Fractalkine	S: GCAGCTCCTAGTCCATTAC	58	167	EST CK464144
CX3CR1A: ACCTTGCCCTTCTGGACTC60182EST BX919199 AS: ACGGTCCGGTTGTCATGGAS: ACGGTCCGGTTGTCATGG60171NM_001001861 AS: TGGCTATGACTTCCGTTTGGCXCL10/IP-10S: CCCACATGTTGAGATCATTGC60168NM_001008691 AS: CATCCTTATCAGTAGTGGCGGFOXP3A: GGTGCCAGTCTCTGGAACAAC60193EST BW971285 AS: GGTGCCAGTCGTCACAATACGATA3A: CCCGTCCTACTACGGAAAC60193EST BW971285 AS: GTGGTGGATGGACGTCTTGGM-CSF/CSF-2S: GAAACCGTAGACGTCGTCTG62150DQ108393AS: GGTGCCCATGGCTACAACTCAAC6091DQ815175 AS: GGGCTGCTTGGTTGGAS: CAGATGTTTCCAAACTCAACHPRT-1S: GGACTTGAATCATGTTTGTG6091DQ815175 AS: CAGATGTTTCCAAACTCAACL1 beta/LAFS: AGAAGAGCCCATCGTCCTTG AS: CACCCTGGTCTGCTTGGTGG62139NM_001005149 AS: GCATGCTCCAGGTCTGGTGTCL2/TCGFS: CCACCTGGTCTGCTTACTG AS: CCTCCCAGAGCTTCGGTGTTCTCTG AS: CCTCTCCGTGGTGTTCTCTG51173NM_214205 AS: CTCTCCGCTGCTTGCTGGATTCL5/EDFS: TGGAGCTGCCTACGTTAGTG AS: TGGTGGGCATCGTTGGTTGCTG62177NM_214399 AS: TGGTGGCTTGCTTGGATTCL8/CXCL-8S: TCCTGCTTTGTCGGATTG AS: TGGTGGCAGAGGTTGGAAGGT AS: TGGTGGGAAGGTGGAAGGT62100NM_213867 AS: GGTGGGAAGGTGGAAAGGTL10/B-TCGFS: ACCAGATGGGCACTTGTGGAATG65123NM_214041 AS: TCTCTGCCTTCGGCATTACG		AS: CACCATTCTGACCCAGAAG			
AS: ACGGTCCGGTTGTTCATGG CXCL2/GRO beta S: TGCTGCTCTAGTG 60 171 NM_001001861 AS: TGCTATGACTTCCGTTTGG CXCL10/IP-10 S: CCCACATGTTGAGATCATTGC 60 168 NM_001008691 AS: CATCCTTATCAGTAGTGCCG FOXP3 A: GGTGCAGTCTCTGGAACAAC 65 148 AY669812 AS: GGTGCCAGTGGCTACAATAC 60 193 EST BW971285 AS: GTGGTGGATGACGTCGTCTG GATA3 A: CCCGTCCTACTAGGAACAAC 60 193 EST BW971285 AS: GTGGTGGATGACGTCGTCTG 62 150 DQ108393 AS: GTGCTGCTCATAGTGCTTGG GM-CSF/CSF-2 S: GAAACCGTAGACGTCGTCTG 60 91 DQ815175 AS: CGACTTGAATCATGTTTGTG 60 91 DQ815175 AS: CGACTTGAATCATGTTGTG 62 139 NM_001005149 AS: GAGAGCCTTCAGCTCATGTG IL1 beta/LAF S: AGAAGAGCCCATCGTCCTTG 62 139 NM_001005149 AS: GCACTTGGGATTTACCAAC IL2/TCGF S: CCAACCTGGTCTGCTGCTG IL4/BCGF S: CAACCCTGGTCTGCTTGC 65 173 NM_214861 AS: CTCTCCCGAGAGGTTTGCTGG IL5/EDF S: TGGACTTGCAGCTCAGTTG IL5/EDF S: TGGACGTGCTACGTTAGTG 62 177 NM_214205 AS: TGGCGTTCAGCTTGCGTAGTG IL5/EDF S: TGGAGCTGCCTACGTTGGT IL6/IFN beta 2 S: ATCAGGAGACCTGCTTGGATTGC IL6/IFN beta 2 S: ATCAGGAGACCTGCTTGGATTGC IL6/IFN beta 2 S: ATCAGGAGACCTGCTTGGATTG IL6/IFN beta 2 S: ATCAGGAGACCTGCTTGGATTG IL6/IFN beta 2 S: ATCAGGAGAGTGCTC IL6/IFN beta 2 S: ATCAGGAGAGCTTGCTGGGATTGC IL6/IFN beta 2 S: ATCAGGAGACCTGCTTGGATG IL6/IFN beta 2 S: ATCAGGAGAGCTGCTTGCTGGATTG IL6/IFN beta 3 S: CCTGCTTTCTGCGAGTTGC IL6/IFN beta 4 S: TCCTGCTTTCTGGATTG AS: TGGTGGCTATGTTGCTGGATTG IL6/IFN beta 5 S: AGAGGGGCTTTGCTGGATTG IL6/IFN beta 6 S: ATCAGGAGACTGGTTGGTTGCTGGATTG IL6/IFN beta 7 S: ATCAGGAGACTGGTTGGATTG IL6/IFN beta 8 S: TCCTGCTTTCTGGAATG IL6/IFN beta 7 S: ATCAGGAGAGGTGTGGAATG IL6/IFN beta 8 S: TCCTGCTTTCTGCAGCTTGC 62 100 NM_213867 AS: TGGTGGGAAAGGTGGGAATG IL10/B-TCGF S: ACCAGATGGCGACTTGGTTGG 65 123 NM_214041 AS: TCTCTGCCTTCGGCATTACG	CX3CR1	A: ACCTTGCCCTTCTGGACTC	60	182	EST BX919199
CXCL2/GRO betaS: TGCTGCTCCTGCTTCAGTG60171NM_001001861 AS: CGCCACATGATGACTTCCGTTTGGCXCL10/IP-10S: CCCACATGTTGAGATCATTGC60168NM_001008691 AS: CATCCTTATCAGTAGTGCCGFOXP3A: GGTGCAGTCTCTGGAACAAC65148AY669812AS: GGTGCCAGTGGCTACAAGGGAAC60193EST BW971285 AS: GTGGTGGAGAGTCTTGGATA3A: CCCGTCCTACTACGGAAAC6091DQ108393AS: GTGGTGGGAGGGCGTCTTG62150DQ108393GM-CSF/CSF-2S: GAACCGTAGACGTCGTCG62139NM_001005149 AS: CAGATGTTTCCAAACTCAACHPRT-1S: GGAGCCATCGTCGTGGATTTAC63159NM_214123AS: CCTCCCAGAGCCTTGCGGATTTAC63159NM_214123IL2/TCGFS: CAACCCTGGTCGTGATTGGTG65173NM_214123AS: CTTCTCCGTGGTGTGTAGTG64105NM_214205IL4/BCGFS: ACACCCTGGCTTGATGGTG62177NM_214205AS: TGGTGGCTACTGGTGGTGTGTG62177NM_214205AS: TGGTGGCTACTGGTTGGTGGGATTG62177NM_214399AS: TGGTGGCTTGTTGTGGATTC62100NM_213867IL8/CXCL-8S: TCCTGCTTTCTGGATTG65123NM_214041AS: TCTGCCTTCGGCATTGGGATTG65123NM_214041		AS: ACGGTCCGGTTGTTCATGG			
AS: TGGCTATGACTTCCGTTTGG CXCL10/IP-10 S: CCCACATGTTGAGATCATTGC 60 168 NM_001008691 AS: CATCCTTATCAGTAGTGCCG 60 168 AY669812 AS: GGTGCCAGTGCTCTGGGAACAAC 65 148 AY669812 AS: GGTGCCAGTGGCTACAATAC 60 193 EST BW971285 AS: GTGGTGGATGGACGTCTTG 62 150 DQ108393 AS: GTGCTGCTCATAGTGGCTTGG 60 91 DQ815175 AS: GGACTGAATCATGTTTGTG 60 91 DQ815175 AS: CAGATGTTTCCAAACTCAACC 7 HPRT-1 S: GGACTTGAATCATGTTTGTG 60 91 DQ815175 AS: CAGATGTTTCCAAACTCAACC 7 H1 beta/LAF S: AGAAGAGCCCATCGTCCTTG 62 139 NM_001005149 AS: GGACTTGCAGCTCAGTGTGTG IL2/TCGF S: GCCATTGCTGGGATTTACC 63 159 NM_213861 AS: CCTCCAGAGCTTTGAGTTC 65 173 NM_214123 AS: CTTCTCCGTCGTGATGATGGTTGG 64 105 NM_214205 AS: TGGCGGCTACGTGGTGGTGG IL5/EDF S: TGGAGGACCTGCTTGATG IL6/IFN beta 2 S: ATCAGGAGACCTGCTTGATG IL8/CXCL-8 S: TCCTGCTTGTTGCTGATGT IL8/CXCL-8 S: TCCTGCTTTGCTTGGATTC IL8/CXCL-8 S: TCCTGCTTGTTGCTGGATTG IL10/B-TCGF S: CCAGATGGTGGGAATGG IL10/B-TCGF S: CCAGATGGTGGGAATGGTG IL10/B-TCGF S: CCAGATGGGCACTTGGTTGTTGTGT AS: CTCTCGCCTTGGTGGTGGTGTGTTGTG IL10/B-TCGF S: ACCAGATGGGCGACTTGGTTG IL10/B-TCGF S: CCCAGATGGTGGGAATGGTG IL10/B-TCGF S: CCCAGATGGTGGGAATGGTG IL10/B-TCGF S: CCCAGATGGTGGGAATGGTG IL10/B-TCGF S: CCCAGATGGGCGACTTGGTTG IL10/B-TCGF S: CCCAGATGGGCGACTTGGTTG IL10/B-TCGF S: CCCAGATGGGCGACTTGGTTG IL10/B-TCGF S: CCCAGATGGGCGACTTGGTTG IL10/B-TCGF S: CCCAGATGGGCACTTGGTTG IL10/B-TCGF S: CCCAGATGGCCACTCGTTGGTGTGTGTGTG IL10/B-TCGF S: CCCAGATGGGCGACTTGGTTG IL10/B-TCGF S: CCCAGATGGCCACTCGCTTGGTGGTGAATGG IL10/B-TCGF S: CCCAGATGGGCGACTTGGTTG IL10/B-TCGF S: CCCAGATGGGCGACTTGGTTG IL10/B-TCGF S: CCCAGATGGGCGACTTGGTTG IL10/B-TCGF S: CCCAGATGGCCACTCCTCGGCATACG	CXCL2/GRO beta	S: TGCTGCTCCTGCTTCTAGTG	60	171	NM_001001861
CXCL10/IP-10S: CCCACATGTTGAGATCATTGC60168NM_001008691AS: CATCCTTATCAGTAGTGCCGAS: GATCCTTATCAGTAGTGCCG65148AY669812FOXP3A: GGTGCCAGTGGCTACAATAC60193EST BW971285GATA3A: CCCGTCCTACTACGGAAAC60193EST BW971285AS: GTGGTGGATGGACGTCGTCG62150DQ108393AS: GTGCTGCTCATAGTGCTTGG6091DQ815175GM-CSF/CSF-2S: GAAACCGTAGACGTCGTCTG6091DQ815175AS: GTGCTGCTCATAGTGCTTGG6091DQ815175HPRT-1S: GGACTGCATGGTCCTTG62139NM_001005149AS: CAGATGTTTCCAAACTCAGTC63159NM_213861L2/TCGFS: GCCATTGCTGCTGGATTTAC63159NM_214205AS: CTCTCCCGTCGTGTTGTGGTTCTTGG64105NM_214205AS: TGGCCATCGCTACGTTAGTG64105NM_214205AS: TGGCGGAAAGGTCGCTTGGTTGCTGGATTC62177NM_214399AS: TGGTGGCTAAGGTGCTGGTTGCTGGATTG62100NM_213867IL8/CXCL-8S: TCCTGCTTTGCAGCTTCCT62100NM_213867AS: GGTGGAAAGGTGTGGAATGIL10/B-TCGFS: ACCAGATGGGCGACTTGTTG65123NM_214041AS: TCTCTGCCTTCGGCATTACG65123NM_214041AS: TCTCTGCCTTCGGCATTACG		AS: TGGCTATGACTTCCGTTTGG			
AS: CATCCTTATCAGTAGTGCCG FOXP3 A: GGTGCAGTGCTCTGGAACAAC 65 148 AY669812 AS: GGTGCCAGTGGGCTACAATAC 60 193 EST BW971285 AS: GTGGTGGATGGACGTCTTG 62 150 DQ108393 AS: GTGCTGCTCATAGTGGCTTGG 62 150 DQ108393 AS: GTGCTGCTCATAGTGCTTGG 60 91 DQ815175 AS: CAGATGTTTCCAAACTCAAC 7 HPRT-1 S: GGACTTGAATCATGTTTGTG 60 91 DQ815175 AS: CAGATGTTTCCAAACTCAAC 7 IL1 beta/LAF S: AGAAGAGCCATCGTCCTTG 62 139 NM_001005149 AS: GGCATGCTGCTGGAGTTACG 63 159 NM_213861 AS: CCTCCCAGAGCTTGCTGAGTCC IL2/TCGF S: CCAACCCTGGTCTGCTGG 65 173 NM_214123 AS: CTCTCCCGTCGTGTGTGTGG IL5/EDF S: TGGAGCTGCCTACGTTAGTG 64 105 NM_214205 AS: TGGCTGCTGCTGCTGATGTG 62 177 NM_214205 AS: TGGCGGAGCCTTCGTGATTG IL5/EDF S: TGGAGGCGCCTACGTTGATG 62 177 NM_214399 AS: TGGTGGCTTGCTGATGTG 62 177 NM_214399 AS: TGGTGGCTTGCTGGATTG IL5/CXCL-8 S: TCCTGCTTTGCGGATTG IL10/B-TCGF S: ACCCGATGGGCGACTTGTG IL10/B-TCGF S: ACCAGATGGGCGACTTGTTG	CXCL10/IP-10	S: CCCACATGTTGAGATCATTGC	60	168	NM_001008691
FOXP3A: GGTGCAGTCTCTGGAACAAC65148AY669812AS: GGTGCCAGTGGCTACAATAC60193EST BW971285GATA3A: CCCGTCCTACACGGAAAC60193EST BW971285AS: GTGGTGGATGGACGTCGTCTG62150DQ108393GM-CSF/CSF-2S: GAAACCGTAGAACGTCGTCTG6091DQ815175AS: GTGCTGCTCATAGTGCTTGG6091DQ815175HPRT-1S: GGACTTGAATCATGTTTGTG62139NM_001005149AS: CAGATGTTTCCAAACTCAACCAS: GAGAGGCCCATCGTCGTGGTGT62139NM_213861L2/TCGFS: GCCATTGCTGCTGGGATTAC63159NM_213861AS: CCTCCCAGAGCTTTGAGGTC65173NM_214123AS: CTTCTCCGTCGTGTTACTG64105NM_214205AS: TCGCCTATCAGCAGAGTTCG62177NM_214399AS: TCGGCGGCTTTGCTGCAGGTGTGCTTGCTG62100NM_213867L8/CXCL-8S: TCCTGCTTTCTGCAGCTCTC62100NM_213867L10/B-TCGFS: ACCAGATGGCGACTTGTTGTG65123NM_214041AS: TCTCTGCCTTCGGCATTACGAS: TCTCTGCCTTCGGCATTACGASAS		AS: CATCCITATCAGTAGTGCCG			
AS: GGTGCCAGTGGCTACAATAC60193EST BW971285AS: GTGGTGGATGGACGTCTG62150DQ108393AS: GTGGTGCTCATAGTGCTTGG62150DQ108393HPRT-1S: GGACTTGAATCATGTTTGTG6091DQ815175AS: CAGATGTTTCCAAACTCAAC62139NM_001005149IL1 beta/LAFS: AGAAGAGCCCATCGTCGTGGATTAC63159NM_213861AS: CCCTCCAGAGCTTTGGGTGGATTAC63159NM_21423IL2/TCGFS: CCAACCCTGGTCTGCTTACTG65173NM_214123AS: CTTCTCCGTCGTGTTTCTGG64105NM_214205AS: TCGCCATCAGCTTAGTG64105NM_214205AS: TCGCCATCAGCTTGATGT62177NM_214399AS: TCGGCTTTCGCTGGATTC62100NM_214399AS: TCGGTGTTTCTCGGAATTG62100NM_213867AS: TCTCTGCTTTCTGCAGCTCC62100NM_213867AS: GGTGGAAAGGTGTGGAATG65123NM_214041AS: TCTCTGCCTTCGGCATTACG65123NM_214041	FOXP3	A: GGTGCAGTCTCTGGAACAAC	65	148	AY669812
GATA3A: CCCGTCTACTACGGGAAAC60193EST BW9/1285 AS: GTGGTGGATGGACGTCTTGGM-CSF/CSF-2S: GAAACCGTAGACGTCGTCTG62150DQ108393 AS: GTGCTGCTCATAGTGCTTGGHPRT-1S: GGACTTGAATCATGTTTGTG AS: CAGATGTTTCCAAACTCAAC6091DQ815175 DQ815175IL1 beta/LAFS: AGAAGAGCCCATCGTCCTTG AS: CAGAGCCTTCAGCTCATGTG62139NM_001005149 NM_010005149 AS: GAGAGCCTTCAGCTCATGTGIL2/TCGFS: GCCATTGCTGCTGGATTAC AS: CCCTCCAGAGCTTTGAGTTC63159NM_213861 AS: CCCTCCGTGGTGTCTCTGGIL4/BCGFS: CAACCCTGGTCTGCTTACTG AS: CTGCCTATCAGCAGAGTTCG65173NM_214123 AS: CTCTCCGTCGTTACTGIL5/EDFS: TGGAGCTGCCTACGTTAGTG AS: TCGCCTATCAGCAGAGTTCG62177NM_214205 AS: TCGCCTATCAGCAGAGTTCGIL6/IFN beta 2S: ATCAGGAGACCTGCTTGATG AS: TGGTGGCTTGCTTGCTGGATTC62100NM_214399 AS: TGGTGGCTTGCTGGATTCIL8/CXCL-8S: TCCTGCTTTCTGCGAGCTCTC AS: GGGTGGAAAGGTGTGGAATG65123NM_214041 AS: TCTCTGCCTTCGGCATTACG	G 1 77 1 9	AS: GGTGCCAGTGGCTACAATAC	<i>(</i> )	102	
AS: GIGGIGGAIGGACGICTIG62150DQ108393AS: GTGCTGCTCATAGTGCTTGG62150DQ815175HPRT-1S: GGACTTGAATCATGTTTGTG6091DQ815175AS: CAGATGTTTCCAAACTCAAC62139NM_001005149AS: GAGAGCCTTCAGCTGGCTGGATTAC63159NM_213861AS: CCCTCCAGAGCTTTGAGTTC63159NM_21423IL2/TCGFS: CCACCTGGTCTGCTTACTG65173NM_214123AS: CTTCTCCGTCGTGTTTCTCTG64105NM_214205AS: TCGCCTATCAGCAGAGTTGGATTG62177NM_214399IL5/EDFS: ATCAGGAGACCTGCTGGATTGATG62177NM_214399AS: TCGCCTATCAGCAGAGTTC7NM_21439913867IL8/CXCL-8S: TCCTGCTTTCTGCGAGCTCC62100NM_213867IL10/B-TCGFS: ACCAGATGGGCGACTTGTTG65123NM_214041AS: TCTCTGCCTTCGGCATTACG65123NM_214041	GATA3	A: CCCGTCCTACTACGGAAAC	60	193	EST BW971285
GM-CSF/CSF-2S: GAAACCGTAGACGTCGTCGTCGG62150DQ108393 AS: GTGCTGCTCATAGTGCTTGGHPRT-1S: GGACTTGAATCATGTTTGTG6091DQ815175AS: CAGATGTTTCCAAACTCAACAS: CAGAGGCCATCGTCCTTG62139NM_001005149H1 beta/LAFS: AGAAGAGCCCATCGTCGTGGATTAC63159NM_213861AS: CCTCCAGAGCTTTGAGTTCAS: CCTCCCAGAGCTTTGAGTTC114/BCGFS: CAACCCTGGTCTGCTTACTG65173NM_214123AS: CTTCTCCGTCGTGTTCTCTGAS: TCGCCTATCAGGCAGAGTTCG64105NM_214205142/14205IL5/EDFS: TGGAGCTGCCTACGTTGATG62177NM_214399AS: TCGCCTATCAGGAGACTTGGATTCAS: TCGCTGCTTGCTGGATTC118/CXCL-8S: TCCTGCTTTGCGGACTCC62100NM_213867IL10/B-TCGFS: ACCAGATGGGCGACTTGTTG65123NM_214041AS: TCTCTGCCTTCGGCATTACG112112/14041		AS: GTGGTGGATGGACGTCTTG	(2)	1.50	50100000
HPRT-1 S: GGACTTGAATCATGTTGTG 60 91 DQ815175 AS: CAGATGTTTCCAAACTCAAC IL1 beta/LAF S: AGAAGAGCCCATCGTCCTTG 62 139 NM_001005149 AS: GAGAGCCTTCAGCTCATGTG 63 159 NM_213861 AS: CCTCCAGAGCTTTGAGTTC 63 159 NM_213861 AS: CCTCCCAGAGCTTTGAGTTC 65 173 NM_214123 AS: CTTCTCCGTCGTGGTTGTTGTG 65 173 NM_214123 AS: CTTCTCCGTCGTGGTTGTTGTG 64 105 NM_214205 AS: TCGCCTATCAGCAGAGTTCG IL5/EDF S: ACAAGGAGACTGCTTGATG 62 177 NM_214399 AS: TCGCCTATCAGCAGAGTTC IL6/IFN beta 2 S: ATCAGGAGACCTGCTTGATG 62 100 NM_213867 AS: TCGTGCTTTGTGCAGCTCTC 62 100 NM_213867 IL8/CXCL-8 S: TCCTGCTTCGGGATTGGGAATG IL10/B-TCGF S: ACCAGATGGGCGACTTGTTG 65 123 NM_214041 AS: TCTCTGCCTTCGGCATTACG	GM-CSF/CSF-2	S: GAAACCGTAGACGTCGTCTG	62	150	DQ108393
HPR1-1S: GGACT1GAATCATGT1TGTG6091DQ815175AS: CAGATGTTTCCAAACTCAACAS: CAGATGTTTCCAAACTCAACCDQ815175IL1 beta/LAFS: AGAAGAGCCCATCGTCCTTG62139NM_001005149AS: GAGAGCCTTCAGCTCATGTGAS: GCATTGCTGCTGGATTTAC63159NM_213861IL2/TCGFS: GCACTGCTGGTTGCTGCTTACTG65173NM_214123AS: CCTCCAGAGCTTTGAGTTCAS: CTTCTCCGTCGTGTTCTCTG105NM_214205IL5/EDFS: TGGAGCTGCCTACGTTAGTG64105NM_214205AS: TCGCCTATCAGCAGAGTTCG100NM_214399AS: TGGTGGCTTTGTTGGAGTCIL6/IFN beta 2S: ATCAGGAGACCTGCTTGATG62100NM_213867AS: TCGTGCTTTGTCGCAGCTCTC62100NM_213867IL8/CXCL-8S: TCCTGCTTCTGCAGCTCTC62100NM_213867IL10/B-TCGFS: ACCAGATGGGCGACTTGTTG65123NM_214041AS: TCTCTGCCTTCGGCATTACG65123NM_214041	UDDT 1	AS: GIGCIGCICAIAGIGCIIGG	(0)	0.1	D0015175
IL1 beta/LAFS: AGAAGAGCCCATCGTCAAC62139NM_001005149AS: GAGAGCCTCAGCTCATGTG62139NM_001005149AS: GAGAGCCTTCAGCTCAGGTCAGTTC63159NM_213861IL2/TCGFS: GCCATTGCTGCTGGATTTAC63159NM_214861AS: CCTCCAGAGCTTTGAGTTC65173NM_214123AS: CTTCTCCGTCGTGGTAGTG64105NM_214205AS: TCGCCTATCAGCAGAGTTCG64105NM_214205IL5/EDFS: ATCAGGAGACCTGCTTGATG62177NM_214399AS: TCGCCTATCAGCAGGATTCG100NM_213867IL6/IFN beta 2S: ATCAGGAAAGGTGTGGAATG62100NM_213867IL8/CXCL-8S: TCCTGCTTTCTGCAGCTCTC62100NM_213867IL10/B-TCGFS: ACCAGATGGGCGACTTGTTG65123NM_214041AS: TCTCTGCCTTCGGCATTACG5123NM_214041	HPK1-1		60	91	DQ815175
IL1 beta/LAPS: AGAAGAGCCCATCGTCCTTG62139NM_001003149AS: GAGAGCCTTCAGCTCAGCTCATGTG11011	II 1 hoto/I AE	AS: CAGAIGITICCAAACICAAC	(2)	120	NIM 001005140
AS: GAGAGCCTTCAGCTCATGTGIL2/TCGFS: GCCATTGCTGCTGCAGAGTTTAC63159NM_213861AS: CCTCCAGAGCTTTGGAGTTCAS: CCTCCCAGAGCTTTGCTGGTTCTCTG113NM_214123IL4/BCGFS: CAACCCTGGTCGCTGCTTACTG65173NM_214123AS: CTTCTCCGTCGTGGTTCTCTGAS: CTCTCCGTCGGCTGGTGGGGGGGGGGGGGGGGGGGGGG	ILI Deta/LAF	S: AGAAGAGCCCAICGICCIIG	62	159	NM_001003149
IL2/TCGFS: GCCATIGCTGCTGGATTIAC63139NM_213881AS: CCTCCAGAGCTTTGAGTTCAS: CCTCCCAGAGCTTTGCGTACTG65173NM_214123AS: CTTCTCCGTCGTGGTTCTCTGAS: CTTCTCCGTCGTGTTCTCTG105NM_214205IL5/EDFS: TGGAGCTGCCTACAGCAGAGTTCG64105NM_214205IL6/IFN beta 2S: ATCAGGAGACCTGCTTGATG62177NM_214399AS: TGGTGGCTTTGTCTGGATTCAS: TGGTGGCTTTGTCTGCAGCTCTC62100NM_213867IL8/CXCL-8S: TCCTGCTTTCTGCAGCTCTG62100NM_213867AS: GGGTGGAAAGGTGTGGAATGIL10/B-TCGFS: ACCAGATGGGCGACTTGTTG65123NM_214041	II ATCCE	AS: GAGAGUUTICAGUTCATGTG	62	150	NIM 212961
IL4/BCGFS: CAACCCTGGTCTGCTTACTG65173NM_214123AS: CTTCTCCGTCGTGTTTCTCGAS: CTTCTCCGTCGTGTTCTCGNM_214205IL5/EDFS: TGGAGCTGCCTACAGCAGAGTTCGAS: TCGCCTATCAGCAGAGTTCGIL6/IFN beta 2S: ATCAGGAGACCTGCTTGATG62177NM_214399AS: TGGTGGCTTTGTCTGGATTCAS: TCCTGCTTTCTGCAGCTCTC62100NM_213867IL8/CXCL-8S: TCCTGCTTTCTGCAGCTCTC62100NM_213867AS: GGGTGGAAAGGTGTGGAATGIL10/B-TCGFS: ACCAGATGGGCGACTTGTTG65123NM_214041	IL2/ICGF	S: GUCAI IGUIGUIGGAI HAU	03	139	NM_213801
IL4/BCGFS. CAACCETGGTEGETTACTG63173NM_214123AS: CTTCTCCGTCGGGTTTCTGGAS: CTTCTCCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGGG	II A/DCCE	AS. CUTCEAGAGUITIGAGITE	65	172	NIM 214122
IL5/EDFS: TGGAGCTGCCTACGTTAGTG64105NM_214205AS: TCGCCTATCAGCAGAGAGTTCGAS: TCGGCTATCAGCAGAGAGTTCG116/JFN beta 2S: ATCAGGAGACCTGCTTGATG62177NM_214399AS: TGGTGGCTTTGTCTGGATTCAS: TCCTGCTTTCTGCAGCTCTC62100NM_213867IL8/CXCL-8S: TCCTGCTTTCTGCAGCTCTC62100NM_213867AS: GGGTGGAAAGGTGTGGAATGIL10/B-TCGFS: ACCAGATGGGCGACTTGTTG65123NM_214041AS: TCTCTGCCTTCGGCATTACGAS: TCTCTGCCTTCGGCATTACGAS: TCTCTGCCTTCGGCATTACGAS: TCTCTGCCTTCGGCATTACG	IL4/BCGF	S. CAACCEIGGICIGEITACIG	05	175	NWI_214125
IL6/IEN     S: TOGGCTATCAGCAGAGTTCG     64     105     NM_214205       IL6/IFN beta 2     S: ATCAGGAGACCTGCTTGATG     62     177     NM_214399       AS: TGGTGGCTTTGTCTGGATTC     AS: TCCTGCTTTCTGCAGCTCTC     62     100     NM_213867       IL8/CXCL-8     S: TCCTGCTTTCTGCAGCTCTC     62     100     NM_213867       AS: GGGTGGAAAGGTGTGGAATG     IL10/B-TCGF     S: ACCAGATGGGCGACTTGTTG     65     123     NM_214041       AS: TCTCTGCCTTCGCGCATTACG     AS: TCTCTGCCTTCGGCATTACG     AS: TCTCTGCCTTCGCAGTACG     AS: TCTCTGCCTTCGGCATTACG	II 5/EDE	S: TGGAGCTGCCTACGTTAGTG	64	105	NM 214205
IL6/IFN beta 2       S: ATCAGGAGACCTGCTTGATG       62       177       NM_214399         AS: TGGTGGCTTTGTCTGGATTC       IL8/CXCL-8       S: TCCTGCTTTCTGCAGCTCTC       62       100       NM_213867         IL8/CXCL-8       S: TCCTGCTTTCTGCAGCTCTC       62       100       NM_213867         AS: GGGTGGAAAGGTGTGGAATG       IL10/B-TCGF       S: ACCAGATGGGCGACTTGTTG       65       123       NM_214041         AS: TCTCTGCCTTCGGCATTACG       AS: TCTCTGCCTTCGGCATTACG       AS: TCTCTGCCTTCGGCATTACG       AS: TCTCTGCCTTCGGCATTACG	11.5/12.01	AS TOCCOTATCAGCAGAGTTCG	04	105	1111_214205
ILIO/INTEGLI 2     SIMICIOSIONETECTETETETETIONE     62     1/1     NM_214037       AS: TGGTGGAAAGGTGTGGAATG     AS: GGGTGGAAAGGTGTGGAATG     62     100     NM_213867       ILIO/B-TCGF     S: ACCAGATGGGCGACTTGTTG     65     123     NM_214041       AS: TCTCTGCCTTCGGCATTACG     AS: TCTCTGCCTTCGGCATTACG     AS: TCTCTGCCTTCGGCATTACG	II.6/IEN beta 2	S: ATCAGGAGACCTGCTTGATG	62	177	NM 214399
IL8/CXCL-8S: TCCTGCTTTCTGCAGCTCTC62100NM_213867AS: GGGTGGAAAGGTGTGGAATGAS: GGGTGGAAAGGTGTGGAATGIL10/B-TCGFS: ACCAGATGGGCGACTTGTTG65123NM_214041AS: TCTCTGCCTTCGGCATTACGAS: TCTCTGCCTTCGGCATTACGAS: TCTCTGCCTTCGGCATTACGAS: TCTCTGCCTTCGGCATTACGAS: TCTCTGCCTTCGGCATTACG	11.0/11 14 Octa 2	AS: TGGTGGCTTTGTCTGGATTC	02	1//	14141_217377
AS: GGGTGGAAAGGTGTGGAATG IL10/B-TCGF S: ACCAGATGGGCGACTTGTTG 65 123 NM_214041 AS: TCTCTGCCTTCGGCATTACG	IL8/CXCL-8	S. TCCTGCTTTCTGCAGCTCTC	62	100	NM 213867
IL10/B-TCGF S: ACCAGATGGGCGACTTGTTG 65 123 NM_214041 AS: TCTCTGCCTTCGGCATTACG	HO, CACL-0	AS: GGGTGGA & AGGTGTGGA ATG	02	100	1111_215007
AS: TCTCTGCCTTCGGCATTACG	IL10/B-TCGF	S' ACCAGATGGGCGACTTGTTG	65	123	NM 214041
		AS: TCTCTGCCTTCGGCATTACG		120	
<b>IL12 p35</b> S: GGCCTGCTTACCACTTGAAC 64 180 NM 213993	IL12 p35	S: GGCCTGCTTACCACTTGAAC	64	180	NM 213993
AS: GCATTCATGGCCTGGAACTC	<b>r</b>	AS: GCATTCATGGCCTGGAACTC			

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## Intestinal response to Salmonella Typhimurium

## Table I. Continued.

Primer name	Primer sequence	Annealing	PCR	Accession
T Timer nume	Timer sequence	temperature	product	number
		(°C)	(hn)	number
II 12 n40	S: CTGA AGA AGACGGCATCACG	62	148	NM 214013
11.12 p40		02	140	1111214015
II 12	AS. ACCACTCACTCACTCAC	67	150	NM 212802
1115	A. IOGCOCICIOGIIGACICIO	07	139	INIVI_213803
H 15/H T	AS. CCATCOLOCUTIOCATAGO	60	164	NIM 214200
1L15/1L-1		00	104	INIM_214390
	AS: GGGAIGAGCAICACITICAG	66	102	A D 102602
IL1/a/CILA8		00	103	AB102095
H 19//CIE	AS: CACIIGGCCICCAGAICAC	(0)	120	FU110262
IL18/IGIF	S: ACATCAAGCCGIGIIIGAGG	60	129	EU118362
11 41	AS: CACIGCACAGAGAIGGIIAC	(2)	104	ND 4 014415
11.21	S: GGCACAGIGGCUCAIAAAIC	62	124	NM_214415
	AS: GCAGCAAIICAGGGICCAAG	(0)	100	11/027220
1L22/1L-11F	S: AAGCAGGICCIGAACIICAC	60	133	AY93/228
<b>XX 62</b> 40	AS: CACCUTTAATACGGCATTGG	-		
1L23 p19	S: CTCCTTCTCCGCCTCAAGATCC	70	82	PEDE Blast
<b>XX AT A</b> A	AS: TIGCIGCICCAIGGGCGAAGAC		1.50	200503228038657
IL27 p28	S: GCCCGCCACTTTGCTGAATC	64	152	EST BP439244
** **	AS: GGGCGAAGTGTCATGGAGAG			
1L33	S: AGCITCGCICIGGCCITAIC	63	126	EST BX924734
	AS: GCTGACAGGCAGCAAGTACC	<i></i>		
IFN gamma	A: GCTCTGGGAAACTGAATGAC	60	167	NM_213948
	AS: TCTCTGGCCTTGGAACATAG			
iNOS	S: GAGAGGCAGAGGCTTGAGAC	62	178	EST BI344008
	AS: TGGAGGAGCTGATGGAGTAG			
LTA/ TNF beta	A: CTCCTCAGCGCTCAGAAGTC	64	172	NM_214453
	AS: GAGCGAAGGCTCCAAAGAAG			
MAdCAM-1	A: AGCCTGGGCTCCGTAAAGTC	68	155	NM_001037998
	AS: TGGTCAGGGAAGGCGAACAC			
NK-lysin	S: ATGCGACGGAGAGCAGTTC	60	156	X85431
	AS: GTGTCCTCGTTGGGTTGTG			
NOD2	A: GAGCGCATCCTCTTAACTTTC	63	66	NM_001105295
	AS: ACGCTCGTGATCCGTGAAC			
PBD-1	S: ACCGCCTCCTCCTTGTATTC	62	150	NM_213838
	AS: CACAGGTGCCGATCTGTTTC			
PBD-2	S: TTGCTGCTGCTGACTGTCTG	62	180	NM_214442
	AS: CTTGGCCTTGCCACTGTAAC			
PMAP-37/cathelicidin	S: GCAGTCCTCGGAAGCTAATC	62	166	L39641
	AS: CCCGTTCTCCTTGAAGTCAC			
PR-39/cathelicidin	S: TAATCTCTACCGCCTCCTGG	62	151	NM_214450
	AS: CCCGTTCTCCTTGAAGTCAC			
RPL-19	S: AACTCCCGTCAGCAGATCC	60	147	AF435591
	AS: AGTACCCTTCCGCTTACCG			
RORC/ROR gamma	A: TTCAGTACGTGGTGGAGTTC	60	141	EST BP164723
	AS: TGTGGTTGTCAGCGTTGTAG			
Secretory	S: ACTGGTGTCGCTGGGAAGAG	64	131	EST CJ025705
component	AS: GACCGTGAAGGTGCCATTGC			
SipA	S: CCAACGCAATGGCGAGTCAC	68	96	NC_003197.1
	AS: GCCGTCTCCGTTTGATGCGT			
SMAD2	A: TGAGTGCCTAAGTGACAGTG	60	143	EST DB812041
	AS: CCAGAAGAGCAGCAAATTCC			
SMAD3	A: CGCAGAACGTCAACACCAAG	62	139	EST BX926114
	AS: AGCTCATGGTGGCTGTGAAG			
STAT3	A: TGCAGCAGAAAGTGAGCTAC	60	166	NM_001044580
	AS: CCGGTCTTGATGACTAATGG			

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#### Table I. Continued.

Primer name	Primer sequence	Annealing temperature (°C)	PCR product (bp)	Accession number
STAT4	A: ACCATTCGCTGACATCCTTC	60	126	AB20984
	AS: TGGGAGCTGTAGTGTTTACC			
STAT5	A: CAGCCATCTGGAGGACTAC	60	109	EST CJ011824
	AS: CATCACGCCATCAAACCAC			
STAT6	A: TCCCAGCTACGATCAAGATG	60	171	EST CN155407
	AS: AGTGAGAGTGTGGTGGATAC			
T-Bet	A: TCAATCCTACTGCCCACTAC	60	151	EST CJ014895
	AS: TTAGGAGACTCTGGGTGAAC			
TBP-1	S: AACAGTTCAGTAGTTATGAGCCAGA	60	153	DQ845178
	AS: AGATGTTCTCAAACGCTTCG			
TGF beta	S: GAAGCGCATCGAGGCCATTC	64	162	NM_214015
	AS: GGCTCCGGTTCGACACTTTC			
TLR2	A: ACGGACTGTGGTGCATGAAG	62	101	NM_213761
	AS: GGACACGAAAGCGTCATAGC			
TLR4	A: TGTGCGTGTGAACACCAGAC	62	136	NM_001113039
	AS: AGGTGGCGTTCCTGAAACTC			
TLR5	A: CCTTCCTGCTTCTTTGATGG	61	124	NM_001123202
	AS: CTGTGACCGTCCTGATGTAG			
TNF alpha/TNFSF2	S: CCAATGGCAGAGTGGGTATG	62	116	X54859
	AS: TGAAGAGGACCTGGGAGTAG			

na: Not available.

was submitted to GenBank (NCBI accession No. NM\_001097430).

## 2.4. Messenger RNA expression analysis using real-time PCR

Many mRNA sequences have already been identified in the pig. When genes were not described in this species, tBLASTn searches of the GenBank and PEDEblast EST databases, using known human and murine amino acid sequences, were performed. This methodology enables the identification of porcine expressed sequence tags (EST) corresponding to human and murine sequences (Tab. I). Then, primers (Tab. I) were designed using Clone Manager 9 (Scientific & Educational Software) and were purchased from Eurogentec (Liège, Belgium).

Quantitative real-time PCR (qPCR) was performed using cDNA synthesized as previously described [29]. Diluted cDNA ( $40 \times$ ) was combined with primer/probe sets and IQ SYBR Green Supermix (Bio-Rad, Hercules, California, USA) according to the manufacturer's recommendations. The qPCR conditions were 95 °C for 3 min, followed by 45 cycles with denaturation at 95 °C for 15 s, annealing temperature (Tab. I) for 30 s and elongation at 72 °C for 30 s. Real time assays were run

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on a Bio-Rad iCycler iQ. The specificity of the qPCR reactions was assessed by analyzing the melting curves of the products and size verification of the amplicons. To minimize sample variation, tissue samples of similar size and location and identical quantities of high quality RNA with no signs of degradation were used. Samples were normalized internally using simultaneously the average cycle threshold (Ct) of Hypoxanthine PhosphoRibosyl-Transferase 1 (HPRT-1), Ribosomal Protein L 19 (RPL-19) and Tata Box Binding Protein 1 (TBP-1) [33] as references in each sample to avoid any artifact of variation in the target gene. HPRT-1, RPL-19 and TBP-1 genes were selected as the reference genes because of their extremely low variation among samples. A standard curve was generated using diluted cDNA. The correlation coefficients of the standard curves were > 0.995 and the concentration of the test samples were calculated from the standard curves, according to the formula  $y = -M \times Ct + B$ , where M is the slope of the curve, Ct the point during the exponential phase of amplification in which the fluorescent signal is first recorded as being statistically significant above background and B the y-axis intercept. All qPCR displayed efficiency between 90% and 110% according to the equation: qPCRefficiency =  $(10^{[-1/M]} - 1) \times 100$ . Expression data

are expressed as relative values after Genex macro analysis (Bio-Rad) [40].

#### 2.5. Statistical analysis

Data for the comparison of differences in mRNA expression between infected and non infected tissues are expressed as relative values. Most of the data were normally distributed as confirmed by the Shapiro-Wilk normality test (using Statistix 7.0<sup>®</sup>, Analytical software, Tallahassee, Florida, USA). When the data were paired and normally distributed, group means were compared using Student's Paired *t*-test (using GraphPad Prism<sup>®</sup> software version 3.00, GraphPad Software Inc., San Diego, California, USA). Paired, non-normally distributed data were analyzed using the Wilcoxon Signed Rank Test (Exact). Differences between groups were considered significant when P < 0.05.

#### 3. RESULTS

#### 3.1. Cloning of porcine CCRL1 gene

The porcine CCRL1 cDNA sequence (Gen-Bank accession number NM 001097430) was found to be 1053 nucleotides in length, encoding a predicted precursor protein of 350 amino acids such as human (GenBank accession number NM\_178445) and murine (GenBank accession number NM 145700) CCRL1. At the protein level, 88 and 85% of identity were found to human and murine homologous sequences, respectively. With the cloning of CCRL1, the sequences of the two receptors of CCL25, CCR9 and CCRL1, are now available. CCRL1 mRNA was expressed (between 21 and 28 Ct) in various tissues such as the duodenum, jejunum, ileum, colon, caecum, liver, thymus and heart (data not shown).

#### 3.2. Cytokine response to *Salmonella* Typhimurium in the gut loop model

To increase our understanding of the early immune response in vivo, we decided to assess the mRNA expression of various Th1, Th2 and newly described Th17 cytokines as well as transcription factors and antimicrobial molecules such as defensins and iNOS (generating Nitric oxide (NO)) after 24 h of infection in the porcine gut loop model.

Among cytokines, the mRNA expression inflammation associated cytokines such as IL6, IL8/CXCL8 and TNF alpha were strongly and significantly up-regulated, particularly in the GW (Tab. II). Similarly, mRNA expressions of Th1 cytokines, IL12 p35, IL12 p40, IL27 p28 and IFN gamma, were upregulated in infected GW and PP (Tab. II and Fig. 2). In contrast, Th2 (IL4, IL5, IL13 and IL33) and Th17 (IL17a, IL21, IL22 and IL23 p19) cytokine mRNA expressions were not upregulated except for IL33 mRNA expression which was significantly up-regulated in the PP (P = 0.022) (Tab. II). Regarding transcription factors associated to Th1 (T-Bet, STAT4), Th2 (GATA3, STAT6) and Th17 (RORC, STAT3) orientations, we did not detect any upregulation except for STAT4 in the infected GW. For regulatory cytokines, IL10 and TGF beta mRNA expressions were increased in infected GW versus control GW while the forkhead transcription factor FOXP3 was significantly more expressed in the PP than in GW and did not show any increase after infection (Tab. II). Antimicrobial peptide production has been assessed in few studies using intestinal epithelial cell lines and the SISP technique with contradictory results. Consequently, the mRNA expressions of PBD-1, PBD-2, PMAP37, PR39 and NK lysine have been assessed in the gut loop model. Only the expression of PBD-2 mRNA was increased in the infected PP (P < 0.05) (Tab. II). Furthermore, iNOS mRNA expression was strongly up-regulated in the infected GW and PP (Tab. II).

GM-CSF, IL1 beta, IL2, IL15, IL18 and LTA mRNA expressions were not up-regulated in the infected tissues (Tab. II).

APRIL and B cell activating factor (BAFF) and their implication in the IgA class-switch recombination (CSR) have been extensively studied in the last few years. In humans and mice, T-cell-dependent IgA CSR is induced by TGF beta and CD40L expressed on activated T cells. On the contrary, APRIL and BAFF directly mediate T-cell-independent IgA CSR. Consequently, we chose to investigate the mRNA expression of these cytokines, which make a link between epithelial cells and

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**Table II.** Statistical comparisons between mRNA levels of expression. Levels of expression are shown in the second column (High: Amplification around 17-24 cycle thresholds (*Ct*), very low more than 33 *Ct*). When the data were paired and normally distributed, group means were compared using Student's Paired *t*-test. Paired, non-normally distributed data were analysed using the Wilcoxon Signed Rank Test (Exact).

Messenger RNA Level of expression		<i>P</i> value					
		cPP vs. iPP	cGW vs. iGW	cPP vs. cGW			
APRIL/TNFSF13	High	0.096 >	0.127	0.069 <			
BAFF/TNFSF13B	High	0.006 ** <	0.058 <	0.009 ** >			
CCL20/MIP3 alpha	High	0.368	0.288	0.510			
CCL25/TECK	High	0.008 ** >	0.009 ** >	0.121			
CCL28/MEC	Moderate	0.381	0.114	0.027 * <			
CCR9	High	0.004 ** >	0.559	0.227			
CCR10	Moderate	0.433	0.213	0.148			
CCRL1/CCR11	High	0.059 >	0.318	0.573			
CD40L	Moderate	0.128	0.382	0.004 ** >			
CX3CL1/Fractalkine	Moderate	0.1727	0.007 ** <	0.083 >			
CX3CR1	Low	0.671	0.236	0.103			
CXCL2/GRO beta	Moderate	0.117	0.013 * <	0.637			
CXCL10/IP-10	High	0.002 ** <	0.010 * <	0.565			
FOXP3	Low	0.609	0.305	0.001 ** >			
GATA3	Low	0.220	0.399	0.048 * >			
GM-CSF/CSF-2	Low	0.452	0.156	0.227			
IL2/TCGF	Low	0.201	0.608	0.038 * >			
IL1 beta/LAF	Very low	na	na	na			
IL4/BCGF	Very low	0.616	0.061 <	0.383			
IL5/EDF	Moderate	0.086 >	0.375	0.639			
IL6/IFN beta 2	Moderate	0.013 * <	0.039 * <	0.592			
IL8/CXCL-8	High	0.149	0.001 ** <	0.843			
IL10/B-TCGF	Moderate	0.118	0.067 * <	0.806			
IL12 p35	Moderate	0.009 ** <	0.001 ** <	0.003 ** >			
IL12 p40	Moderate	0.003 ** <	0.027 * <	0.018 * >			
IL13	Very low	0.151	0.091 <	0.415			
IL15/IL-T	Moderate	0.263	0.142	0.813			
IL17a/CTLA8	Low	0.541	0.062 <	0.239			
IL18/IGIF	High	0.150	0.869	0.187			
IL21	Low	0.319	0.553	0.238			
IL22/IL-TIF	Moderate	0.100	0.761	0.684			
IL23 p19	Moderate	0.325	0.922	0.036 * <			
IL27 p28	Moderate	0.019 * <	0.105	0.239			
IL33	Moderate	0.022 * <	0.703	0.322			
IFN gamma	Moderate	0.010 * <	0.009 ** <	0.237			
iNOS	Moderate	0.009 ** <	0.015 * <	0.831			
LTA/TNF beta	Low	0.972	0.715	0.141			
MAdCAM-1	High	0.244	0.148	0.410			
NK-lysin	High	0.164	0.354	0.181			
NOD2	Moderate	0.013 * <	0.004 ** <	0.102			
PBD-1	Moderate	0.213	0.051 >	0.065 >			
PBD-2	Moderate	0.017 * <	0.280	0.025 * >			
PMAP-37/cathelicidin	Very low	0.013 * >	0.289	0.134			
PR-39	Very low	0.106	0.089 >	0.027 * >			
RORC/ROR gamma	Moderate	0.340	0.201	0.035 * <			

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Messenger RNA	Level of expression	P value			
		cPP vs. iPP	cGW vs. iGW	cPP vs. cGW	
Secretory component	High	0.026 * >	0.037 * >	0.122	
SMAD2	High	0.648	0.613	0.052 >	
SMAD3	Moderate	0.070 >	0.849	0.644	
STAT3	High	0.606	0.224	0.856	
STAT4	Moderate	0.363	0.015 * <	0.018 * >	
STAT5	Moderate	0.828	0.846	0.629	
STAT6	Moderate	0.828	0.691	0.155	
T-Bet	Low	0.710	0.207	0.127	
TGF beta	Moderate	0.253	0.002 ** <	0.005 ** >	
TLR2	Moderate	0.002 ** <	0.044 * <	0.797	
TLR4	Moderate	0.001 ** <	0.020 * <	0.021 * >	
TLR5	Moderate	0.020 * >	0.001 ** >	0.514	
TNF alpha/TNFSF2	Moderate	0.007 ** <	0.002 ** <	0.004 ** >	

cPP: Control Peyer's Patch (PP); iPP: Infected PP; cGW: Control Gut Wall (GW); iGW: Infected GW. \* P < 0.050, \*\* P < 0.010. na: Not available, expression too low. Higher (>) or lower (<) mRNA expression in the control than in the infected tissues.



**Figure 2.** (A) Schematic presentation of the T-helper type-1 (Th1) mediated immune response. (B) Relative mRNA expression of Th1 cytokines and transcription factors. Data were presented as mean  $\pm$  S.E.M. for a total of 8 control and 12 infected loops. \* P < 0.05, \*\* P < 0.01, ns: not significant (Student's Paired *t*-test or Wilcoxon Signed Rank Test (Exact)). DC: Dendritic Cell; cPP: Control Peyer's Patch (PP); iPP: Infected PP; cGW: Control Gut Wall (GW); iGW: Infected GW.

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**Figure 3.** Relative mRNA expression of CCL25 and its two receptors, CCR9 and CCRL1. Data were presented as mean  $\pm$  S.E.M. for a total of 8 control and 12 infected loops. \* P < 0.05, \*\* P < 0.01, ns: not significant (Student's Paired *t*-test or Wilcoxon Signed Rank Test (Exact)).

IgA secretion by B cells. While the mRNA expression of BAFF was significantly upregulated in the PP, there was no increase of its expression in the GW after 24 h of infection with *S*. Typhimurium (Tab. II). In contrast to BAFF, APRIL and CD40L were not up-regulated in infected tissues (Tab. II). Regarding TGF beta, which is more expressed as CD40L and BAFF in the PP, the mRNA expression was higher in the infected GW than in the control while there was no significant difference between control and infected PP (Tab. II).

#### 3.3. Transcript expression of intestinal chemokines and their receptors and mucosal addressin cellular adhesion molecule 1 in the gut following *Salmonella* Typhimurium inoculation

CCL25 and CCL28 play a crucial role in lymphocyte trafficking to the gut. Since little is known about their expression in the context of an intestinal infection, we decided to assess mRNA expression of these chemokines and their receptors (CCR9, CCR10 and CCRL1) in GW and PP collected 24 h after inoculation of S. Typhimurium in the gut loops. Surprisingly, important statistically significant down-regulations (P < 0.01) of CCL25 mRNA expression were detected in both PP and GW (Tab. II and Fig. 3). Similarly, but to a lower extent, the mRNA expressions of the two receptors of CCL25, CCR9 and CCRL1 were down-regulated, particularly in the PP which is rich in lymphocytes (Fig. 3). Regarding CCL28 and its receptor, CCR10, we did not observe any down- or up-regulations (Tab. II). Then, we investigated the expression of two chemokines (CX3CL1 and CCL20) involved in dendritic cell recruitment. CX3CL1 was up-regulated at the mRNA level (P < 0.01) in the infected GW while CCL20 mRNA expression did not increase in the infected PP nor in the infected GW (Tab. II). The receptor of CX3CL1, CX3CR1, was not up-regulated in infected PP as well as infected GW (Tab. II). Interestingly, CXCL10, a potent chemoattractant of Th1 CD4<sup>+</sup> and Natural Killer (NK) cells, was strongly up-regulated at the mRNA level in both infected PP and GW (Tab. II). Moreover, mRNA expression of CXCL2, a neutrophil chemoattractant was up-regulated in the infected GW (Tab. II). Regarding the mucosal addressin cellular adhesion molecule 1 (MAdCAM-1), controlling with its ligand, integrin  $\alpha 4\beta 7$ , the first steps of B and T cell migration to the gut, we did not observe any up-regulation in infected PP and GW (Tab. II).

#### 3.4. Pattern-recognition receptor mRNA expression in the gut following *Salmonella* Typhimurium inoculation

Intestinal epithelial cells and immune cells are able to sense the presence of pathogens via pattern-recognition receptors such as Toll-like receptors (TLR) and intracellular Nod-like receptors. TLR2, 4 and 5 and the nucleotide-binding oligomerization domain-2 (NOD2) are known for their implication in

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**Figure 4.** Relative mRNA expression of four pattern recognition receptors (NOD2, TLR2, TLR4 and TLR5) usually associated with *Salmonella*. Data were presented as mean  $\pm$  S.E.M. for a total of 8 control and 12 infected loops. \* *P* < 0.05, \*\* *P* < 0.01, ns: not significant (Student's Paired *t*-test or Wilcoxon Signed Rank Test (Exact)).

the recognition of *Salmonella* spp. Therefore, we assessed their mRNA expression in the gut loop model after inoculation of *S*. Typhimurium. Expressions of NOD2, TLR2 and TLR4 mRNA were significantly up-regulated both in PP and GW in response to *Salmonella* infection while the mRNA expression of TLR5 was significantly down-regulated in both tissues (Fig. 4).

#### 4. DISCUSSION

In the current article, the cloning of the porcine CCRL1/CCX-CKR, the second receptor of the intestinal chemokine CCL25 is reported and the mRNA expression of CCL25 and its two receptors is assessed for the first time in the context of *S*. Typhimurium infection. Moreover, a first broad assessment at the mRNA level of the innate immune response and the Th orientation was carried out in response to *S*. Typhimurium in a porcine gut loop model. Several genes involved in

the innate response and the establishment of the adaptive response displayed changed expression patterns, demonstrating the complex interactions between the facultative intracellular bacteria *S*. Typhimurium and the intestinal mucosa.

Several papers have focused recently on "scavenger receptors" which are "atypical" receptors playing a role in scavenging or altering the localization of chemoattractant molecules such as chemokines and complement molecules [8, 10, 25]. The "atypical" receptor family comprised the receptors D6, the Duffy Antigen/Receptor for Chemokines and CCRL1/CCX-CKR [8, 10]. CCRL1 was described in mice and humans but there was no data about this receptor in the pig [9, 16, 34, 37]. As previously shown, this receptor of CCL19, CCL21 and CCL25 is expressed in various tissues such as the duodenum, jejunum, ileum, colon, caecum, liver, thymus and heart [20, 34, 37]. At the protein level, the sequence of porcine CCRL1 is very close

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to the murine and human sequences with 85-88% identity suggesting similar function in swine. Data from in vitro studies suggest that CCRL1 may be able to act as a chemokine scavenger, at least with one of its chemokine ligands, CCL19 [9]. Further experiments are required to characterize the function of this receptor in the pig and its relation with CCL25. Interestingly, in the current study, parallel decreases were observed for the mRNA expression of CCR9 and CCRL1 receptors and the chemokine CCL25 in the context of an infection with S. Typhimurium confirming a previously observed influence of intestinal bacteria on this chemokine mRNA expression [29]. The drastic and significant reduction in the mRNA expression of CCL25 suggests a potential strategy of S. Typhimurium to reduce lymphocyte homing to the intestine. Indeed, it is well known that Salmonella enterica is able to develop many strategies to escape T cell immunity (for a review see [4]). In contrast to the CCL25/CCR9-CCRL1 axis, the CCL28/CCR10 axis was not induced. The induction of CCL28 is different from that of CCL25 which is atypical with the involvement of caudal-related homeobox 2 [13, 29].

Regarding the cytokines and the Th orientation, a clear up-regulation of the mRNA expression has been observed for IL27 p28, IL12 p35 and p40 and IFN gamma which are Th1 cytokines and for STAT4, a Th1-associated transcription factor. This profile further confirms and completes the Th1 orientation of the immune response to S. Typhimurium in the pig [7, 21, 32]. To our knowledge, this is the first broad assessment at the mRNA level of Th orientations in the pig. Indeed, IL27 p28, T-Bet and STAT4 (for Th1 orientation) have not been assessed in this context before. The Th1 response orientation with a strong up-regulation of the mRNA expression of CXCL10 has been previously observed in both PP and GW [39]. The expression of CXCL10, a potent chemoattractant of Th1-type CD4<sup>+</sup> and NK cells [31], has been shown recently to be controlled by TLR4 in the context of Salmonella infection [22] and TLR4 mRNA expression was strongly up-regulated in our conditions. With IL2 and

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IL18 mRNA expression, we did not observe any up-regulation. This could be explained by the time when the tissues were collected since up-regulation or down-regulation could take place earlier or later. Similarly, it was also probably too early or too late to detect any up-regulation in most of the transcription factor mRNA expressions. Only STAT4 mRNA, in the IL-12 signaling pathway, was more expressed in the infected GW. For genes associated with inflammation, an induction of IL6, IL8 and TNF alpha mRNA was observed at 24 h as previously shown in the pig [39]. However, in our conditions, IL1 beta and GM-CSF mRNA expressions were at very low levels with no increase in the infected tissues. In contrast to Th1 genes, Th2 and Th17 genes did not show any induction except for IL10 in the GW and IL33 in the PP. IL10 mRNA expression increase was associated with TGF beta mRNA expression increase in the same tissue. This double increase could be associated with the induction of T regulatory cells at this location. However, this hypothesis could not be sustained by an increase in FOXP3 mRNA expression which was high in PP and low in GW. IL33 mRNA expression is not documented for the pig in the literature and it is very difficult, so far, to explain this, such as IL33 mRNA expression variation in the context of a Salmonella infection. Nevertheless, we can make the hypothesis that this induction, if biologically significant, could be related to the establishment of a humoral response in the PP. BAFF which is involved in the IgA CSR in humans and mice in both PP and lamina propria [6, 24, 26] was also up-regulated at the mRNA level in PP. The role of IL33 and BAFF in a humoral response to Salmonella in the pig has to be confirmed and further studies are required.

Concerning the recruitment of dendritic cells, we identified a significant up-regulation of CX3CL1 mRNA expression (P < 0.01) in infected GW while CCL20 mRNA expression was not altered. These results are a little bit surprising considering reports in mice [18] showing that, at least, two main populations of dendritic cells are located in the gut: One of the sentinels of the intestinal lumen – responding

to CX3CL1 –, sampling and presentating harmless commensal micro-organisms and one of fully competent cells – responding to CCL20 – able to respond quickly and properly to pathogens. This discrepancy could be due to a sequential recruitment of dendritic cells through CCL20 earlier in the infection course.

Regarding the first steps in the recognition of the pathogens by the immune system, a strong up-regulation of NOD2, TLR2 and TLR4 mRNA expression was observed in the PP and the GW. The up-regulation was particularly obvious in the PP where NOD2 is highly expressed in the adult pig [36]. This observation is not surprising since PP M cells constitute a preferential site of entry for the bacteria [17, 19]. Surprisingly, TLR5 mRNA expression was down-regulated in both tissues. Abasht et al. made a similar observation with Salmonella enterica serovar Enteritidis in chicken caecum and liver [1]. They hypothesized that the down-regulation of TLR5 RNA expression might be beneficial to protect host cells from over-stimulation by bacterial flagellin [1]. An explanation to this down-regulation could be, as observed with Borrelia burgdorferi, that the pathogen lipoprotein-mediated TLR2 stimulation could cause the down-regulation of TLR5 to escape the immune response [5]. TLR2 can act synergistically with NOD2 and TLR4 while there is no evidence of such a synergism with TLR5 [38].

Antimicrobial peptides, NO and NK-lysin are known to be active against Salmonella [2, 3, 35, 43, 46]. In our conditions, we detected iNOS up-regulations in both PP and GW (P <0.01) and PBD-2 and PMAP-37 only in PP. The up-regulation of iNOS sustains the anti-Salmonella role of NO [2]. In vitro, PBD-2 mRNA expression was increased 10-fold upon infection with S. Typhimurium [41] while in vivo PBD-2 was only slightly increased in some infected intestinal segments [42]. In our conditions, PBD-2 mRNA was moderately induced only in the infected PP (P < 0.05) in accordance with Veldhuizen et al. observations [42]. However, the in vivo significance of these differences is difficult to appreciate because of the low magnitude of the observed

differences and the quite low starting level of expression. Further experiments are needed to definitely elucidate the role of PBD-2 in the protection against *S*. Typhimurium. For PMAP-37 a similar conclusion can be made since levels of expression are low. NK-lysin, PBD-1 and PR-39 genes were not induced in both PP and GW 24 h after the inoculations. This observation for PBD-1 is similar to previous reports [41, 42].

For the current study, one-month-old piglets were used. The mRNA expressions of the different genes were similar to other studies using young piglets [32, 39, 41]. Nevertheless, mRNA expressions could be different in adults as observed previously [12, 28] explaining differences in the response to the infection between young and adult pigs.

In conclusion, the inoculation of *S*. Typhimurium in the gut loop model has enabled a first broad assessment at the mRNA level of the T-helper orientation as well as many newly described genes in response to *Salmonella* in the pig. In the future, all the data collected here should help in the development of improved prophylactic and therapeutic approaches against porcine intestinal *S*. Typhimurium infections.

Acknowledgements. This work was supported by grants from the French National Institute for Agricultural Research (INRA). Conseil Régional du Centre. the Natural Science and Engineering Research Council of Canada (NSERC) and the Saskatchewan Agriculture Development Fund Strategic Research Program as well as travel grant from the French Embassy in Canada. We are thankful to VIDO Animal Care Staff for their invaluable help with housing animals and collecting tissues, especially Dr Don Wilson, Dr Kuldip Mirakhur, Amanda Giesbrecht, Jan Erickson, Sherry Tetland and Lucas Wirth. We are very grateful to Dr Hugh Townsend for his valuable assistance to carry out the statistical analysis. We are grateful to Drs Robert Brownlie and to Neil Rawlyk, Jérôme Trotereau, Stacy Strom and Jill Van Kessel for their help and advice.

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