Transcriptomics Data Integration Reveals Jak-STAT as a Common Pathway Affected by Pathogenic Intracellular Bacteria in Natural Reservoir Hosts

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Abstract

The study of the host-pathogen interface in natural reservoir hosts is essential to identify host-cell mechanisms affected by bacterial infection and persistence. Herein we used the Database for Annotation, Visualization and Integrated Discovery (DAVID) to integrate transcriptomics data and find common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria of the genera Anaplasma, Brucella and Mycobacterium during infection and persistence in two natural reservoir hosts, wild boar and sheep. The results showed that the upregulation of host innate immune pro-inflammatory genes and signaling pathways constitutes a general antibacterial mechanism in response to intracellular bacteria. Pathway focused analysis revealed a role for the Jak-STAT pathway during bacterial intracellular infection, a fact reported before in Mycobacterium infected cells but not during Brucella spp. and A. phagocytophilum infection. A clear activation of the Jak-STAT pathway was observed in A. phagocytophilum infected wild boar and sheep when compared to uninfected controls. Brucella spp. infection resulted in a balanced regulation of the Jak-STAT signaling and M. bovis infection of wild boar clearly produced a downregulation of some of the Jak-STAT effectors such as IL5 and TKY2. These results suggested that mycobacteria and brucellae induce host innate immune responses while manipulating adaptive immunity to circumvent host-cell defenses and establish infection. In contrast, A. phagocytophilum infection induces both innate and adaptive immunity, those suggesting that this pathogen uses other mechanisms to circumvent host-cell defenses by downregulating other adaptive immune genes and delaying the apoptotic death of neutrophils through activation of the Jak-STAT pathway among other mechanisms.

Keywords: Transcriptomics; Anaplasma; Mycobacterium; Brucella; Systems biology

Introduction

Pathogenic bacteria have to interact with host cells and reprogram the complex molecular and cellular networks of these cells to allow bacterial infection, replication and spread, while countering host-defense mechanisms [1,2]. This process is likely to involve genes from both pathogens and hosts, all of which are probably subject to complex regulation [1-3].

Molecular biology and in particular recent advances in genomics, transcriptomics and proteomics have allowed the characterization of host-pathogen interactions [2,3]. However, these studies have focused on the response of particular hosts to one or multiple pathogens, mostly using in vitro systems (see for example, [2]). Moving from in vitro studies in cultured cells to relevant animal disease models and natural reservoir hosts is crucial for understanding host-pathogen interactions, yet such studies are often neglected because cell culture-based systems are easier to manipulate. However, the study of the host-pathogen interface in natural reservoir hosts infected with different pathogens is now possible and essential to identify host-cell mechanisms affected by bacterial infection and persistence, which may be different from those identified in vitro [3,4].

Herein, the Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to integrate transcriptomics data and find common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria of the genera Anaplasma, Brucella and Mycobacterium during infection and persistence in two natural reservoir hosts, wild boar (Sus scrofa) and sheep (Ovis aries).

Materials and Methods

Transcriptomics data

Transcriptomics data was obtained from previously published studies on infected and uninfected matching control animals using microarray hybridization and real-time RT-PCR in wild boar infected with Mycobacterium bovis, Anaplasma phagocytophilum and Brucella suis [4-6] (NCBI Gene Expression Omnibus (GEO) platform accession numbers GPL3533, GPL3533, GSE15766, GSE17492) and in sheep infected with A. phagocytophilum and Brucella ovis [7-9] (GPL4456, GPL6954, GSE11928 and GSE10286). In these studies, the infection with M. bovis, A. phagocytophilum, B. ovis or B. suis strains was characterized in experimentally or naturally infected animals during acute or chronic infection (Table 1).

Transcriptomics data integration and analysis

Microarray data from all host-pathogen interactions were filtered to select significant (P < 0.05) differentially expressed genes with an infected/uninfected fold change (FC) ≥ 1.2. These genes were analyzed using DAVID V6.7 (http://david.abcc.ncifcrf.gov/) [10,11] to select

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the highest enrichment score (ES), which is the geometric mean of all enrichment P values (EASE scores) for each gene ontology (GO) term [11] and clustering for host cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria was analyzed by real-time RT-PCR using primers designed based on sequences available in the GenBank (Table 2). The real-time RT-PCR was performed on pooled RNA samples from infected and uninfected wild boar and sheep (wild boar infected with \textit{A. phagocytophilum}, \textit{N}=2; wild boar infected with \textit{Brucella} spp., \textit{N}=3; wild boar infected with \textit{M. bovis}, \textit{N}=6; sheep infected with \textit{A. phagocytophilum}, \textit{N}=2; sheep infected with \textit{Brucella} spp., \textit{N}=6; wild boar uninfected controls, \textit{N}=12; sheep uninfected controls, \textit{N}=5) with gene specific primers using the iScript One-Step RT-PCR Kit with SYBR Green and the iQ5 thermal cycler (Bio-Rad, Hercules, CA, USA) following manufacturer’s recommendations. The mRNA levels were normalized against cyclophillin and beta-actin using the genNorm method (ddCT method as implemented by Bio-Rad iQ5 Standard Edition, Version 2.0) [12]. In all cases, the mean of triplicate values was used and data from infected and uninfected animals were compared using the Student’s t-test (P < 0.05). Correlation analysis between microarray and RT-PCR results was conducted in Excel by calculating (a) percent of values with similar tendency (i.e. no variation, upregulated or downregulated) and (b) correlation coefficients (R²) between all values independently of the statistical analysis for RT-PCR results which were affected by the low number of samples used in the analysis.

**Results and Discussion**

The analysis conducted here focused on wild boar infected with \textit{M. bovis}, \textit{A. phagocytophilum} and \textit{B. suis} [4-6] and sheep infected with \textit{A. phagocytophilum} and \textit{B. ovis} [7-9]. These pathogens represent intracellular bacteria that infect and replicate within host immune cells and were selected because of their impact as zoonotic pathogens in many regions of the world.

An analysis pipeline was developed using DAVID to integrate data and find common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria of the genera \textit{Anaplasma}, \textit{Brucella} and \textit{Mycobacterium} during infection and persistence in two natural reservoir hosts, Eurasian wild boar and sheep (Figure 1). Because transcriptomics data were obtained from different experiments with tissue samples collected at different infection times and conditions [4-9] (Table 1), differences between various host-pathogen interactions could be explained by different factors. These factors include differences in the transcriptomics methods employed (microarray and data analysis platforms), experimental conditions (natural or experimental infections), host tissues used for RNA extraction (peripheral blood mononuclear cells (PBMC) or spleen) and individual variability of both pathogens and hosts. However, we hypothesized that statistically significant common factors emerging despite all these differences, have a particular relevance in identifying host-pathogen interactions of different pathogenic intracellular bacteria in different hosts, thus allowing the identification of common mechanisms that may be used for infection characterization, control and prevention. Therefore, the analysis focused on common mechanisms affected by these bacteria in all host-pathogen interactions.

**Common host-cell biological processes, molecular functions and pathways affected by \textit{Anaplasma}, \textit{Brucella} and \textit{Mycobacterium} infection in wild boar and sheep**

The results showed that it is possible to integrate data from different transcriptomics experiments to find common mechanisms affected by pathogenic intracellular bacteria in natural reservoir hosts. Common

| Strain            | Origin                                                                 | Host     | Tissue examined | Infection type               | Characterization of infection                                                                 |
|-------------------|------------------------------------------------------------------------|----------|----------------|----------------------------|------------------------------------------------------------------------------------------------|
| \textit{A. phagocytophilum} | Isolated from infected sheep in the Basque Country, Spain [30] (Genbank accession number EU436164) | Sheep    | PBMC           | Experimental acute infection | Infection was confirmed by microscopic examination of stained blood smears and \textit{msp4} PCR [7,30] |
|                   | Isolated from infected Eurasian wild boar hunter-killed in Slovenia, genotically identical to strains isolated from humans, dogs and \textit{I. ricinus} ticks [31] (Genbank accession numbers AY055469, AF033101 and EU246961) | Wild boar | PBMC           | Natural chronic infection   | Infection was confirmed by 16S rDNA and \textit{groESL} PCRs and sequence analysis [31] |
| \textit{B. ovis}  | R virulent PA strain Provided by Dr. J.M. Verger, Unite´ d’Infectiologie Animale et Sante’ Publique, INRA, Nouzilly, France [32,33] | Sheep    | PBMC           | Experimental acute infection | Infection was confirmed at necropsy by bacterial culture, morphology, Gram staining, oxidase and urease tests, \textit{CO}_2 requirements and phage typing [8,34] |
| \textit{B. suis}  | biovar 2 Isolated from infected Eurasian wild boar in Navarra, Spain [34,35] | Wild boar | Spleen         | Natural chronic infection   | Infection was confirmed by bacterial culture and seroconversion [5,36] |
| \textit{M. bovis} | Isolated from infected Eurasian wild boar in Southwestern Spain [3]    | Wild boar | Spleen         | Natural chronic infection   | Infection was confirmed at necropsy by pathology, bacterial culture and spoligotyping [3,4] |

Table 1: Bacterial strains and experimental animals.
host-cell biological processes affected by *Anaplasma*, *Brucella* and *Mycobacterium* infection in wild boar and sheep included regulation of immune system and immune system with 33 genes represented (Tables 3 and 4). The common host-cell molecular functions affected included 28 genes with receptor binding, cytokine activity and growth factor activity (Tables 3 and 4). The common host-cell pathways affected by these bacteria were cytokine-receptor interaction, hematopoietic cell lineage and Janus Kinase-Signal Transducer and Activator of Transcription (Jak-STAT) signaling pathway (Table 3). A good correlation was obtained between microarray and RT-PCR results for genes in common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria (Table 4). Correlation between microarray and RT-PCR results was 0.36 ($R^2=0.74$), 0.55 ($R^2=0.78$) and 0.77 ($R^2=0.81$) for wild boar infected with *A. phagocytophilum*; *B. suis*; *M. bovis*, respectively, and 0.64 ($R^2=0.79$) and 0.70 ($R^2=0.80$) for sheep infected with *A. phagocytophilum* and *B. ovis*, respectively.

### Effect of *Anaplasma*, *Brucella* and *Mycobacterium* infection on wild boar and sheep innate and adaptive immunity

These results showed that *Anaplasma*, *Brucella* and *Mycobacterium* infection of wild boar and sheep affect the expression of genes involved in host innate and adaptive immunity. However, not surprisingly, the way in which host immune response was affected varied between different host-bacteria interactions. Differences in host immune response between different host-pathogen interactions could be related to host/pathogen-specific factors and/or differences in gene expression between early (acute) and late (chronic) infections. Nevertheless, common to all bacteria-host interactions was the induction of innate immunity through upregulation of pro-inflammatory cytokines such as interleukins IL1B and/or IL6 that are induced in phagocytes after toll-like receptor (TLR) recognition resulting in activation of the complement system and pathogen opsonization for phagocytosis by macrophages and neutrophils [13]. As in previous experiments with cultured human macrophages infected with Gram-positive bacteria, Gram-negative bacteria and *M. tuberculosis* [2], shared responses included genes encoding receptors and signal transduction molecules affecting the cytokine-receptor interaction, hematopoietic cell lineage and Jak-STAT signaling pathways. However, adaptive immunity was induced through upregulation of genes such as cluster differentiation 4 (CD4) and IL21 only in wild boar and sheep infected with *A. phagocytophilum*.

The results obtained herein showed that the upregulation of host innate immune pro-inflammatory genes and signaling pathways constitute a general antibacterial mechanism in response to pathogenic intracellular bacteria of the genera *Anaplasma*, *Brucella* and *Mycobacterium*, a finding previously suggested in other studies with *Brucella* spp. [5,8,14], *Mycobacterium* spp. [2,4,15-20] and *A. phagocytophilum* [7,21].

### Role for the Jak-STAT pathway during *Anaplasma*, *Brucella* and *Mycobacterium* infection of wild boar and sheep

Pathway-focused analysis revealed a role for the Jak-STAT pathway during bacterial intracellular infection, a fact reported before in
| GenBank accession number1 | Gene symbol | Upstream/downstream primer sequences (5´-3´) |
|--------------------------|-------------|---------------------------------------------|
| NM_213844.2/ NM_001144097.1 | CRP | Ss-CRPF: GTTGTGTCACCGGAGAGGATT<br>Ss-CRPR: CCAGAGAACAGGGGAGTGA<br>Oa-CRPF: AGGATCGCGGTACCAAGAGA<br>Oa-CRPR: TTTGCTTGACAGTTGAC |
| NM_214155.2/ NM_001009417.1 | CD247 | Ss-CD247F: TGGGGAACAGAAGTGATGA<br>Ss-CD247R: TTGACTGCCTCCAGGACAG<br>Bi-CD247F: TTGAGGTGGAACAGGGAGT<br>Bi-CD247R: AAGCCTGCAACACTGACTG |
| NM_213775.2/ NM_0010009382.1 | CD3D | Ss-CD3DF: TTGGAGGACCAAGAGGAGT<br>Ss-CD3DR: TGGAGGACCAAGAGGAGT<br>Oa-CD3DF: TTGGAGGACCAAGAGGAGT<br>Oa-CD3DR: GCTGACTGCCTCCAGGACAG |
| NM_0001901908.1/ NM_001129960.1 | CD4 | Ss-CD4F: GCTTGGGAGGGGAGTAGAT<br>Ss-CD4R: AAATGGCTCACAGTCTGGA<br>Oa-CD4F: AAGCCTGCAACACTGACTG<br>Oa-CD4R: CTTGTTGACAGTTGAC |
| NM_213774.1/ NM_001034735.1 | CD74 | Ss-CD74F: CTTGCTGGCTCCAGGAGAG<br>Ss-CD74R: GTAGCTGACCTAGGACACAG<br>Bi-CD74F: TTGAGGTGGAACAGGGAGT<br>Bi-CD74R: GCTGACTGCCTCCAGGACAG |
| NM_214269.2/ NM_174375.2 | KITLG | Ss-KITLGF: AATGCAATCTGAGGAGT<br>Ss-KITLGR: ATGGTTGACAGTTGAC<br>Bi-KITLGF: GTTGGTACAGTTGAC<br>Bi-KITLGR: TGGAGGACCAAGAGGAGT |
| NM_213434.1/ NM_001076269.1 | C4 | Ss-C4F: CATGGCTGTTCACAGGAAAA<br>Ss-C4R: TATGTCTGCCCATTCCTTCC<br>Oa-C4F: TATGTCTGCCCATTCCTTCC<br>Oa-C4R: TATGTCTGCCCATTCCTTCC |
| NM_0001924460.1/ NM_001100293.1 | CCR4 | Ss-CCR4F: TACCAAGGAGTGCGGCTTTC<br>Ss-CCR4R: GCATGGCTGTTCACAGGAAAA<br>Oa-CCR4F: TACCAAGGAGTGCGGCTTTC<br>Oa-CCR4R: TACCAAGGAGTGCGGCTTTC |
| NM_0001909580.1/ NM_001113174.1 | CXCL12 | Ss-CXCL12F: CATGCTGCCCGTGGTTGGAC<br>Ss-CXCL12R: CTGCTAAGCAATCGGAGAG<br>Bt-CXCL12F: GAAGTACAGTCTGCTGGCT<br>Bt-CXCL12R: GAAGTACAGTCTGCTGGCT |
| U61139.1/ L07939.1 | CSF2 | Ss-CSF2F: TACAATCCCCTTCTTTGAC<br>Ss-CSF2R: AGTGCTGACCTAGGACACAG<br>Oa-CSF2F: TACAATCCCCTTCTTTGAC<br>Oa-CSF2R: AGTGCTGACCTAGGACACAG |
| NM_0001003924 / NM_001014945 | C1QA | Ss-C1QAF: TTCCTGAGGAGTAGTTGGA<br>Ss-C1QAR: ATGGAATCTGAGGAGT<br>Bi-C1QAF: GTTTGAGGATGCTAGGACAG<br>Bi-C1QAR: ATGGAATCTGAGGAGT |
| AY394920.1/ NM_001046599 | C1qB | Ss-C1QB: GGCAATGCCGAGGACTCAACAG<br>Ss-C1qBR: AGGAATCTGAGGAGT<br>Bi-C1QB: GTTTGAGGATGCTAGGACAG<br>Bi-C1qBR: AGGAATCTGAGGAGT |
| NM_0001001645.1/ NM_001166616.1 | C5 | Ss-C5F: GCGAGTGGCAGAGGACTG<br>Ss-C5R: AGGAATCTGAGGAGT<br>Bi-C5F: GTTTGAGGATGCTAGGACAG<br>Bi-C5R: AGGAATCTGAGGAGT |
| NM_214282.2/ NM_001045966.1 | C7 | Ss-C7F: TCAATCTGCCCTTCTCTCTCT<br>Ss-C7R: GCTGATGGTGACTGCTTTAC<br>Bi-C7F: GCGAGTGGCAGAGGACTG<br>Bi-C7R: GCTGATGGTGACTGCTTTAC |
| NM_213975.1/ NM_001009786.1 | FTH1 | Ss-FTH1F: TGCTTCAAGCAGTCTGCT<br>Ss-FTH1R: TCTCAAGACAGCATCTAGC<br>Oa-FTH1F: TGCTTCAAGCAGTCTGCT<br>Oa-FTH1R: TCTCAAGACAGCATCTAGC |
| NM_000104027.1/ NM_001014912.1 | HMOX1 | Ss-HMOX1F: ATGGCTGATCGACACCT<br>Ss-HMOX1R: GGCTGATGGTGACTGCTTTAC<br>Bi-HMOX1F: ACTTACACCCCTTCTCTCCT<br>Bi-HMOX1R: ACTCAGGCTACCAACCT |
| NM_001123124.1/ NM_174339.3 | HIF1A | Ss-HIF1AF: ATGGCATGCATGGACTG<br>Ss-HIF1AR: GCTGATGGTGACTGCTTTAC<br>Bi-HIF1AF: ATGGCATGCATGGACTG<br>Bi-HIF1AR: GCTGATGGTGACTGCTTTAC |
| NM_214055.1/ NM_001009465.2 | IL1B | Ss-IL1BF: CAGCAGATGGCGGAGGAT<br>Ss-IL1BR: CGAGGACTAGATGGACTG<br>Oa-IL1BF: CAGCAGATGGCGGAGGAT<br>Oa-IL1BR: CGAGGACTAGATGGACTG |
| AY552750.1 / NM_001009734.1 | IL15 | Ss-IL15F: TTGGTCTGGTGTGTTGCT<br>Ss-IL15R: GCCAAGACTGATGACAG<br>Oa-IL15F: TTGGTCTGGTGTGTTGCT<br>Oa-IL15R: GCCAAGACTGATGACAG |
| NM_214415.1/ NM_198832.1 | IL21 | Ss-IL21F: CCGGGGACAGCAGGAGAATT<br>Ss-IL21R: CAAGGCTAGGAGACTGAGAT<br>Bi-IL21F: CCGGGGACAGCAGGAGAATT<br>Bi-IL21R: CAAGGCTAGGAGACTGAGAT |
| BUS46820.1/ NM_001195219.1 | IL25 | Ss-IL25F: CTACGACTGGCGGAGGCT<br>Ss-IL25R: TCTCGGAAAGGCGGAGG<br>Oa-IL25F: GCGGGTACAGGCTACGA<br>Oa-IL25R: AAGGACGCTACGACGAGG |
| NM_214340.1/ NM_001075142.1 | IL4R | Ss-IL4RF: CCGGGTACAGGCTACGA<br>Ss-IL4RR: TGACAGGCACTCGCTCAT<br>Bi-IL4RF: CCGGGTACAGGCTACGA<br>Bi-IL4RR: TGACAGGCACTCGCTCAT |
| NM_214205.1/ NM_001009783.1 | IL5 | Ss-IL5F: TGGGCAAGACCTGGAGG<br>Ss-IL5R: CCGGGTACAGGCTACGA<br>Oa-IL5F: TGGGCAAGACCTGGAGG<br>Oa-IL5R: CCGGGTACAGGCTACGA |
| M80258.1/ NM_001009392.1 | IL8 | Ss-IL8F: CACCGAGAAGCAGGAGG<br>Ss-IL8R: GTGAGGCACTCGCTCAT<br>Oa-IL8F: CACCGAGAAGCAGGAGG<br>Oa-IL8R: CACCGAGAAGCAGGAGG |
| NM_001166043.1/ E1184569.1 | IL9 | Ss-IL9F: TATGGTCTGGGACAGTCTTTCC<br>Ss-IL9R: CATGGCTTGGTACAAGAGAA<br>Oa-IL9F: CACCGAGAAGCAGGAGG<br>Oa-IL9R: CACCGAGAAGCAGGAGG |
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**Abstract:**

Mycobacterium-infected cells [22-24] but not during Brucella spp. and A. phagocytophilum infection. This result highlighted the importance of integrating data from different transcriptomics experiments to discover common host-cell mechanisms affected by pathogenic intracellular bacteria.

In mammals, the Jak-STAT pathway is the principal signaling mechanism for a wide array of cytokines and growth factors such as CSF2, IL15, IL21, IL4R, IL5, IL6, IL9, TK2, EPOR, IL15RA shown here to be differentially expressed in infected animals [25]. Jak activation stimulates cell proliferation, differentiation, cell migration and apoptosis resulting in hematopoiesis and immune development among other processes [25]. Predictably, downregulation of the Jak-STAT pathway activity affects these processes but failure to properly regulate Jak signaling cause inflammation, erythrocytosis and leukemia among other diseases [25]. Herein, a clear activation of the Jak-STAT pathway activity is noted in A. phagocytophilum-infected cells [22-24] but not during Mycobacterium spp. and Brucella spp., infection resulted in the upregulation of some ligands and the corresponding receptors (Table 4). For example, the upregulation of MIF and MIFR was observed in A. phagocytophilum-infected cells [22-24] but not during Mycobacterium spp. and Brucella spp., infection.

**Table 2:** Primer sets used for analysis of differential gene expression by real-time RT-PCR.

| Gene Symbol | Forward Primer | Reverse Primer |
|-------------|----------------|----------------|
| SS-MIF | CTTCTCCTCCAGGTCAAGC | AAGCTAGGACTCCCTCTCCAGG |
| SS-MIFR | TTCTAGATCGTGTGAAGTAGG | TACAGATTACAGACCCCAAG |
| SS-MSFN | GAGAGAGCAACATG | GAGAGGAGCTGAGGAGT |
| SS-MSNR | TTCTAGATCGTGTGAAGTAGG | TACAGATTACAGACCCCAAG |
| SS-PPARG | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |
| SS-PSME1 | GAGAGAGCAACATG | GAGAGGAGCTGAGGAGT |
| SS-PSME1F | CTTCTCCTCCAGGTCAAGC | AAGCTAGGACTCCCTCTCCAGG |
| SS-PSME1R | TTCTAGATCGTGTGAAGTAGG | TACAGATTACAGACCCCAAG |
| SS-RLSR | GAGAGAGCAACATG | GAGAGGAGCTGAGGAGT |
| SS-RGL1 | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |
| SS-SCG2 | CTTCTCCTCCAGGTCAAGC | AAGCTAGGACTCCCTCTCCAGG |
| SS-SCG2R | TTCTAGATCGTGTGAAGTAGG | TACAGATTACAGACCCCAAG |
| SS-TK2F | GAGAGGAGCTGAGGAGT | TACAGATTACAGACCCCAAG |
| SS-TK2R | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |
| SS-TGFB3F | CTTCTCCTCCAGGTCAAGC | AAGCTAGGACTCCCTCTCCAGG |
| SS-TGFB3R | TTCTAGATCGTGTGAAGTAGG | TACAGATTACAGACCCCAAG |
| SS-STC1F | GAGAGGAGCTGAGGAGT | TACAGATTACAGACCCCAAG |
| SS-STC1R | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |
| SS-FGFr | GAGAGGAGCTGAGGAGT | TACAGATTACAGACCCCAAG |
| SS-FGFr2 | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |
| SS-DMPFR | GAGAGGAGCTGAGGAGT | TACAGATTACAGACCCCAAG |
| SS-DMPFR2 | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |
| SS-EPORF | GAGAGGAGCTGAGGAGT | TACAGATTACAGACCCCAAG |
| SS-EPORR | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |
| SS-IL15RF | GAGAGGAGCTGAGGAGT | TACAGATTACAGACCCCAAG |
| SS-IL15RR | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |
| SS-IL15RF2 | GAGAGGAGCTGAGGAGT | TACAGATTACAGACCCCAAG |
| SS-IL15RR2 | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |
| SS-TCGF | GAGAGGAGCTGAGGAGT | TACAGATTACAGACCCCAAG |
| SS-TCGF2 | GAAGGAGCTGAGGAGGAG | TACAGATTACAGACCCCAAG |

GenBank accession numbers are shown for wild boar/sheep-cattle sequences.
Table 3: Common host-cell biological processes, molecular functions and pathways affected by pathogenic intracellular bacteria.

| Gene symbol | Gene description                                      | WB-A   | WB-B   | WB-M   | S-A    | S-B   |
|-------------|-------------------------------------------------------|--------|--------|--------|--------|--------|
| CRP         | C-reactive protein, pentraxin-related                 | 1.6 (ns)| ns (ns)| -2.0 (-3.3 ± 0.01)| ns (ns)| ns (ns) |
| CD247       | CD247 molecule                                        | 1.6 (ns)| -2.2 (ns)| ns (ns)| ns (ns)| ns (ns) |
| CD3D        | CD3d molecule, delta (CD3-TCR complex)                | ns (ns)| ns (ns)| 2.1 (ns)| -2.2 (ns)| ns (ns) |
| CD4         | CD4 molecule                                          | 1.4 (ns)| ns (ns)| ns (ns)| 1.3 (ns)| ns (ns) |
| CD74        | CD74 molecule, major histocompatibility complex       | ns (ns)| -4.1 (ns)| -3.3 (-5.3 ± 0.2)| ns (ns)| ns (ns) |
| KITLG       | KIT ligand                                            | 3.2 (ns)| ns (ns)| ns (ns)| -1.2 (ns)| ns (ns) |
| CALCR       | Calcinonin receptor                                   | 5.3 (ns)| 3.7 (ns)| ns (ns)| ns (ns)| ns (ns) |
| CCR4        | Chemokine (C-C motif) receptor 4                     | ns (1.9±0.02)| ns (ns)| -2.3 (-8.1±0.05)| -1.4 (ns)| 3.0 (ns) |
| CXCL12      | Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1) | 1.4 (ns)| -4.8 (ns)| -9.8 (-2.5±0.02)| -2.7 (-4.5±2E-3)| ns (-11.1±8E-6) |
| CSF2        | Colony stimulating factor 2 (granulocyte-macrophage)  | 1.4 (ns)| ns (ns)| ns (ns)| 1.6 (6.1±4E-4)| ns (-4.2±4.5-6) |
| C1QA        | Complement component 1, q subcomponent, A chain      | 1.3 (ns)| -2.8 (ns)| ns (-3.7 ± 0.2)| ns (ns)| ns (ns) |
| C1qB        | Complement component 1, q subcomponent, B chain      | 1.3 (ns)| -3.9 (ns)| -9.0 (ns)| ns (ns)| ns (ns) |
| C5          | Complement component 5                               | 1.5 (ns)| ns (ns)| ns (ns)| 1.3 (8.0±4E-4)| ns (-10.0±2E-6) |
| C7          | Complement component 7                               | 2.5 (ns)| ns (ns)| ns (ns)| 1.3 (ns)| ns (ns) |
| FTH1        | Ferritin, heavy polypeptide 1                         | ns (ns)| -3.6 (ns)| -4.1 (-2.5±0.7)| ns (ns)| ns (ns) |
| HMOX1       | Heme oxygenase (decycling) 1                          | ns (ns)| -2.5 (ns)| -1.7 (-2.5±0.1)| ns (ns)| ns (ns) |
| HIF1A       | Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) | 1.3 (ns)| -2.0 (-3.0±0.02)| ns (ns)| ns (ns)| ns (ns) |
| IL1B        | Interleukin 1, Beta                                   | ns (ns)| 2.9 (ns)| ns (ns)| 1.3 (2.3±3E-4)| 2.1 (1.4±5E-5) |
| IL15        | Interleukin 15                                        | ns (ns)| ns (ns)| ns (ns)| 1.2 (ns)| 2.7 (ns) |
| IL21        | Interleukin 21                                        | 1.3 (ns)| ns (ns)| ns (ns)| 1.2 (ns)| ns (ns) |
| IL25        | Interleukin 25                                        | ns (ns)| ns (ns)| ns (ns)| 1.3 (ns)| 1.6 (ns) |
| IL4R        | Interleukin 4 receptor                                | 1.2 (ns)| ns (ns)| ns (ns)| -2.0 (ns)| ns (ns) |
| IL5         | Interleukin 5 (colony-stimulating factor, eosinophil) | 1.3 (ns)| 10.8 (± 0.5) | ns (-3.1±3E-4)| ns (ns)| 2.6 (ns) |
| IL6         | Interleukin 6 (interferon, beta 2)                    | 2.0 (ns)| ns (ns)| ns (ns)| 1.2 (ns)| (1.1±4E-6) |
| IL9         | Interleukin 9                                         | ns (ns)| ns (ns)| ns (ns)| 1.3 (ns)| 1.3 (ns) |
| MIF         | Macrophage migration inhibitory factor (glycosylation-inhibiting factor) | ns (ns)| ns (ns)| ns (ns)| -2.0 (ns)| 10.4 (ns) |
| MSN         | Moesin                                               | ns (ns)| -2.6 (ns)| -3.4 (ns)| ns (ns)| ns (ns) |
| PPARG       | Peroxisome proliferator-activated receptor gamma     | 1.2 (ns)| -3.5 (ns)| ns (ns)| ns (ns)| ns (ns) |
downregulation of others that may result in a balanced regulation of the Jak-STAT signaling to prevent negative effects associated with improper regulation of this pathway (Table 4). As previously reported [22-24], *M. bovis* infection of wild boar clearly produced a downregulation of some of the Jak-STAT effectors such as IL5 and TKY2 (Table 4).

**Conclusions**

These results suggested that mycobacteria and brucellae induce host innate immune responses while manipulating adaptive immunity through Jak-STAT pathway and other mechanisms to circumvent host-cell defenses and establish infection. In contrast, *A. phagocytophilum* infection induces both innate and adaptive immunity, those suggesting that this pathogen uses other mechanisms to circumvent host-cell defenses. These mechanisms may include downregulation of other adaptive immune genes such as IL2 and IL4 [7,26] and delaying the apoptotic death of neutrophils [7,21,27,28] through activation of the Jak-STAT pathway among other mechanisms.

These results improved our understanding of host-pathogen interactions by characterizing common host-cell mechanisms affected by pathogenic intracellular bacteria of the genera *Anaplasma*, *Brucella* and *Mycobacterium* in natural reservoir hosts and provided insights into mechanisms of pathogenesis that could be used as targets for therapeutic intervention and vaccine development. In fact, some of the cytokine-receptor interactions described here such as those involving IL4 and IL6 have already been used to characterize the immune response to parenteral and oral *Bacillus Calmette-Guérin* (BCG) vaccination to prevent *M. bovis* infection in wild boar [6,29] and the protective response to the *B. melitensis* Rev 1 vaccine in sheep for the control of *B. ovis* [9], respectively.

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