Johne's disease in cattle: an in vitro model to study early response to infection of *Mycobacterium avium* subsp. *paratuberculosis* using RNA-seq.

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Abbreviations: ARG2, arginase 2; CIITA, class II major histocompatibility complex transactivator; CD, Crohn's disease; CSFs, colony-stimulating factors; CISH, cytokine inducible SH2-containing protein; CSF2, granulocyte-macrophage colony-stimulating factor; CSF3, granulocyte colony-stimulating factor; DE, differentially expressed genes; γδ T cells, gamma delta T cells; GO, gene ontology; IFNG, interferon gamma; IFNGR1, interferon gamma receptor 1; IFNGR2, interferon gamma receptor 2; IL, interleukin; iNOS, nitric oxide synthase 2; ITGA2, integrin subunit alpha 2; ITGB3, integrin subunit beta 3; JD, Johne's disease; MAC, membrane attack complex; MAP, *Mycobacterium avium* subsp. *paratubercolosis*; MDM, monocyte-derived macrophages; MHC, major histocompatibility complex ; OLR1, oxidized low density lipoprotein receptor 1; OSM, oncostatin M; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PI, post infection; RBC, red blood cells; SOCS3, suppressor of cytokine signaling 3; TNFα, tumor necrosis factor.

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Abstract

Johne's disease is a chronic granulomatous enteritis caused by *Mycobacterium avium* subsp. *paratubercolosis* (MAP) which affects ruminants worldwide and has a significant economic impact. MAP has also been associated with human Crohn's disease, although this connection is not well established. MAP is highly adapted for survival within host macrophages and prevents macrophage activation, blocks phagosome acidification and maturation, and attenuates presentation of antigens to the immune system. The consequence is a very long silent infection before clinical signs are observed.

The present work examined the transcriptome of bovine monocyte-derived macrophages (MDM) infected with the L1 strain of MAP at 2h, 6h and 24h post infection using RNA-Seq. Pathway over-representation analysis of genes differentially expressed between infected *vs.* control MDM identified that immune related pathways were affected. Genes belonging to the c*ytokine-cytokine receptor interaction pathway* and members of the *JAK-STAT pathway*, which is involved in the regulation of immune response, were up-regulated. However, in parallel inhibitors of immune functions were activated, including suppressor of cytokine signalling (*SOCS*) and cytokine-inducible SH2-containing protein (*CISH*), which most likely suppresses IFNγ and the JAK/STAT signaling cascade in infected MDM, which may favour MAP survival.

After exposure, macrophages phagocytise pathogens, activate the complement cascade and the adaptive immune system through the antigen presentation process. However, data presented here suggest that genes related to phagocytosis and lysosome function are down regulated in MAP infected MDM. Genes of MHC-II complex and complement pathway were also down-regulated. This study therefore shows that MAP infection is associated with changes in expression of genes related to the host immune response that may affect its ability to survive and multiply inside the host cell.

Keywords: Bovine, Johne's disease, macrophage, *Mycobacterium avium* subsp. *paratubercolosis*, paratuberculosis, RNA-sequencing.

1. Introduction

Mycobacterium avium subsp. *paratubercolosis* (MAP) is the causative agent of a granulomatous, inflammatory chronic bowel disease in ruminants (cattle, goats, sheep, buffalo and deer), known as Johne's disease (JD) or paratuberculosis. JD was first described in 1895 by Johne and Frothingham, who demonstrated the presence of mycobacteria in the intestines of affected cattle in Germany (Chiodini 1993). MAP is a gram-positive, aerobic, non-spore-forming, non-motile, acid-fast

bacillus. It has a complex relatively impermeable cell wall composed of 60% lipids, including mycolic acids that are characteristic of the Mycobacteriaceae family. This cell wall enhances MAP resistance to extremes of pH, chemicals and heat, and promotes its survival in the environment. The thick cell wall also restricts the uptake of nutrients, hence the organism is very slow growing, with a generation time of over 20 hours even in ideal conditions, making it difficult to culture in the laboratory.

JD is common worldwide and it has now been reported on every continent. In Europe, although many epidemiological studies have been carried out, true MAP prevalence estimations are difficult to obtain (Garcia and Shalloo 2015; Nielsen and Toft 2009). Most animals in an infected herd will be in the silent preclinical phase of the disease and infection is only likely to be detected when an animal reaches the clinical stage, typically between 2 and 5 years of age in cattle. This long latent phase is a problem for monitoring and controlling the disease. Nevertheless, during the period between infection and clinical manifestation of JD, infected animals may shed MAP in faeces and milk, contaminating the environment and infecting other animals, and possibly humans. JD has a considerable impact on the global economy, e.g. in dairy cattle herds it results in a decrease in milk production, which when corrected for lactation number and herd of origin can be 1.87 kg/d, or equivalent to 5.9% of the yield (McAloon et al 2016). JD is also associated with herd health problems, especially mastitis and low fertility, leading to premature culling, and a reduced slaughter value of the carcass (Beaudeau et al. 2007; Gonda et al. 2007; Hendrick et al. 2005; Villarino et al. 2011). At an international level, JD causes considerable damage to commerce resulting from the limitation of trade of animals from infected farms.

It has been suggested that MAP is involved in Crohn's disease (CD). There are similarities in symptoms and lesions between JD in ruminants and CD in humans, and MAP has been isolated from the tissues of many CD patients (Abubakar et al. 2008; Sechi et al. 2005; Singh et al. 2008). However, MAP has also found in samples from healthy humans. MAP could be the causative agent of CD, a co-factor in development of the disease, or may be more prevalent in CD patients as the result of immune dysfunction related to the disease (Rosenfeld and Bressler 2010). Despite considerable research, the evidence linking MAP with Crohn's disease remains inconclusive (Atreya et al. 2014; Bach 2015; Das 2012; Feller et al. 2007; Hermon-Taylor 2009; Liverani et al. 2014; Rosenfeld and Bressler 2010).

Animals are most susceptible to MAP in the first months of life, when they ingest contaminated colostrum, milk and faecal material. Infection *in utero* has also been reported (Whittington and Windsor 2009). This early susceptibility to infection may be due to the limited ability of young animals to control intracellular pathogens (Windsor and Whittington 2010), while adult cattle seem

to be refractory to infection (Begg and Whittington 2008; Rankin 1961). After the ingestion of MAP, the pathogen colonizes the ileum. It was then thought to cross the intestinal mucosa through M-cells (specialized non-villous epithelial cells), located in Peyer's patches (Plattner et al. 2011). However, it has recently been demonstrated that MAP can cross the gut wall in areas with and without Peyer's patches, suggesting that it can enter the body through enterocytes in addition to M cells (Bermudez et al. 2010; Sigurðardóttir et al. 2005). Once on the submucosal side of the intestinal epithelium MAP organisms are phagocytised by macrophages. MAP is able to survive and proliferate within phagosomes and inhibits apoptosis and phagosomal maturation in infected macrophages. In this phase, macrophages need to become activated to enhance their ability to kill intracellular MAP and control infection. Activation is achieved through the production of gamma interferon (IFNγ) and other cytokines by Th1 type T-helper lymphocytes (Khalifeh and Stabel 2004; Zurbrick et al. 1988). It is likely that some exposed cattle are successful in eliminating the MAP infection, however MAP persists within macrophages of many infected animals (Hestvik et al. 2005). This early "silent" infection period (stage I) can last for 2 or more years, during which time the infection is contained. The presence of the pathogen may trigger a local inflammatory response, which results in granulomatous lesions, usually in the gut and gut associated lymphoid tissue, which disrupt the mucosal structure and function. When the infection overcomes the host defences there is usually a switch from the Th1 to a Th2-dominated immune response where cytokines, including IL-4 and IL-10, trigger antibody production. At this point infected cattle enter into stage II, in which the pathogen spreads to other tissues and large numbers of MAP are shed in the faeces.

Several *in vitro* and *in vivo* studies in monocyte-derived macrophages (MDM) and peripheral blood mononuclear cells (PBMC) have investigated how the immune system responds to MAP infection (Bannantine and Talaat 2010; Borrmann et al. 2011; MacHugh et al. 2012; Purdie et al. 2012; Weigoldt et al. 2011). However, knowledge of the control of the host immune response to MAP as well as the genetic properties of the bacterium affecting its virulence remains incomplete (Gollnick et al. 2007). It is now possible to investigate MAP-host interaction at the level of the whole transcriptome by deep sequencing. In the present study RNA-seq was used to explore the response of bovine MDM cultured *in vitro* to MAP infection. The genes differentially expressed between infected and control MDM were analysed to identify host cellular pathways involved in the interaction with this pathogen.

2. Material and methods

2.1. Experimental animals

Animals used were housed on CERZOO experimental farm, in San Bonico (CERZOO - Research Centre for Animal Production and Environment, Italy). The farm adheres to a high standard of veterinary care and is under the control of the Italian Official Veterinary Service. The study was carried out in 2011-2012 and complied with Italian laws on animal experimentation (DL n. 116, 27/01/1992) and ethics. Blood was collected from 68 Italian Holstein cows ranging in age from 24 to 50 months during veterinary screening for paratuberculosis by indirect absorbed ELISA (Id Screen® Paratuberculosis Indirect-ID.vet). All animals on the farm were negative to the ELISA screening. The immune status with regard to MAP infection is repeatedly monitored by ELISA and there have been no JD affected animals on the farm. Monocytes used in the study were prepared from blood collected from four control animals involved in the search for blood biomarkers to assess rumen functions (prot. N. 31425 of 31/05/2010). All the blood samples used were verified serum negative to Johne's disease by ELISA.

2.2. Isolation of bovine MDM and MAP infection

PBMC were isolated from 350 ml of blood collected in acid citrate dextrose as anticoagulant in repeated blood collections. Blood was centrifuged for 15 min at 750 g in 50 ml conical tubes. Buffy coats were collected and phosphate buffered saline (PBS) (Sigma-Aldrich) added to 30 ml final volume, then transferred to ACCUSPIN System-Histopaque-1077 tubes (Sigma-Aldrich) and centrifuged for 30 min at 750 g at room temperature. PBMC were collected from the PBS-Histopaque interface. Contaminating red blood cells (RBC) were removed by lysis in RBC buffer (10 mM KHCO3, 150 mM NH4Cl, 0.1 mM EDTA pH 8.0) and PBMC were washed three times with PBS (Sigma-Aldrich). PBMC were diluted to $5x10^6$ cells/ml in RPMI 1640 culture medium (Gibco) supplemented with 12% foetal bovine serum, 2mM L-GLN, 0.1% 2-b-mercaptoethanol, transferred into six well plates ($5\text{-}7x10^6$ cells per well) and incubated for 12 h at 37°C and 5% CO_2, to allow the cells to adhere. Following incubation, the medium was changed and non-adhered cells removed. Adherent monocytes were left to differentiate into macrophages undisturbed for 7 days as reported in Weiss et al 2001. Differentiation into MDM and purity was assessed by examining cell morphology under a light microscope. Mononuclear cells viability was assessed by Trypan Blue exclusion. The L1 strain of MAP used for the *in vitro* infection is a field strain isolate and is a classical *M. avium* subsp. *paratuberculosis* type II group, also known as C type. It was classified using the genotyping method reported in Amosin et al. 2004.

L1 MAP was grown for 2 months in Middlebrook 7H9 (Difco) broth with Tween 80 (4% wt/vol, Difco), mycobactin J (Allied Monitor) and OADC growth supplement (Difco) at 37°C with

constant agitation (60 rpm). Bacterial cells were harvested by centrifugation (2,500 x g for 20 minutes), washed once and resuspended in sterile PBS (Sigma-Aldrich). The bacterial suspension was declumped using the procedure described by Odumeru et al. (2001). Briefly, the suspension was forced through a 21-gauge needle several times and allowed to stand at room temperature for 20 min in upright screw capped tubes. The supernatant was carefully removed and the cell density was standardised spectrophotometrically (OD 600nm).

On day 7 of culture, MDM cells were washed three times with warm PBS (37°C) to remove non-adherent and dead cells. All cell infections were grown in RPMI 1640 culture medium (Gibco) supplemented with 12% foetal bovine serum, 2mM L-GLN, 0.1% 2-b-mercaptoethanol.

Three six well plates of MDM cells (5-7x10^5 cells per well) per sample were incubated with the L1 MAP strain at a multiplicity of infection (MOI) 10:1 (10 bacilli/macrophage). Cells were washed with warm PBS (37°C) two hours after infection to remove non-phagocytized MAP. Three six well plates for parallel uninfected MDM cultures were also set up at the same cell density as controls. Total RNA was extracted from samples at 2, 6, 24 hours post-infection (PI) with TriReagent (Sigma) as recommended by the manufacturer. Concentration and quality of total RNA was measured using a Nanodrop Spectrophotometer and an Agilent Bioanalyzer 2100. All samples had an RNA integrity value of 7.6 or greater.

2.3. RNA-Seq: library preparation and NGS sequencing

RNA samples were processed using TruSeq RNA-seq sample prep kit from Illumina (Illumina, Inc., CA, USA). Briefly, poly-A containing mRNA was purified using poly-T oligo- magnetic beads, and cDNA was synthesized by reverse transcription according to manufacturer's protocol. Illumina TruSeq adapters with indexes were ligated to the cDNA fragments. Samples were amplified by PCR to selectively enrich those cDNA fragments in the library having adapters at both ends. Samples were sequenced at 12 samples per lane on an Illumina HiSeq2000 (Illumina Inc.). On average 20 million 100bp paired-end reads were obtained per sample.

2.4. Bioinformatic analysis

The raw reads were processed using Trimmomatic software (Bolger et al. 2014) to filter low quality bases and sequencing adapters. The filtered reads were aligned with the bovine genome (assembly version UMD3.1) using STAR software version 2.3.0e (Dobin et al. 2013). The resulting SAM file was converted into BAM format, sorted by coordinates and indexed using Samtools v1.1. (Li et al. 2009).

Read counts per gene, annotated on the bovine genome, were calculated using HTSeq-Count utility (Anders et al. 2015) and the GTF file provided by Ensembl database v68. The raw read counts were

imported into R and processed using the Bioconductor package edgeR (Robinson et al. 2010) and normalized using the TMM method. Genes having less than 1 count per million in a minimum of two samples were discarded. The remaining set of genes was used to assess differential expression between MAP infected cells and controls, using a generalized linear model (GLM) and a design matrix built according to the experimental conditions.

Results of the differential expression analysis were corrected for multiple testing using the Benjamini-Hochberg method (Benjamini and Hochberg 1995) and the functions provided by the edgeR package. Genes showing a False Discovery Rate below 5% were considered as differentially expressed and used in the biological interpretation. Pathway analysis, and Gene Ontology (GO) analysis (Benjamini & Hochberg adjusted *P* values) of the differentially expressed genes were performed using the online tool of Reactome Database in InnateDB (Breuer et al. 2013).

5. Results and discussion

5.1. Identification of differentially expressed genes in response to *in vitro* MAP infection and gene functional analysis

Statistical analysis of the RNA-seq data at 2 hours, 6 hours and 24 hours PI revealed that transcription levels for 3212 unique genes differed between MAP infected and control MDM (FDR $\leq$0.05). The number of differentially expressed (DE) genes increased along the time course of the infection. There were 258 DE genes at 2 hours PI (185 up-regulated; 73 down-regulated), 1328 at 6 hours PI (698 up-regulated; 630 down-regulated) and 2566 at 24 hours PI (1247 up-regulated; 1319 down-regulated). Interestingly, the proportion of down-regulated genes progressively increased over the 24 hours PI.

A core set of 130 genes were differentially expressed across all three time points. In addition, 40 genes were in common between 2 hours and 6 hours PI, 617 genes between 6 hours and 24 hours PI, and 23 genes between 2 hours and 24 hours PI. All other DE genes were time point specific: 65, 541, 1796 at 2 hours, 6 hours and 24 hours PI, respectively (Fig 1). Only a few DE genes shared between time points changed direction along the time course (Fig S1 in Supplementary Material). Many of the top ten up-regulated genes at the three time points were related to immune response (See Table 1) and included colony stimulating factor 2 (granulocyte-macrophage) (*CSF2*), oncostatin M (*OSM*), interleukin 11 (*IL11*), interleukin 17A and F (*IL17A, IL17F*), interleukin 9 (*IL9*), interferon gamma (*IFNG*), interleukin 12B (*IL12B*). Conversely, two chemokines (*CXCL11, CXCL13*) were down-regulated. A complete list of DE genes is given in Tables S1, S2 and S3.

Functional classification of DE genes showed that the top ranked *molecular functions* at 2 hours PI

were "cytokine activity" (GO:0005125) and "growth factor activity" (GO:0008083), while at 6 hours and 24 hours PI they were "protein binding" (GO:0005515) and "cytokine activity" (GO:0005125) (Table 2). Top *biological processes* were "immune response" (GO:0006955) at all time points PI, while "cellular response to extracellular stimulus" (GO:0031668) was top at 2 hours PI, "cellular response to lipopolysaccharide" (GO:0071222) and "inflammatory response" (GO:0006954) at 6 hours and 24hours PI, and "antigen processing and presentation" (GO:0019882) at 24 hours PI (Table 3). "Cytoplasm" (GO:0005737) was the top *cellular component* at 2 hours and 6 hours PI, while "extracellular vesicular exosome" (GO:0070062) was top at 24 hours PI (Table 4). "Early phagosome" (GO:0032009) was significant only at 6 hours PI. "Lysosomal membrane" (GO:0005765), "lysosome" (GO:0005764), "external side of plasma membrane" (GO:0009897), "cell surface" (GO:0009986), "endoplasmic reticulum" (GO:0005783) were overrepresented only at 24 hours PI, the last time point assessed post infection (Table 4). For full details see Tables S4, S5, S6.

Pathway Analysis was used to determine which biological pathways were significantly over-represented in the list of genes affected by MAP infection (Breuer et al. 2013). All 3212 unique genes significantly DE were first analysed using the Reactome Database of *Bos taurus.* Eighty biological pathways were significantly enriched in MAP infected *vs* control MDM (p-value<0.05). The top pathway was *Innate immune system,* with 32% of the genes in this pathway DE. Other enriched immune related pathways were *Chemokine receptors bind chemokines, Signalling by Interleukins, Immune System, Cytokine Signalling in Immune system, Interleukin-1 signalling, Classical antibody mediated complement activation* (Table 5). Phagocytosis, apoptosis, detoxification related pathways were also identified. The up- and down-regulated genes were then analyzed separately at each time point. The most involved pathways for up-regulated genes were related to immune response, while for down-regulated genes pathways were more related to metabolism (Fig S2-S7).

5.2. Activation of *Cytokine-cytokine receptor interaction* pathway in response to MAP infection

The list of DE genes annotated by orthology to human genes were analysed using the KEGG database in InnateDB Pathway Analysis (Breuer et al. 2013) in order to better understand their significance. *Cytokine-cytokine receptor interaction* was the top KEGG pathway significantly associated with mainly up-regulated genes (Fig. S8). This pathway has previously been reported to be perturbed in the intestinal mucosa of MAP infected cattle (Khare et al. 2012). Genes involved included chemokines, hemapoietins, platelet-derived growth factors, transforming growth factor beta family members, tumor necrosis factors and interferon (see Table 6 for the complete list). At

269 the transcriptional level, the results presented here are consistent with classical pro-inflammatory
270 macrophage activation (M1) in response to MAP infection, which is characterized by high
271 expression levels of interleukin 12 (*IL12*), interleukin 23 (*IL23*) and tumor necrosis factor (*TNFα*)
272 (Mantovani et al. 2002). IL12 and TNFα stimulate IFNγ secretion, which results in a further
273 increase in IL12 and TNFα production in a pro-inflammatory positive feed-back. It is noteworthy
274 that the increase of *IL12* expression was evident at 6 hours and 24 hours PI, but not at 2 hours PI
275 (Table 6). This may reflect a delayed Th1-type immune response that is essential for the control of
276 infections of infected macrophages. Additional typical markers of macrophage activation were
277 detected as DE including: nitric oxide synthase 2 (*iNOS*), arginase 2 (*ARG2*) (Yang and Ming 2014)
278 and interleukin 1 alpha and beta (*IL1A, IL1B*) (Table S1, S2 and S3 in Supplementary Material).
279 Other *in vitro* studies have shown enhanced production of the anti-inflammatory cytokine IL10
280 following MAP infection, that antagonizes the pro-inflammatory immune response by down-
281 regulating the production of *IL12, TNFα* and *IFNG,* which leads to the inhibition of the host innate
282 response resulting in enhanced pathogen survival (reviewed in Abendaño et al. 2013). A slight up-
283 regulation of the anti-inflammatory cytokine *IL10* was seen at 6 hours PI compared with the non-
284 infected control as reported in Table 6. Differences in the pattern of expressed cytokines may be
285 result from the use of different pathogen strains and MOI (Borrmann et al. 2011; Janagama et al.
286 2006; Kabara et al. 2010). In the present study MDM were prepared from whole blood monocytes,
287 and not differentiated from CD14+ enriched monocytes. The presence of residual non-differentiated
288 lymphocytes during MAP infection may have modified the response to the pathogen. A recent study
289 investigated the role of gamma delta T cells (γδ T) during *in vitro* MAP infection of macrophages
290 and showed that the presence of lymphocytes affected MDM responses to MAP (Baquero and
291 Plattner 2016). In particular, the secretion of IFNγ and the pro-inflammatory cytokine IL17A was
292 enhanced by the presence of T lymphocytes, while IL10 was not detected in the supernatants from
293 cultured cells in response to MAP when undifferentiated lymphocytes were present. In the present
294 study, the expression of *IL17A*, *IL17F* and *IFNG* was increased in MAP infected MDM, indeed
295 *IL17A* and *IL17F* were among the genes that showed the greatest increase in expression at 24 hours
296 PI (Tables 1 and 6). Much of the IL-17 released during an inflammatory response is produced by
297 innate immune cells, although IL-17 is usually considered to be a T cell-secreted cytokine. The
298 production of IL17A by macrophages has been shown to play a central role in the
299 immunopathology of chronic diseases (Reynolds et al., 2010). IL17A has been shown to be
300 involved in the protective response against *M. tuberculosis* in vaccinated animals (Khader and
301 Cooper 2008). Furthermore the expression of *IL17* has been associated with the formation of
302 granuloma in a bovine model of tuberculosis: animals with macroscopic lesions have higher levels

of expression of *IL17A* (Blanco et al. 2011). Impaired granuloma formation is also observed in the lungs of *IL17*-deficient mice infected with Mycobacterium bovis bacille Calmette-Guérin (BCG). This highlights the role of IL17 in the formation of tubercular granulomas (Umemura et al. 2007; Okamoto Yoshida et al. 2010). IL17 members may also be involved in the response of macrophage to MAP infection, promoting the recruitment of other immune factors and controlling the granuloma formation in JD. It is well established that IL17A promotes the generation, recruitment, and activation of neutrophils by inducing the expression of the colony-stimulating factors: particularly granulocyte colony-stimulating factor (*CSF3*) and granulocyte-macrophage colony-stimulating factor (*CSF2*). These two cytokines, which induce the expression of pro-inflammatory cytokines, were expressed at high levels following MAP infection in the present study (Table 6). Previous reports have shown that *CSF2* is up-regulated in bovine MDM incubated with MAP (MOI 10) at 6h and 24h PI (Weiss et al. 2002). *CSF3* expression has been found to increase in MAP infected MDM isolated from cattle (Casey et al. 2015) and red deer (Marfell et al. 2013). Expression of *CSF2* and *CSF3* has also been shown to increase in cells following *in vitro* challenge with *M. bovis* (Lin et al. 2015). CSFs stimulate immune functions, including the antibacterial capability of phagocytes against *Leishmania tropica, Candida albicans, M. avium complex* (Blanchard et al. 1991). While involved in the protective immune response, increased CSFs expression promotes the survival of macrophages, which may be a mechanism that mycobacteria use for long term survival in the host.

Mycobacterial infection has been shown to modulate the expression of genes encoding chemokines (Méndez-Samperio et al. 2003; Rhoades et al. 1995), which was also found here. Several chemokines were significantly up-regulated in MDM after MAP infection, although others (*CXCL9, CXCL11, CXCL13, CCL8*) were down-regulated (Table 6 and Fig. S8). Representative members of the four subfamilies of chemokines (CXC, CC, CX3C and XC) were identified, although the CXC and CC motifs were most represented. This is in accordance with earlier studies, which showed that mycobacteria infection induces increased expression of CC and CXC members (Méndez-Samperio 2008). The CC chemokines, particularly *CCL5* (*RANTES*), which was up-regulated at 24 hours PI (Table 6), are potent leucocyte activators which have a role in granuloma formation. The expression of *CCL3* (also known as macrophage inflammatory protein *MIP-1*α), *CCL4* (*MIP*-1β) and *CCL5* is affected by *M. tuberculosis* infection (Méndez-Samperio 2008). *CCL4* expression has been reported to increase in MAP infected MDM compared with non-activated control macrophages at 16 hours PI (Weiss et al. 2004). Analysis of bovine MDM gene expression in response to *in vitro* infection with MAP showed increased expression of the chemokines *CXCL2* and *CCL20* at 2 hours PI and *CXCL2,CCL4, CCL5, CCL20* at 6 hours PI (MacHugh et al. 2012). *CCL20, CXCL2*, and in addition

CXCL3 was also shown to be highly up-regulated at 2 hours PI in an *in vitro* study of the bovine MDM transcriptome response during MAP infection (Casey et al. 2015). All these chemokines showed increased expression following MAP infection of MDM in the present study (Table 6 and Fig. S8). *CXCL8* (also known as IL8) was up-regulated at 6h and 24h PI. This chemokine is involved in inflammatory response to mycobacteria infections (Méndez-Samperio 2008). The results presented here suggested that an early *in vitro* response of MDM to MAP infection is the expression and release of molecules acting to attract other immune cells to the site of the infection.

5.3. MAP infection modulates the *Janus kinase/signal transducers and activators of transcription (JAK/STAT)* signalling cascade

The role of cytokines to modulate and coordinate the immune response is mediated by cytokine receptors that generally form a stable association with a cytoplasmic tyrosine kinase known as a JAnus Kinase. The *JAK/STAT* signaling pathway is the main signaling cascade involved in activation and regulation of host immune response by cytokines and growth factors. Signal transduction by IFNγ is classically associated with this pathway. A large number of genes found to be differentially expressed in the present study are associated with the JAK-STAT pathway, including *IFNG, LIF, OSM, IL11, IL12B, IL23A, CSF2, CSF3* which were up-regulated in MAP infected MDM (Table 6). However, it has previously been shown that MAP infection inhibits JAK-STAT signaling via increased expression of suppressor of cytokine signaling (*SOCS*) as well as decreased expression of the IFNγ receptor genes (Arsenault et al. 2014). In the present study, the cytokine inducible SH2-containing protein (*CISH*) and the suppressor of cytokine signaling 3 (*SOCS3*) were highly expressed in infected MDM at 6h and 24h PI, while the IFNγ receptor 1 (*IFNGR1*) expression was reduced in MDM at 24hours PI (Table 6 and Fig. S9). Thus MAP may alter, or even shut down, this signaling cascade to increase its survival inside the macrophage. Many virus and bacteria have evolved mechanisms to reduce IFNγ response following infection by inhibiting JAK/STAT signaling at various levels. It has been shown that *M. avium* increases expression of the suppressor of cytokine signaling (*SOCS*) to diminish IFNγ responsiveness in infected human macrophages (Vazquez et al. 2006). Increased expression of *SOCS1* and *SOCS3*, as well as decreased expression of *IFNGR1* and *IFNGR2* in MAP infected monocytes has also been reported (Arsenault et al. 2012). Reduced expression of IFNγ-inducible genes was observed in mouse macrophages infected with *M. avium* (Hussain et al. 1999), while *SOCS3* was up-regulated in MAP infected MDM (Casey et al. 2015).

Given the importance of IFNγ for the control and clearance of intracellular pathogens, this seems to be a significant host defense mechanism that MAP seeks to avoid (Arsenault et al. 2014).

5.4. MAP infection regulates genes involved in antigen presentation

Antigen processing and presentation pathway was one of the most down-regulated KEGG pathways seen in this study (Table 7 and Fig. S10). Previous reports have shown that mycobacteria reduce the expression of the major histocompatibility complex (MHC) class II genes *in vitro* and *in vivo,* thus diminishing the capacity of antigen presentation and activation of T helper cells (Baquero and Plattner 2016; Berger and Griffin 2006; Grace and Ernst 2016; Hussain et al. 1999; Khare et al. 2012; Noss et al. 2000; Pecora et al. 2009; Weiss et al. 2001).

MHC class II gene expression is finely regulated in macrophages and dendritic cells, and the expression of MHC class II molecules is induced by IFNγ. This activates JAK–STAT signaling via its *IFNGRs*, and induces expression of genes related to the antigen processing, including the MHC class II trans-activator (*CIITA).* CIITA has been referred to as "the master control factor" of the transcription of MHC class II genes and regulates the expression of the genes encoding the classical MHC class II proteins (*DR, DP* and *DQ*), and several genes encoding accessory proteins required for MHC class II-restricted antigen-presentation (*Ii, DM* and *DO*) (LeibundGut-Landmann et al. 2004). In the present study, *CIITA* was down-regulated at 6 hours and 24 hours PI (Table 7). This is consistent with lower expression level of classical components of the MHC class II complex (*BOLA-DRA, BOLA-DRB3, BOLA-DQB, BOLA-DQA2, BOLA-DQA5*) in infected MDM at 24 hours PI. Subunits of MHC-II DM, alpha and beta (*BOLA-DMA, BOLA-DMB*), and the subunit alpha of MHC-II DO (*BOLA-DOA*) which are key regulator of MHC class II antigen presentation to T cells (Mellins and Stern 2014; Pos et al. 2013) were also down regulated. Given the key role of the MHC class II in focusing the immune response, the down-regulation of the MHC class II during the establishment of MAP infection may delay, or even block, activation of an immune response against infecting pathogens. The reduced expression of MHC class II genes has been reported in the blood of calves exposed to MAP while expression of the MHC class I genes was increased (Purdie et al. 2012). MHC class II molecules are involved in the development of the humoral immune response while the MHC class I molecules are responsible for T cytotoxic cell recognition of infected cells. Therefore, in modulating expression of MHC class I and class II antigens, MAP may affect the type and specificity of the host immune response. A correlation between a variation at residue 53 (Val53Leu) in Bola DRB3 exon 2 and increased susceptibility to MAP infection has been described (Rastislav and Mangesh 2012) further suggesting a functional role of MHC class II antigens in regulating the progression of infection.

5.5. Regulation of genes involved in MAP phagocytosis

Genes within the KEGG p*hagosome* and *lysosome* pathways were down regulated following MAP infection of MDM (Tables 8-9; Fig. S11 and S12). The interaction of mycobacteria with MDM and their subsequent uptake is mediated by receptors including complement receptors CR1, CR3, and CR4, immunoglobulin receptor FcR, the mannose receptor, and scavenger receptors (Woo and Czuprynski 2008). Many receptors involved in the process of phagocytosis were down-regulated in MAP infected MDM, with the exception of *ITGB3* (integrin subunit beta 3), *ITGA2* (integrin subunit alpha 2) and *OLR1* (oxidized low density lipoprotein) which were up-regulated (Table 8 and Fig. S11). Several genes encoding lysosomal enzymes, including glycosydases, lypases, sulphatases, phosphatases were down regulated in MAP infected MDM at 24h PI (Table 9 and Fig. S12). Down-regulation of the majority of key effector lysosomal enzymes has been reported for *M. bovis* infection of alveolar macrophages (Nalpas et al. 2015). The suppression of lysosomal function may be a general defense mechanism used by mycobacteria to promote its survival following phagocytosis by the macrophage (Podinovskaia et al. 2014). A feature of phagosome maturation is the acidification of the lumen, which is an important antimicrobial process. Interestingly, slight, but significant, up-regulation of genes mediating acidification (*ATP6V0B, ATP6V0A2*) was seen in MDM at 6h and 24h PI (Table 9). This was also seen in previous studies of MAP and *M. bovis* infected macrophages (Weiss et al. 2002; Murphy et al. 2006; Nalpas et al. 2015). MAP secretes a tyrosine phosphatase (PtpA) that binds a specific subunit of the macrophage v-ATPase. Therefore MAP may inhibit phagosome acidification by decreasing energy supplies to enzymes involved in the process, rather than regulating their expression directly (Arsenault et al. 2014).

5.6. MAP infection alters complement cascade

Although macrophages play a minor role, compared with liver, in the production of complement proteins, it is interesting to note that key proteins of the complement system were down-regulated in MDM following MAP infection (Table 10; Fig. S13). The reduced expression of various complement associated genes suggest that the complement system is partially suppressed by infection of macrophages which may favour MAP survival.

6. Conclusions

Transcription changes in MDM used here as a cellular model for MAP infection suggests that MAP subverts the macrophage response in order to survive and multiply inside the cell and to avoid detection by the immune system. MAP infection affects the cytokine signaling pathway and down regulates the major histocompatibility complex class II, potentially altering antigen presentation which may allow the bacterium to partly avoid detection by regulation of the immune response.

MAP also interferes with the formation of the mature phagosomes and therefore once inside the macrophage is able to survive. Finally, MAP down-regulates the complement immune pathway, hence increasing the chance of survival of bacteria when outside the host cells. It is not known if the changes in gene expression described here in MAP infected MDM are driven by the pathogen or the host or both. It would be interesting to hypothesize that the differences in expression may facilitate the persistence of the bacterium within the infected macrophage during the preclinical stage of JD, however, further research will be required to explore the role of the potential target genes identified and the mechanisms involved.

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Figures

Fig 1 Venn diagram showing transcription changes following infection of monocyte derived macrophages (MDM) with *M. avium* subsp. *paratuberculosis* at 2 hours post infection (PI), 6 hours PI and 24 hours PI based on RNA-seq data analysis.

Tables

Table 1 Top ten genes up and down regulated at 2, 6 and 24 hours post infection in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* (FDR ≤ 0.05).

Table 2 The 10 top ranked overrepresented molecular functions in monocyte-derived macrophages infected with M*ycobacterium avium* subsp. *paratuberculosis* at 2 hours, 6 hours and 24 hours post infection identified using the Gene Ontology (GO) over-representation analysis (ORA) in InnateDB.

Table 3 The 10 top ranked overrepresented biological processes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* at 2 hours, 6 hours and 24 hours post

infection identified using the Gene Ontology (GO) over-representation analysis (ORA) in InnateDB.

Table 4 The 10 top ranked overrepresented cellular components in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* at 2 hours, 6 hours and 24 hours post infection identified using the Gene Ontology (GO) over-representation analysis (ORA) in InnateDB.

Table 5 Pathways (Path) overrepresented in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis.*

Table 6 Differentially transcribed genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* belonging to the Cytokine-cytokine receptor interaction pathway and JAK-STAT signalling cascade.

Table 7 Differentially transcribed genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* participate to the MHC class II antigen processing and presentation pathway.

Table 8 Differentially transcribed genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* involved in the Phagocytosis process.

Table 9 Differentially transcribed genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* located in the Lysosome compartment.

Table 10 Differentially transcribed genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* belonging to the Coagulation and Complement Cascade.

Supplementary Material

505 Fig. S1 Venn diagram drawn by VennPlex program (Cai et al. 2013). The number of factors in each
506 set or intersection that are up-regulated are represented in italic, down-regulated are underlined or
507 contra-regulated are in red colour.
508
509 Fig. S2 Bar chart of significantly enriched pathways resulting analyzing the list of up-regulated
510 genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp.
511 *paratuberculosis* at 2 hours post infection (PI) using the REACTOME database of *Bos taurus*
512 (InnateDB).
513
514 Fig. S3 Bar chart of significantly enriched pathways resulting analyzing the list of down-regulated
515 genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp.
516 *paratuberculosis* at 2 hours post infection (PI) using the REACTOME database of *Bos taurus*
517 (InnateDB).
518
519 Fig. S4 Bar chart of significantly enriched pathways resulting analyzing the list of up-regulated
520 genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp.
521 *paratuberculosis* at 6 hours post infection (PI) using the REACTOME database of *Bos taurus*
522 (InnateDB).
523
524 Fig. S5 Bar chart of significantly enriched pathways resulting analyzing the list of down-regulated
525 genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp.
526 *paratuberculosis* at 6 hours post infection (PI) using the REACTOME database of *Bos taurus*
527 (InnateDB).
528
529 Fig. S6 Bar chart of significantly enriched pathways resulting analyzing the list of up-regulated
530 genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp.
531 *paratuberculosis* at 24 hours post infection (PI) using the REACTOME database of *Bos taurus*
532 (InnateDB).
533
534 Fig. S7 Bar chart of significantly enriched pathways resulting analyzing the list of down-regulated
535 genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp.
536 *paratuberculosis* at 24 hours post infection (PI) using the REACTOME database of *Bos taurus*
537 (InnateDB).
538

Fig. S8 Cytokine-cytokine receptor interaction KEGG pathway. In red up-regulated genes in monocyte-derived macrophages (MDM) infected with *Mycobacterium avium* subsp. *paratuberculosis* . In green down-regulated genes in infected MDM. No indication is reported about the time point.

Fig. S9 Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling. In red up-regulated genes in monocyte-derived macrophages (MDM) infected with *Mycobacterium avium* subsp. *paratuberculosis*. In green down-regulated genes in infected MDM. No indication is reported about the time point.

Fig. S10 Antigen processing and presentation pathway. In red up-regulated genes in monocyte-derived macrophages (MDM) infected with *Mycobacterium avium* subsp. *paratuberculosis*. In green down-regulated genes in infected MDM. No indication is reported about the time point.

Fig. S11 Phagosome. In red up-regulated genes in monocyte-derived macrophages (MDM) infected with *Mycobacterium avium* subsp. *paratuberculosis*. In green down-regulated genes in infected MDM. No indication is reported about the time point.

Fig. S12 Lysosome. In red up-regulated genes in monocyte-derived macrophages (MDM) infected with *Mycobacterium avium* subsp. *paratuberculosis*. In green down-regulated genes in infected MDM. No indication is reported about the time point.

Fig. S13 Complement and coagulation cascades. In red up-regulated genes in monocyte-derived macrophages (MDM) infected with *Mycobacterium avium* subsp. *paratuberculosis*. In green down-regulated genes in infected MDM. No indication is reported about the time point.

Table S1. List of significantly (P value ≤0.05) regulated genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* at 2 hours post infection.

Table S2. List of significantly (P value ≤0.05) regulated genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* at 6 hours post infection.

Table S3. List of significantly (P value ≤0.05) regulated genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* at 24 hours post infection.

Table S4. List of enriched GO resulted analyzing the list of regulated genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* at 2 hours post infection using the Gene Ontology (GO) over-representation analysis (ORA) in InnateDB.

Table S5. List of enriched GO resulted analyzing the list of regulated genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* at 6 hours post infection using the Gene Ontology (GO) over-representation analysis (ORA) in InnateDB.

Table S6. List of enriched GO resulted analyzing the list of regulated genes in monocyte-derived macrophages infected with *Mycobacterium avium* subsp. *paratuberculosis* at 24 hours post infection using the Gene Ontology (GO) over-representation analysis (ORA) in InnateDB.

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Figure

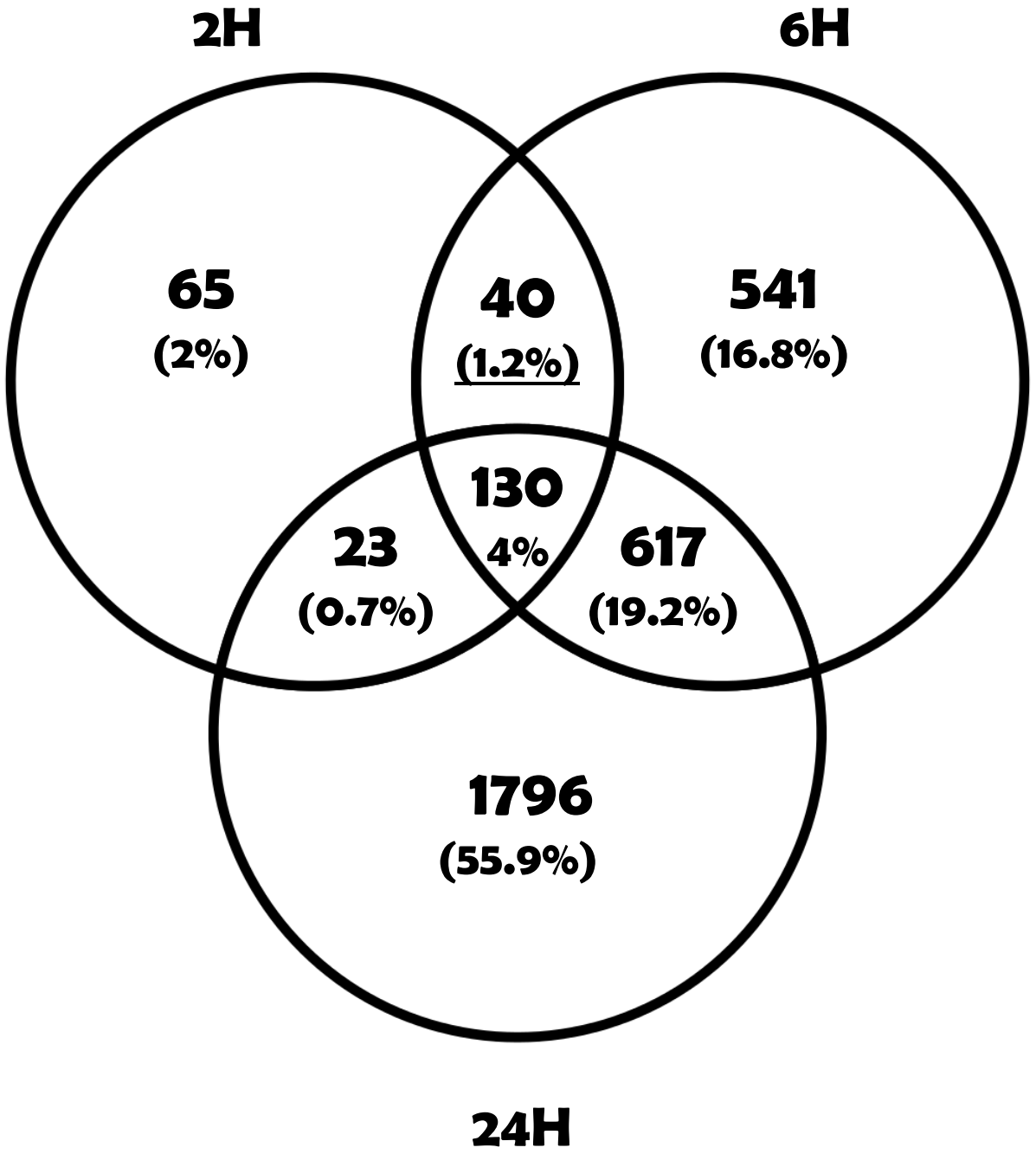

Table 1

Time point	GeneID	Gene Symbol and Name	logFC	PValue	FDR
2h	**ENSBTAG00000001570**	***CSF2*, colony stimulating factor 2 (granulocyte-macrophage)**	**5,88**	**4,02E-05**	**0,004683**
2h	**ENSBTAG00000009393**	***GAL*, galanin/GMAP prepropeptide**	**5,46**	**3,15E-11**	**3,55E-08**
2h	**ENSBTAG00000002362**	***APOLD1*, apolipoprotein L domain containing 1**	**5,21**	**3,09E-06**	**0,000629**
2h	**ENSBTAG00000016163**	***OSM*, oncostatin M**	**4,73**	**2,82E-08**	**1,06E-05**
2h	**ENSBTAG00000006367**	***CTGF*, connective tissue growth factor**	**4,67**	**0,000121**	**0,010779**
2h	**ENSBTAG00000043738**	**Non-coding RNAs**	**4,67**	**0,000242**	**0,018387**
2h	**ENSBTAG00000047400**	***IL11*, interleukin 11**	**4,61**	**0,000453**	**0,028641**
2h	**ENSBTAG00000019892**	***HAS2*, hyaluronan synthase 2**	**4,34**	**1,29E-06**	**0,000314**
2h	**ENSBTAG00000002150**	***IL17A*, Interleukin-17A**	**4,32**	**0,000705**	**0,038868**
2h	**ENSBTAG00000010273**	***EREG*, epiregulin**	**4,28**	**3,59E-09**	**2,02E-06**
2h	ENSBTAG00000017648	*TIFAB*, TRAF-interacting protein	-3,78	3,41E-08	1,24E-05
2h	ENSBTAG00000011638	Uncharacterized protein	-3,66	4,81E-13	1,49E-09
2h	ENSBTAG00000011195	Uncharacterized protein	-3,53	1,85E-05	0,002631
2h	ENSBTAG00000038415	*SLC6A12*, solute carrier family 6 member 12	-3,51	7,41E-08	2,3E-05
2h	ENSBTAG00000012715	*KIF26B*, kinesin family member 26B	-2,86	0,000737	0,040096
2h	ENSBTAG00000031397	*P2RY13*, purinergic receptor P2Y, G-protein coupled, 13	-2,85	0,00039	0,025857
2h	ENSBTAG00000005603	*CXCL11*, chemokine (C-X-C motif) ligand 11	-2,83	0,000152	0,012694
2h	ENSBTAG00000016344	*PIK3R6*, phosphoinositide-3-kinase, regulatory subunit 6	-2,68	5,28E-06	0,000926
2h	ENSBTAG00000009055	*RNF144B*, ring finger protein 144B	-2,59	4,04E-05	0,004683
2h	ENSBTAG00000039012	*MFSD6L*, major facilitator superfamily domain containing 6-like	-2,22	0,000518	0,031962
6h	**ENSBTAG00000024058**	***EGR4* ,early growth response 4**	**7,53**	**4,26E-08**	**3,89E-06**
6h	**ENSBTAG00000047400**	***IL11*, interleukin 11**	**7,41**	**3,63E-06**	**0,000154**
6h	**ENSBTAG00000018290**	***IL9*, interleukin 9**	**7,22**	**1,51E-15**	**1,39E-12**
6h	**ENSBTAG00000021717**	***BDKRB2*, bradykinin receptor B2**	**7,06**	**8,81E-11**	**2,1E-08**
6h	**ENSBTAG00000001570**	***CSF2*, colony stimulating factor 2 (granulocyte-macrophage)**	**7,00**	**3,46E-06**	**0,00015**
6h	**ENSBTAG00000021699**	***RORB*, RAR-related orphan receptor B**	**6,90**	**6,95E-08**	**5,87E-06**
6h	**ENSBTAG00000000783**	***TGFA*, transforming growth factor, alpha**	**6,53**	**2,74E-06**	**0,000124**
6h	**ENSBTAG00000007424**	***LIF*, leukemia inhibitory factor**	**6,17**	**4,71E-12**	**1,77E-09**

Table 1 (continued)

Time point	GeneID	Gene Symbol and Name	logFC	PValue	FDR
6h	**ENSBTAG00000008182**	***FOSB*, FBJ murine osteosarcoma viral oncogene homolog B**	**6,11**	**1,87E-18**	**4,71E-15**
6h	**ENSBTAG00000009393**	***GAL*, galanin/GMAP prepropeptide**	**5,88**	**1,25E-12**	**6,45E-10**
6h	ENSBTAG00000020433	*NLRP1*, NLR family, pyrin domain containing 1	-5,40	7,14E-16	8,85E-13
6h	ENSBTAG00000008479	*CXCL13*, chemokine (C-X-C motif) ligand 13	-4,59	8,95E-08	7,16E-06
6h	ENSBTAG00000012715	*KIF26B*, kinesin family member 26B	-4,33	5,07E-07	3,14E-05
6h	ENSBTAG00000011638	Uncharacterized protein	-4,27	1,04E-15	1,07E-12
6h	ENSBTAG00000005424	*KCNC1*, potassium channel, voltage gated Shaw related subfamily C, member 1	-4,08	6,66E-05	0,001708
6h	ENSBTAG00000015296	*PTPRB*, protein tyrosine phosphatase, receptor type, B	-3,93	6,96E-09	8,38E-07
6h	ENSBTAG00000009975	*PBX4*, pre-B-cell leukemia homeobox 4	-3,89	3,48E-06	0,000151
6h	ENSBTAG00000004680	*SLC13A5*, solute carrier family 13 (sodium-dependent citrate transporter), member 5	-3,79	9,28E-12	3,11E-09
6h	ENSBTAG00000002773	Uncharacterized protein	-3,56	9,22E-09	1,05E-06
6h	ENSBTAG00000005828	*MERTK*, MER proto-oncogene, tyrosine kinase	-3,51	2,47E-20	3,06E-16
24h	**ENSBTAG00000016835**	***IL17F*, interleukin 17F**	**10,13**	**5,39E-19**	**3,93E-16**
24h	**ENSBTAG00000002150**	***IL17A*, Interleukin-17A**	**8,66**	**2,06E-11**	**2,3E-09**
24h	**ENSBTAG00000012529**	***IFNG*, interferon, gamma**	**8,50**	**9,18E-09**	**4,48E-07**
24h	**ENSBTAG00000000437**	***FFAR4*, free fatty acid receptor 4**	**8,34**	**8,53E-14**	**1,73E-11**
24h	**ENSBTAG00000001570**	***CSF2*, colony stimulating factor 2 (granulocyte-macrophage)**	**8,14**	**2,78E-07**	**9,2E-06**
24h	**ENSBTAG00000039080**	***SLC22A3*, solute carrier family 22 (organic cation transporter), member 3**	**8,12**	**3,07E-13**	**5,37E-11**
24h	**ENSBTAG00000046375**	**LOC100850808**	**8,05**	**5,89E-13**	**1,01E-10**
24h	**ENSBTAG00000004741**	***IL12B*, interleukin 12B**	**7,53**	**1,8E-07**	**6,39E-06**
24h	**ENSBTAG00000039028**	***PI3*, Peptidase inhibitor 3**	**7,26**	**1,22E-13**	**2,4E-11**
24h	**ENSBTAG00000010085**	***SLC7A2*, solute carrier family 7 (cationic amino acid transporter, y+ system), member 2**	**7,17**	**7,76E-07**	**2,18E-05**
24h	ENSBTAG00000014365	Uncharacterized protein	-10,17	2,3E-07	7,82E-06
24h	ENSBTAG00000013185	*TIMD4*, T-cell immunoglobulin and mucin domain containing 4	-9,81	2,21E-19	1,83E-16
24h	ENSBTAG00000046977	*PLA2G2D4*, Phospholipase A(2)	-9,62	3,37E-11	3,45E-09
24h	ENSBTAG00000019960	*TM4SF18*, transmembrane 4 L six family member 18	-8,65	1,07E-08	5,11E-07
24h	ENSBTAG00000014313	*PYROXD2*, pyridine nucleotide-disulphide oxidoreductase domain 2	-8,43	3,51E-18	1,98E-15
24h	ENSBTAG00000020499	*PLA2G2D1*, Putative calcium-dependent phospholipase A2	-8,35	2E-05	0,000322
24h	ENSBTAG00000034323	*SLC2A5*, solute carrier family 2, facilitated glucose transporter member 5	-8,33	1,22E-15	3,98E-13

Table 1 (continued)

Time point	GeneID	Gene Symbol and Name	logFC	PValue	FDR
24h	ENSBTAG00000047776	Uncharacterized protein	-8,28	3,93E-06	8,45E-05
24h	ENSBTAG00000016997	LOC511180	-8,24	9,1E-15	2,3E-12
24h	ENSBTAG00000004560	*CLEC4F*, C-type lectin domain family 4, member F	-8,01	6E-09	3,11E-07

Bold indicates significance up-regulation

Table 2

GO Term ID	GO Term Name	Uploaded Genes Associated with GO Term	Genes Associated with GO Term in InnateDB	GO Term ORA P-Value	GO Term ORA P-Value (Corrected)	Time point
GO:0005125	cytokine activity	14	149	<1.0E-5	0.00001	2h
GO:0008083	growth factor activity	11	120	<1.0E-5	0.00014	2h
GO:0003700	sequence-specific DNA binding transcription factor activity	28	861	<1.0E-5	0.00106	2h
GO:0000977	RNA polymerase II regulatory region sequence-specific DNA binding	8	77	<1.0E-5	0.00113	2h
GO:0005515	protein binding	144	8713	<1.0E-5	0.00106	2h
GO:0017017	MAP kinase tyrosine/serine/threonine phosphatase activity	4	12	0.00001	0.00176	2h
GO:0043565	sequence-specific DNA binding	20	631	0.00017	0.01214	2h
GO:0055106	ubiquitin-protein ligase regulator activity	2	3	0.00048	0.02342	2h
GO:0043426	MRF binding	2	5	0.00157	0.04406	2h
GO:0001228	RNA polymerase II transcription regulatory region sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription	4	41	0.00177	0.04731	2h
GO:0005515	protein binding	699	8713	<1.0E-5	<1.0E-5	6h
GO:0005125	cytokine activity	33	149	<1.0E-5	<1.0E-5	6h
GO:0016772	transferase activity, transferring phosphorus-containing groups	65	534	<1.0E-5	0.00054	6h
GO:0004713	protein tyrosine kinase activity	57	456	<1.0E-5	0.00084	6h
GO:0000166	nucleotide binding	145	1538	<1.0E-5	0.00192	6h
GO:0005524	ATP binding	124	1273	<1.0E-5	0.00192	6h
GO:0016773	phosphotransferase activity, alcohol group as acceptor	18	85	<1.0E-5	0.00206	6h
GO:0004672	protein kinase activity	58	510	0.00004	0.00577	6h

Table 2 (continued)

GO Term ID	GO Term Name	Uploaded Genes	Genes Associated with	GO Term	GO Term ORA P-	Time

		Associated with GO Term	GO Term in InnateDB	ORA P-Value	Value (Corrected)	point
GO:0001206	RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity involved in negative regulation of transcription	4	5	0.00009	0.01061	6h
GO:0004725	protein tyrosine phosphatase activity	17	95	0.00015	0.01513	6h
GO:0005515	protein binding	1317	8713	<1.0E-5	<1.0E-5	24h
GO:0005125	cytokine activity	46	149	<1.0E-5	<1.0E-5	24h
GO:0005524	ATP binding	227	1273	<1.0E-5	0.00015	24h
GO:0005164	tumor necrosis factor receptor binding	15	28	<1.0E-5	0.00015	24h
GO:0016491	oxidoreductase activity	105	505	<1.0E-5	0.00021	24h
GO:0008009	chemokine activity	17	39	<1.0E-5	0.00077	24h
GO:0005178	integrin binding	20	55	0.00001	0.00231	24h
GO:0015179	L-amino acid transmembrane transporter activity	5	5	0.00004	0.0065	24h
GO:0016787	hydrolase activity	174	1024	0.00013	0.01642	24h
GO:0035091	phosphatidylinositol binding	21	69	0.00013	0.01637	24h

Table 3

GO Term ID	GO Term Name	Uploaded Genes Associated with GO Term	Genes Associated with GO Term in InnateDB	GO Term ORA P-Value	GO Term ORA P-Value (Corrected)	Time point
GO:0006955	immune response	16	217	<1.0E-5	0.00001	2h
GO:0035914	skeletal muscle cell differentiation	8	43	<1.0E-5	0.00003	2h
GO:0045944	positive regulation of transcription from RNA polymerase II promoter	27	739	<1.0E-5	0.00025	2h
GO:0000188	inactivation of MAPK activity	5	20	<1.0E-5	0.00106	2h
GO:0045893	positive regulation of transcription, DNA-templated	18	451	0.00002	0.00268	2h
GO:0031668	cellular response to extracellular stimulus	4	15	0.00003	0.00392	2h
GO:0006469	negative regulation of protein kinase activity	6	50	0.00004	0.00457	2h
GO:0046888	negative regulation of hormone secretion	3	6	0.00004	0.00427	2h
GO:0045672	positive regulation of osteoclast differentiation	4	17	0.00005	0.00536	2h
GO:0045651	positive regulation of macrophage differentiation	3	8	0.00011	0.01026	2h
GO:0006955	immune response	40	217	<1.0E-5	<1.0E-5	6h
GO:0071222	cellular response to lipopolysaccharide	19	59	<1.0E-5	<1.0E-5	6h
GO:0006954	inflammatory response	29	134	<1.0E-5	0.00001	6h
GO:0045944	positive regulation of transcription from RNA polymerase II promoter	88	739	<1.0E-5	0.00004	6h
GO:0035556	intracellular signal transduction	47	312	<1.0E-5	0.00006	6h
GO:0050728	negative regulation of inflammatory response	16	55	<1.0E-5	0.00016	6h
GO:0045672	positive regulation of osteoclast differentiation	9	17	<1.0E-5	0.00016	6h
GO:0045893	positive regulation of transcription, DNA-templated	58	451	<1.0E-5	0.00038	6h
GO:0032755	positive regulation of interleukin-6 production	12	36	<1.0E-5	0.00066	6h

Table 3 (continued)

GO Term ID	GO Term Name	Uploaded Genes Associated with GO Term	Genes Associated with GO Term in InnateDB	GO Term ORA P-Value	GO Term ORA P-Value (Corrected)	Time point
GO:0032729	positive regulation of interferon-gamma production	12	37	<1.0E-5	0.00085	6h
GO:0006955	immune response	83	217	<1.0E-5	<1.0E-5	24h
GO:0006954	inflammatory response	51	134	<1.0E-5	<1.0E-5	24h
GO:0008152	metabolic process	226	1208	<1.0E-5	<1.0E-5	24h
GO:0071222	cellular response to lipopolysaccharide	25	59	<1.0E-5	0.00001	24h
GO:0019882	antigen processing and presentation	27	76	<1.0E-5	0.00019	24h
GO:0030335	positive regulation of cell migration	34	109	<1.0E-5	0.00022	24h
GO:0050728	negative regulation of inflammatory response	21	55	<1.0E-5	0.00076	24h
GO:0055114	oxidation-reduction process	127	673	<1.0E-5	0.00243	24h
GO:0048661	positive regulation of smooth muscle cell proliferation	11	20	0.00001	0.00238	24h
GO:0002504	antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	9	14	0.00001	0.00251	24h

Table 4

GO Term ID	GO Term Name	Uploaded Genes Associated with GO Term	Genes Associated with GO Term in InnateDB	GO Term ORA P-Value	GO Term ORA P-Value (Corrected)	Time point
GO:0005737	cytoplasm	69	3807	0.00079	0.03452	2h
GO:0005634	nucleus	77	4394	0.00096	0.03159	2h
GO:0005615	extracellular space	21	797	0.0013	0.04028	2h
GO:0005737	cytoplasm	327	3807	<1.0E-5	0.00005	6h
GO:0005925	focal adhesion	17	94	0.00013	0.01385	6h
GO:0043234	protein complex	33	254	0.00016	0.01583	6h
GO:0031234	extrinsic component of cytoplasmic side of plasma membrane	7	20	0.0002	0.01733	6h
GO:0005634	nucleus	342	4394	0.00031	0.01933	6h
GO:0030175	filopodium	9	37	0.00055	0.02905	6h
GO:0032009	early phagosome	3	4	0.00111	0.04447	6h
GO:0070062	extracellular vesicular exosome	392	2047	<1.0E-5	<1.0E-5	24h
GO:0005737	cytoplasm	624	3807	<1.0E-5	<1.0E-5	24h
GO:0005765	lysosomal membrane	53	163	<1.0E-5	<1.0E-5	24h
GO:0005764	lysosome	47	146	<1.0E-5	<1.0E-5	24h
GO:0009897	external side of plasma membrane	48	171	<1.0E-5	0.00008	24h
GO:0009986	cell surface	72	300	<1.0E-5	0.00008	24h
GO:0005783	endoplasmic reticulum	128	639	<1.0E-5	0.00016	24h
GO:0005925	focal adhesion	31	94	<1.0E-5	0.0002	24h
GO:0005615	extracellular space	148	797	<1.0E-5	0.00123	24h
GO:0005739	mitochondrion	222	1290	<1.0E-5	0.00161	24h

(*) At 2 and 6 hours we reported the GO term (<10) significantly overrepresented after correction for multiple testing.

Table 5

Path Name	Path Id	Regulated genes % in the Path	Path p-value	Gene Symbols
Innate Immune System	19515	32	0,000	*ACTG1; ACTR3; ADCY7; APP; BAIAP2; C1QA; C1QB; CALM; CFD; DNM1; DUSP6; FGF1; FOS; GAB1; HSP90AA1; HSP90B1; IRAK2; IRF1; LGMN; MAP2K6; MYD88; MYO1C; NFATC1; NLRP3; NOD2; NR4A1; PAK1; PDE1B; PIK3R2; POLR3GL; PRKACA; PRKAR1A; PROS1; RIPK2; S100B; SHC1; TICAM2; TMEM173; TRIB3; TRIM21; TXN; UBE2D1; UBE2N; WASF2;*
Chemokine receptors bind chemokines	19574	53	0,001	*CCL20; CCL4; CCL5; CCRL2; CXCL11; CXCL2; CXCL2; CXCL9; CXCR4; IL8;*
Signaling by Interleukins	19663	40	0,003	*CSF2; IL18; IL1A; IL1B; IL1RN; IL2RG; IRAK2; MAP2K6; MYD88; NOD2; PIK3R2; PRKACA; RIPK2; SHC1; SOCS3; UBE2N;*
Immune System	17907	26	0,005	*ACTG1; ACTR3; ADCY7; AP1S1; AP2A2; APP; BAIAP2; C1QA; C1QB; CALM; CD200R1; CD81; CFD; CISH; CSF2; DNM1; DUSP6; DYNLL1; EIF4A1; EIF4E; FGF1; FOS; GAB1; HSP90AA1; HSP90B1; IFNG; IL18; IL1A; IL1B; IL1RN; IL2RG; IRAK2; IRF1; KIF3C; LGMN; MAP2K6; MYD88; MYO1C; NFATC1; NLRP3; NOD2; NR4A1; PAK1; PDE1B; PIK3R2; POLR3GL; PRKACA; PRKAR1A; PROS1; PSMA1; PSMD5; RIPK2; S100B; SHC1; SOCS3; TICAM2; TMEM173; TRIB3; TRIM21; TUBA1B; TUBB4B; TXN; UBE2D1; UBE2N; VASP; WASF2;*
Cytokine Signaling in Immune system	17637	33	0,009	*CISH; CSF2; EIF4A1; EIF4E; IFNG; IL18; IL1A; IL1B; IL1RN; IL2RG; IRAK2; MAP2K6; MYD88; NOD2; PIK3R2; PRKACA; RIPK2; SHC1; SOCS3; UBE2N;*
Interleukin-1 signaling	19605	43	0,014	*IL1A; IL1B; IL1RN; IRAK2; MAP2K6; MYD88; NOD2; RIPK2; UBE2N;*
Classical antibody-mediated complement activation	18280	100	0,040	*C1QA; C1QB;*
Clathrin derived vesicle budding	18426	47	0,017	*AP1S1; ARRB1; NECAP1; RAB5C; SNX5; STX4; VAMP8;*
trans-Golgi Network Vesicle Budding	18678	47	0,017	*AP1S1; ARRB1; NECAP1; RAB5C; SNX5; STX4; VAMP8;*
Regulation of actin dynamics for phagocytic cup formation	18471	44	0,026	*ACTG1; ACTR3; BAIAP2; HSP90AA1; MYO1C; PAK1; WASF2;*

Table 5 (continued)

Path Name	Path Id	Regulated genes % in the Path	Path p-value	Gene Symbols
Fcgamma receptor (FCGR) dependent phagocytosis	17005	40	0,031	*ACTG1; ACTR3; BAIAP2; HSP90AA1; MYO1C; PAK1; PIK3R2; WASF2;*
Apoptotic execution phase	18699	56	0,019	*DBNL; DNM1L; H1F0; KPNA1; VIM;*
Death Receptor Signalling	19726	75	0,027	*FAS; TNF; TRADD;*
Extrinsic Pathway for Apoptosis	16978	75	0,027	*FAS; TNF; TRADD;*
Activation of caspases through apoptosome-mediated cleavage	17461	100	0,040	*CYCS; CYCS;*
Detoxification of Reactive Oxygen Species	17995	44	0,015	*CAT; CYCS; CYCS; PRDX3; PRDX6; SOD1; TXN; TXNRD1;*
Biological oxidations	18212	33	0,040	*AHCY; ALDH1A1; ALDH2; BPNT1; COMT; FDX1; GGCT; GSTA4; MAOA; MAT2B; TPMT; UGDH;*

Table 6

Ensembl ID	Gene name	Gene descriptiopn	2h PI	6h PI	24h PI
CHEMOKINES					
ENSBTAG00000027513	*CXCL2*	chemokine (C-X-C motif) ligand 2	n.s.	3.69	4.76
ENSBTAG00000019716	*CXCL8 (IL8)*	chemokine (C-X-C motif) ligand 8	n.s.	3.36	4.36
ENSBTAG00000037778	*CXCL3*	chemokine (C-X-C motif) ligand 3	n.s.	2.57	2.82
ENSBTAG00000038639	*CXCL9*	chemokine (C-X-C motif) ligand 9	n.s.	n.s.	-2.60
ENSBTAG00000005603	*CXCL11*	chemokine (C-X-C motif) ligand 11	-2.83	n.s.	-3.74
ENSBTAG00000008479	*CXCL13*	chemokine (C-X-C motif) ligand 13	n.s.	n.s.	-4.46
ENSBTAG00000009943	*XCL2*	chemokine (C motif) ligand 2	n.s.	n.s.	3.66
ENSBTAG00000024869	*CX3CL1*	chemokine (C-X3-C motif) ligand 1	n.s.	n.s.	3.40
ENSBTAG00000025250	*CCL3*	chemokine (C-C motif) ligand 3	2.91	3.88	6.04
ENSBTAG00000017718	*CCL22*	chemokine (C-C motif) ligand 22	n.s.	n.s.	5.81
ENSBTAG00000025257	*CCL4*	chemokine (C-C motif) ligand 4	n.s.	2.90	5.26
ENSBTAG00000021326	*CCL20*	chemokine (C-C motif) ligand 20	n.s.	2.61	4.73
ENSBTAG00000007191	*CCL5*	chemokine (C-C motif) ligand 5	n.s.	n.s.	2.27
ENSBTAG00000026275	*CCL24*	chemokine (C-C motif) ligand 24	n.s.	n.s.	2.12
ENSBTAG00000014113	*CCL8*	chemokine (C-C motif) ligand 8	n.s.	n.s.	-1.81
HEMAPOIETINS					
ENSBTAG00000001570	*CSF2*	colony stimulating factor 2	5.88	7.00	8.14
ENSBTAG00000004741	*IL12B*	interleukin 12B	n.s.	4.36	7.53
ENSBTAG00000007424	*LIF*	leukemia inhibitory factor	3.95	6.17	6.91
ENSBTAG00000021462	*CSF3*	colony stimulating factor 3	n.s.	3.52	4.01
ENSBTAG00000047400	*IL11*	interleukin 11	4.61	7.41	3.65
ENSBTAG00000018290	*IL9*	interleukin 9	3.97	7.22	3.53
ENSBTAG00000046110	*BSF3*	cardiotrophin-like cytokine factor 1	n.s.	3.21	3.15
ENSBTAG00000018015	*IL27 **	interleukin-27 subunit alpha precursor	n.s.	2.58	2.33
ENSBTAG00000004378	*IL23A*	interleukin 23 subunit alpha	3.41	3.30	2.27
ENSBTAG00000016163	*OSM*	oncostatin M	4.73	5.35	n.s.

Table 6 (continued)

Ensembl ID	Gene name	Gene descriptiopn	2h PI	6h PI	24h PI
ENSBTAG00000020892	*IL2RA*	interleukin 2 receptor subunit alpha	n.s.	2.79	3.84
ENSBTAG00000009455	*IL12RB2*	interleukin 12 receptor subunit beta 2	n.s.	2.18	3.02
ENSBTAG00000006078	*IL15RA*	interleukin 15 receptor subunit alpha	n.s.	n.s.	1.60
ENSBTAG00000014907	*IL11RA*	interleukin 11 receptor subunit alpha	n.s.	n.s.	-1.19
ENSBTAG00000033107	*OSMR*	oncostatin M receptor	n.s.	n.s.	-1.60
ENSBTAG00000008197	*EPOR*	erythropoietin receptor	n.s.	n.s.	-1.75
PDGF FAMILY					
ENSBTAG00000000283	*CSF1*	colony stimulating factor 1	3.63	3.16	4.80
ENSBTAG00000005339	*VEGFA*	vascular endothelial growth factor A	2.19	1.88	n.s.
ENSBTAG00000017664	*HGF*	hepatocyte growth factor (hepapoietin A; scatter factor)	n.s.	n.s.	-4.59
ENSBTAG00000043959	*PDGFC*	platelet derived growth factor C	n.s.	n.s.	-3.54
ENSBTAG00000016915	*FLT1*	fms related tyrosine kinase 1	n.s.	n.s.	4.04
ENSBTAG00000006161	*MET*	MET proto-oncogene, receptor tyrosine kinase	n.s.	3.13	3.38
ENSBTAG00000012771	*CSF1R*	colony stimulating factor 1 receptor	n.s.	n.s.	-2.64
INTERFERON FAMILY					
ENSBTAG00000012529	*IFNG*	interferon, gamma	n.s.	4.10	8.50
ENSBTAG00000012544	*IFNGR1*	interferon gamma receptor 1	n.s.	n.s.	-1.53
IL-10 FAMILY					
ENSBTAG00000006685	*IL10*	interleukin-10 precursor	n.s.	1.42	n.s.
ENSBTAG00000001101	*IFNLR1 (IL28RA)*	interferon, lambda receptor 1	n.s.	n.s.	3.94
TNF FAMILY					
ENSBTAG00000025471	*TNFA (TNF)*	tumor necrosis factor alpha	n.s.	4.98	4.54
ENSBTAG00000018069	*TNFSF15*	tumor necrosis factor superfamily member 15	n.s.	n.s.	4.49
ENSBTAG00000012223	*TNFSF14*	tumor necrosis factor superfamily member 14	3.34	3.12	3.93
ENSBTAG00000002894	*TNFSF4*	tumor necrosis factor superfamily member 4	n.s.	n.s.	3.23
ENSBTAG00000046266	*TNFSF9*	tumor necrosis factor superfamily member 9	2.06	2.98	3.15
ENSBTAG00000032808	*FASLG*	Fas ligand	n.s.	n.s.	2.03
ENSBTAG00000025782	*TNFSF8*	tumor necrosis factor superfamily member 8	n.s.	n.s.	1.84

Table 6 (continued)

Ensembl ID	Gene name	Gene descriptiopn	2h PI	6h PI	24h PI
ENSBTAG00000000130	*TNFSF13*	tumor necrosis factor superfamily member 13	n.s.	n.s.	-2.78
ENSBTAG00000012543	*EDA*	ectodysplasin A	n.s.	n.s.	-3.98
ENSBTAG00000015632	*TNFRSF18 (SF18)*	tumor necrosis factor receptor superfamily member 18	n.s.	2.59	5.12
ENSBTAG00000039937	*TNFRSF8 (SF8)*	tumor necrosis factor receptor superfamily member 8	n.s.	n.s.	4.38
ENSBTAG00000003313	*TNFRSF9 (SF9)*	tumor necrosis factor receptor superfamily member 9	n.s.	1.41	2.84
ENSBTAG00000012082	*TNFRSF12A (SF12A)*	tumor necrosis factor receptor superfamily member 12A	n.s.	1.94	2.74
ENSBTAG00000015635	*TNFRSF4 (SF4)*	tumor necrosis factor receptor superfamily member 4	n.s.	n.s.	2.22
ENSBTAG00000010785	*FAS*	Fas cell surface death receptor	n.s.	n.s.	1.87
TGF-β FAMILY					
ENSBTAG00000002912	*INHBA*	inhibin beta A	4.26	4.40	5.66
ENSBTAG00000018105	*ACVR2B*	activin A receptor type IIB	n.s.	n.s.	-2.51
IL-17 FAMILY					
ENSBTAG00000016835	*IL17F **	interleukin 17F	n.s.	3.15	10.13
ENSBTAG00000002150	*IL17A*	interleukin 17A	4.32	5.03	8.66
IL-1 FAMILY					
ENSBTAG00000002085	*IL36G **	interleukin 36, γ	n.s.	3.21	4.79
ENSBTAG00000001321	*IL1B*	interleukin 1 β	n.s.	3.10	4.67
ENSBTAG00000027676	*IL18BP **	interleukin 18 binding protein	n.s.	1.71	3.84
ENSBTAG00000010349	*IL1A*	interleukin 1 α	n.s.	3.36	3.35
ENSBTAG00000000277	*IL18*	interleukin 18	n.s.	n.s.	-2.24
ENSBTAG00000019665	*IL1RN **	interleukin 1 receptor antagonist	1.66	3.31	5.52
ENSBTAG00000033748	*IL18RAP*	interleukin 18 receptor accessory protein	n.s.	n.s.	4.04
ENSBTAG00000001034	*IL18R1*	interleukin 18 receptor 1	n.s.	n.s.	2.39
ENSBTAG00000013205	*IL1RAP*	interleukin 1 receptor accessory protein	n.s.	n.s.	1.89
JAK/STAT MEMBERS					
ENSBTAG00000022622	*CISH (CIS)*	cytokine inducible SH2-containing protein	n.s.	2.85	3.01
ENSBTAG00000008441	*SOCS3 (SOCS)*	suppressor of cytokine signaling 3	n.s.	1.51	1.42
ENSBTAG00000019456	*SPRED2 **	sprouty-related, EVH1 domain containing 2	1,81	n.s.	1,96

Table 6 (continued)

Ensembl ID	Gene name	Gene descriptiopn	2h PI	6h PI	24h PI
ENSBTAG00000020294	*PTPN6 (SHP1)*	protein tyrosine phosphatase, non-receptor type 6	n.s.	-1.92	n.s.
ENSBTAG00000002350	*PIK3R2 (PI3K)*	Phosphatidylinositol 3-kinase regulatory subunit β	n.s.	-1.28	-1.75
ENSBTAG00000020848	*PIK3CG (PI3K)*	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic sub γ	n.s.	-1.90	n.s.

Underlined genes are involved in both Cytokine-cytokine receptor interaction and JAK/STAT pathways.

Receptors are presented in gray.

* Indicates genes which are not represent in figure S8.

() Indicates the gene name or the protein complex name as shown in figure S8 or S9.

Table 7

Ensembl ID	Gene name	Gene descriptiopn	2h PI	6h PI	24h PI
ENSBTAG00000012529	*IFNG*	interferon, gamma	n.s.	4.10	8.50
ENSBTAG00000025471	*TNFA*	tumor necrosis factor alpha	n.s.	4.98	4.54
ENSBTAG00000038128	*BOLA-DQA5 (MHCII)*	major histocompatibility complex, class II, DQ alpha 5	n.s.	n.s.	-2.27
ENSBTAG00000010645	*BOLA-DRA (MHCII)*	major histocompatibility complex, class II, DR alpha	n.s.	n.s.	-2.37
ENSBTAG00000013919	*BOLA-DRB3 (MHCII)*	major histocompatibility complex, class II, DRB3	n.s.	n.s.	-1.93
ENSBTAG00000015730	*BOLA-DMA (HLA-DM and MHCII)*	major histocompatibility complex, class II, DM alpha	n.s.	n.s.	-3.52
ENSBTAG00000012451	*BOLA-DMB (HLA-DM and MHCII)*	major histocompatibility complex, class II, DM beta	n.s.	n.s.	-3.66
ENSBTAG00000006490	*BOLA-DOA (MHCII)*	major histocompatibility complex, class II, DO alpha	n.s.	n.s.	-2.43
ENSBTAG00000046979	*LGMN (AEP)*	legumain	n.s.	n.s.	-2.48
ENSBTAG00000020649	*CIITA*	major histocompatibility complex,class II, transactivator	n.s.	-1.38	-2.07

()Indicates the gene name or the protein complex name as shown in figure S4.

Table 8

Ensembl ID	Gene name	Gene descriptiopn	2h PI	6h PI	24h PI
Fc RECEPTORS					
ENSBTAG00000002096	*FCGR3A (FcγR)*	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)	n.s.	n.s.	-2.39
ENSBTAG00000008592	*FCGR1A (FcγR)*	Fc gamma receptor I	n.s.	n.s.	-1.70
INTEGRINS					
ENSBTAG00000009987	*ITGB3 (αVβ3)*	integrin subunit beta 3	n.s.	2.20	3.44
ENSBTAG00000019289	*ITGA2 (α2β1)*	integrin subunit alpha 2	n.s.	n.s.	2.49
ENSBTAG00000013755	*ITGB5 (αVβ5)*	integrin beta-5 precursor	n.s.	n.s.	-3.76
TOLL-LIKE RECEPTORS					
ENSBTAG00000015032	*CD14*	CD14 molecule	n.s.	n.s.	-1.40
C-LECTIN RECEPTORS					
ENSBTAG00000014546	*CLEC7A (*dectin1*)*	C-type lectin domain family 7 member A	n.s.	n.s.	-7.02
ENSBTAG00000030424	*CLEC1A **	C-type lectin domain family 1, member A	n.s.	n.s.	-6.93
ENSBTAG00000015739	*MRC2 (MR)*	C-type mannose receptor 2 precursor	n.s.	n.s.	-5.46
ENSBTAG00000032515	*PLA2R1 (MR)*	phospholipase A2 receptor 1	n.s.	n.s.	-1.89
SCAVENGER RECEPTORS					
ENSBTAG00000002885	*MRS1 (SRA1)*	macrophage scavenger receptor 1	n.s.	n.s.	-3.08
ENSBTAG00000014269	*SCARB1 (SRB1)*	scavenger receptor class B member 1	n.s.	n.s.	-2.29
ENSBTAG00000004547	*OLR1 (Lox1)*	oxidized low density lipoprotein (lectin-like) receptor 1	n.s.	2.38	n.s.
IRON UPTAKE					
ENSBTAG00000032719	*TFRC (TfR)*	transferrin receptor protein 1	n.s.	n.s.	1.56
NADPH OXIDASE					
ENSBTAG00000007531	*NCF4 (p40phox)*	neutrophil cytosolic factor 4	n.s.	n.s.	-2.20
OPSONINS					
ENSBTAG00000002006	*THBS1 (TSP)*	thrombospondin 1	n.s.	-1.73	n.s.

* Indicates genes which are not represent in figure S6.

() Indicates the gene name or the protein complex name as shown in figure S6.

Table 9

Ensembl ID	Gene name	Gene descriptiopn	2h PI	6h PI	24h PI
PROTEASES					
ENSBTAG00000007622	*CTSD*	cathepsin D precursor	n.s.	n.s.	-1.66
ENSBTAG00000010994	*CTSF*	cathepsin F	n.s.	n.s.	-2.27
ENSBTAG00000011100	*CTSC*	cathepsin C	n.s.	n.s.	-1.24
ENSBTAG00000046979	*LGMN*	legumain	n.s.	n.s.	-2.48
ENSBTAG00000015403	*TPP1*	tripeptidyl peptidase I	n.s.	n.s.	-1.55
GLYCOSIDASES					
ENSBTAG00000030434	*FUCA1*	fucosidase, alpha-L- 1, tissue	n.s.	n.s.	-1.62
ENSBTAG00000019256	*GLA*	galactosidase alpha	n.s.	n.s.	-1.77
ENSBTAG00000001124	*GALC*	galactosylceramidase	n.s.	-0.99	-1.84
ENSBTAG00000006241	*MAN2B1 (LAMAN)*	mannosidase alpha class 2B member 1	n.s.	n.s.	-1.79
SULFATASES					
ENSBTAG00000015267	*SGSH*	N-sulfoglucosamine sulfohydrolase	n.s.	n.s.	-1.65
PHOSFATASE					
ENSBTAG00000021002	*ACP2*	acid phosphatase 2, lysosomal	n.s.	-2.14	-1.47
ENSBTAG00000004826	*ACP5*	acid phosphatase 5, tartrate resistant	n.s.	n.s.	-1.55
OTHER ENZYMES AND ACTIVATORS					
ENSBTAG00000017201	*AP4S1 (AP-4)*	adaptor related protein complex 4, sigma 1 subunit	n.s.	2.15	n.s.
ENSBTAG00000013367	*PPT1 (CLN1)*	palmitoyl-protein thioesterase 1	n.s.	n.s.	-2.01
ENSBTAG00000001189	*AP1B1 (AP-1)*	adaptor related protein complex 1 beta 1 subunit	n.s.	n.s.	-1.80
ENSBTAG00000039855	*SUMF1 (FGE)*	sulfatase modifying factor 1	n.s.	n.s.	-1.64
ENSBTAG00000021499	*PSAP* (saposin)	prosaposin	n.s.	n.s.	-1.47
LYSOSOMAL MEMBRANE PROTEINS					
ENSBTAG00000018889	*ATP6V0B (ATPeV)*	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit b	n.s.	1.03	1.13
ENSBTAG00000007272	*ATP6V0A2 (ATPeV)*	ATPase, H+ transporting, lysosomal V0 subunit a2	n.s.	n.s.	0.73
ENSBTAG00000000831	*CTNS* (cystinosin)	lysosomal cystine transporter	n.s.	n.s.	-1.97
ENSBTAG00000016959	*LAPTM4B (LAPTM)*	lysosomal protein transmembrane 4 beta	n.s.	n.s.	-1.86

Table 9 (continued)

Ensembl ID	Gene name	Gene descriptiopn	2h PI	6h PI	24h PI
ENSBTAG00000010956	*SCARB2 (LIMP)*	scavenger receptor class B member 2	n.s.	n.s.	-1.54
ENSBTAG00000044053	*SLC17A5* (sialin)	solute carrier family 17 (acidic sugar transporter), member 5	n.s.	n.s.	-1.48

() Indicates the gene name or the protein complex name as shown in figure S7.

Table 10

Ensembl ID	Gene name	Gene descriptiopn	2h PI	6h PI	24h PI
ENSBTAG00000019741	*C3AR1*	complement component 3a receptor 1	n.s.	-2.10	-4.95
ENSBTAG00000007153	*C1QA (C1qrs)*	complement component 1, q subcomponent, A chain	n.s.	n.s.	-4.55
ENSBTAG00000011196	*C1QB (C1qrs)*	complement component 1, q subcomponent, B chain	n.s.	n.s.	-3.91
ENSBTAG00000015815	*CFP (FB)*	complement factor properdin	n.s.	n.s.	-3.87
ENSBTAG00000011193	*C1QC (C1qrs)*	complement component 1, q subcomponent, C chain	n.s.	n.s.	-3.80
ENSBTAG00000034501	*CFI (FI)*	complement factor I	n.s.	n.s.	-2.80
ENSBTAG00000008612	*C1R (C1qrs)*	complement component 1, r subcomponent	n.s.	n.s.	-2.44
ENSBTAG00000002302	*CD59*	CD59 molecule, complement regulatory protein	n.s.	n.s.	-2.44
ENSBTAG00000048122	*CFD (FD)*	complement factor D	n.s.	n.s.	-2.33
ENSBTAG00000007450	*C2*	complement component 2	n.s.	n.s.	-2.08
ENSBTAG00000017280	*C3*	complement component 3	n.s.	n.s.	-1.80
ENSBTAG00000010520	*C8G (C6, C7, C8, C9)*	complement component 8, gamma polypeptide	n.s.	n.s.	-1.73
ENSBTAG00000020872	*C5AR1*	complement component 5a receptor 1	n.s.	-1.45	-1.64
ENSBTAG00000037735	*C5AR2 **	complement component 5a receptor 2	n.s.	-2.06	-1.64
ENSBTAG00000021717	*BDKRB2 (B1/B2)*	bradykinin receptor B2	n.s.	7.06	5.01
ENSBTAG00000002758	*THBD (TM)*	thrombomodulin	4.07	5.33	5.63
ENSBTAG00000007101	*F3*	coagulation factor III	4.15	5.24	5.19
ENSBTAG00000014465	*SERPINE1 (PAI)*	serpin peptidase inhibitor, clade E	n.s.	n.s.	2.77
ENSBTAG00000001244	*PLAT (tPA)*	tissue-type plasminogen activator	n.s.	n.s.	2.74
ENSBTAG00000005947	*PLAU (uPA)*	plasminogen activator, urokinase	2.41	n.s.	2.39
ENSBTAG00000007268	*F13A1 (F13)*	coagulation factor XIII, A1 polypeptide	n.s.	n.s.	-4.40
ENSBTAG00000023652	*PROS1 (PS)*	protein S (alpha)	n.s.	n.s.	-3.76
ENSBTAG00000018137	*A2M*	alpha-2-macroglobulin	n.s.	n.s.	-3.60
ENSBTAG00000003572	*F11*	coagulation factor XI	n.s.	-2.82	-2.49

* Indicates genes which are not represent in figure S5.

()Indicates the gene name or the protein complex name as shown in figure S5.