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ABSTRACT

Invasive meningococcal disease (IMD) is usually associated with intense inflammatory response that is correlated with severe infection. Corticosteroids may regulate this inflammatory response through an early but transient induction of IL-10 that is suggested to improve the outcome of IMD. We explored the mechanism of action of corticosteroids as an adjuvant treatment to antibiotics. Transgenic mice expressing the human transferrin were infected by a hyperinvasive meningococcal strain and transcriptomic analysis were then performed in the blood for all conditions of infection and treatment. Infected untreated mice, infected antibiotic-treated mice and infected amoxicillin and dexamethasone-treated mice were compared. Treatment using both corticosteroids and antibiotics was associated with differential gene expression in the blood especially in Monocytes-Macrophages pathways. Depletion of these cells in infected mice was associated with a more severe bacterial infection and uncontrolled production of both pro-inflammatory and anti-inflammatory cytokines. Accordingly, children suffering from severe IMD had low counts of monocytes at admission. Our data are in favor of a role of corticosteroids in enhancing a polarization from pro-inflammatory to anti-inflammatory phenotypes of Monocytes-Macrophages axis that may help controlling meningococcal invasive infections.

Key words:

Neisseria meningitidis; corticosteroids; monocytes/macrophages; sepsis; inflammation; mice.

HIGHLIGHTS

- Use of corticosteroid in addition to antibiotics for invasive meningococcal disease is debated.
- RNAseq in experimental infection in mice was used.
- Mechanistic evidences for a positive role of corticosteroids as an adjuvant treatment.
- The effect is mediated by inflammatory cells such as Monocytes-Macrophages.

1. INTRODUCTION

Neisseria meningitidis (Nm) is a Gram-negative bacterium that usually colonizes the mucosa of the upper respiratory tract in human. Up to 10 to 15% of the general population carry Nm [1] and the highest carriage rate is observed among adolescents and young adults [2, 3]. Under certain conditions, Nm can be responsible for invasive meningococcal disease (IMD) including meningitis and septic shock (*purpura fulminans*, PF) [4]. IMD is associated with significant morbidity and mortality with a fatality rate of 11.3% [5] and sequelae among 20% of survivors [6]. Several meningococcal components such as the lipooligosaccharide (LOS) are involved in the induction of inflammatory response [7]. The intensity of the inflammatory reaction seems to be involved in the bad outcomes [8]. In fact, the ability of Nm to grow rapidly in the bloodstream leads to an exaggerated, uncontrolled and destructive inflammatory response in the vasculature, heart, kidneys, and lungs with the production of high quantity of pro- and anti-inflammatory cytokines [9].

Moreover, the genotype of the bacterial isolates also impacts on the outcome of IMD. Studies using molecular methods such as multilocus sequence typing (MLST) [10] have shown that IMD is frequently provoked by isolates belonging to a limited number of genotypes (also called clonal complexes cc), known as the hyperinvasive lineages [11]. Among these hyperinvasive lineages, cc11 isolates have remarkably a high association with IMD with important induction of apoptosis, inflammatory reaction and death [12-15]. Children with IMD caused by cc11 isolates had significantly higher fatality rates compared to children with IMD due to other cc [16]. However, this difference disappeared in children who received corticosteroids, strong anti-inflammatory drugs, altogether with appropriate antibiotics. In adults with septic shock, low-dose corticosteroids may favorably impact all-cause 28-days mortality among patients with sepsis [17] but these molecules were not specifically tested in IMD at higher doses.

In a previous study, we explored the impact of corticosteroids in an experimental infection in transgenic mice expressing the human transferrin. We have previously reported the use of these mice as a relevant model for invasive meningococcal infection. Human transferrin is used in this model as an iron source by meningococci that can only acquire iron from human resources (lactoferrin, transferrin, hemoglobin and haptoglobin) [18, 19]. The adjunction of dexamethasone to antibiotic treatment significantly improved the clinical outcome of infected mice through a modulation of the inflammatory response [20]. Antibiotic-dexamethasone-treated mice had lower levels of Crp, Lcn2, TNF-α and lower bacterial loads that were associated with early and temporary secretion of IL-10 without secondary immune suppression [20]. However, the mechanism of the positive effect of dexamethasone remains to be defined. We aimed in this work to address this mechanism using transcriptomic approach to define the differentially expression of mouse genes in the blood under antibiotic-dexamethasone treatment.

2. MATERILAS AND METHODS

2.1. Ethics statement

This study was carried out in strict accordance with the European Union Directive 2010/63/EU (and its revision 86/609/EEC) on the protection of animals used for scientific purposes. Our laboratory has the administrative authorization for animal experimentation (Permit Number 75-1554) and the protocol was approved by the Institut Pasteur Review Board that is part of the Regional Committee of Ethics of Animal Experiments of Paris Region (Permit Number: 75-1554). All the invasive procedures were performed under anesthesia and all possible efforts were made to minimize animal suffering.

2.2. Bacterial strain and mice infection

We used the strain 24198LUX that is derived from the strain 24198 (serogroup C strain that belongs to ST-11 clonal complex) [20, 21] by the insertion of the luciferase operon (*luxCDABE*) on the chromosome under the control of the meningococcal promoter of *porB* gene as previously described [22]. Experimental meningococcal infections were performed in 8 week-old female congenic BALB/c transgenic mice expressing human transferrin (hTf). This animal model was previously published for meningococcal meningitis and sepsis as it allows bacterial growth in mice by providing the human transferrin as an iron source [18]. Mice were infected by intra-peritoneal injection (IPI) of 1.5×10^7 CFU diluted in 0.5 ml of saline.

2.3. Transcriptomic analysis

BALB/c transgenic mice expressing the hTf were separated into four groups: a group of infected mice which didn't receive any treatment (non-treated, NT), a group of infected mice treated with amoxicillin three hours after intra-peritoneal injection (AMX at 50 mg/kg dose in

0.1 ml), a group of infected mice treated with AMX and dexamethasone (DXM at 0.15 mg/kg dose in 0.1 ml in one thigh and AMX at 50 mg/kg dose in 0.1 ml in the other thigh) three hours after IPI and a group of non-infected mice homogeneously distributed within the three previous groups. Blood samples were then collected six hours after infection. Animals for RNA preparation (uninfected and infected mice) were anesthetized by IPI of 10 mg/kg of xylazine and 100 mg/kg of ketamine and were exsanguinated by intra-cardiac puncture. RNA preparation was performed using Qiagen Rneasy protect animal blood kits® and cDNA were then obtained from those samples. We sponsored Beckman GATC Biotec (Konstanz, Germany) to perform transcriptomic analysis and gene expression profiling by RNAseq with Genome Sequencer Illumina HiSeq2500®. The raw RNA-Seq reads were analyzed using tools that were previously reported [23]. The reads were aligned to the reference genome or reference transcriptome, assembled and annotates. And finally, merged transcripts from two or more samples / conditions are compared to determine the differential expression levels including a measure of significance between samples/conditions. All combinations were compared together. Data are available on NCBI under the accession number PRJNA412849. The functional and pathway analysis (Genontology) was performed using The PANTHER (Protein ANalysis Through Evolutionary Relationships) classification system (v.8.0) [24, 25] and the DAVID (Database for Annotation, Visualization and Integrated Discovery) Bioinformatics Resources [26].

2.4. Data from paediatric patients with IMD

To explore the reliability of the impact of monocyte-macrophage in the evolution of IMD, we reviewed retrospectively the files of pediatric patients who were admitted to the Robert-Debré University Hospital in Paris for IMD between 1999 and 2016. Data regarding age, sex, serogroup of meningococcal strain, biological parameters on hospital admission (CRP, PCT,

FG, Leucocytes, PMN, lymphocytes, monocytes, platelets, hemoglobin) and evolution data (death or sequelae) were collected as a part of routine management of cases.

2.5. Mice, experimental infection and Monocytes-Macrophages depletion

Eight week-old congenic BALB/c transgenic mice expressing human transferrin were injected with 300µg of CSF1R – CD115 monoclonal antibody in the peritoneum (BioXCell) 12 hours before the infection in order to deplete Monocytes-Macrophages (mice MONO-, N=5) [27]. These mice were compared to mice that were infected and injected with PBS instead of the CSF1R – CD115 monoclonal antibody (MONO+, N=5). Mice were infected intra-peritonealy as described above. Two non-infected mice were also followed for each condition (Monocytes-Macrophages depleted and non-depleted). Mice were followed during 24 hours. The experiment was repeated twice with 5 more mice in each group for a total of 10 MONO- mice and 10 MONO+ mice. In another set of experiment, the depletion was performed three hours after infection to evaluate the timing of Monocytes-Macrophages depletion on the outcome of mice in two experiments as previously (8 MONO-, 8 MONO+ and two non infected mice). Clinical assessment was performed before bacteria inoculation at 30 minutes, 3 hours, 6 hours and 24 hours post-infection. Weight was recorded and body temperature was measured transcutaneously using an infrared thermometer (BIOSEB, Vitrolles, France). Two clinical scores were used: the vitality score (VS) (ranging from 0 to 6 and adapted from those previously published [28, 29] and the murine sepsis score (MSS) [30]. The VS included fur aspect that ranged from 0 to 2 (0 = erection of the hair, 1 = hair coat appears soiled, 2 = hair coat appears glossy and smooth) and strength of mice that ranged from 0 to 4 (0 to 4 steel chain that could be lifted by the mice as described previously [29]. Meningococcal infection was followed by dynamic imaging [31]. Quantification was performed using total photons per second emitted by each mouse after 30 min, 3 h, 6 h and 24 h post-infection by defining

regions of interest (total body and skull). At 24 h post-infection, blood was withdrawn from the retro-orbital plexus into heparinized tubes for cytokine analysis.

2.6. Control of monocyte depletion by Flow cytometric analyses

Monocyte depletion was controlled by flow-cytometry of peritoneal lavages that were obtained by injecting 2ml of PBS in the peritoneal cavity that were retrieved after gentle peritoneum massage. A six color staining protocol was used. Fluorophore-conjugated antibodies against CD11c, MHCII, Ly6G, Siglec-F, Ly6C and F4-80 were used (CD11c/APC, MHCII/eFluor450 and F4-80/PE-Cy7 (eBioscience™), Ly6G/APC-Cy7 and Ly6C/FITC (BD pharmingen™) and Siglec-F/PE-CF594 BD Horizon™). Pellets from 2ml of peritoneal lavage were resuspended in 100µL of Hank's balanced salt solution (HBSS) added with 1% of fetal bovine serum (Thermo Fisher). Incubation with labelled antibodies was performed for 20 minutes at 37°C, 5% CO2. Antibodies were then washed-out by centrifugation and the pellets were resuspended in 500µL of HBSS added with 1% of fetal bovine serum and formalin 10%. Samples were processed by LSR Fortessa™ analyzer (BD Biosciences) and further analyzed with FlowJo™ software (Tree Star). Live singlets were analyzed and monocytes populations were gated as follows: CD11c -/ CMHII - (leukocytes without B-cells); Ly6G - / Siglec - (exclusion of polymorphonuclear cells) and F4-80 + (including all F4-80 expressing monocytes). Monocytes were then sorted in inflammatory monocytes (Ly6C high) and resident monocytes (Ly6C low).

2.7. Cytokine immunoassays

IL-6, KC, IL-10 and TNF-α quantification were performed on blood samples using ELISA kit (R&D Systems Europe) according to the manufacturer's recommendations.

2.8. Detection of mouse Lipocalin 2 by Western blot

Heparinized plasmas from infected mice were analyzed using Western blot to detect the Lipocalin 2 (Lcn2), an acute phase protein that was previously reported to increase in meningococcal invasive infection [22]. The levels of Lcn2 were relatively quantified from the blots by assigning arbitrary units after comparison to a set of controls with known amount of Lcn2 using ImageJ 1.43u (NIH, USA).

2.9. Histological analysis

Mice were euthanatized by an overdose of IPI of xylazine and ketamine as described above. At necropsy, brain were sampled, fixed in 4% buffered formalin and embedded in paraffin. 3µm sections were performed and stained with hematoxylin and eosin (H&E).

2.10. Statistical analysis

The results of the descriptive analysis were expressed as numbers and percentages for qualitative variables and as means and standard deviations for quantitative variables. Two-tailed Fisher's exact test for quantitative variables and Chi-square test for qualitative variables were used. Reproducibility of each experiment was confirmed with a separated set of experiment. Statistical analyses were made using Prism 6 software (GraphPad Inc. San Diego, CA, USA).

3. RESULTS

3.1. Differentially expressed genes in mice during experimental meningococcal infection

Differentially expressed transcripts (DETs) in the blood were determined for all combinations (blood from mice that were: non-infected, non-infected but treated with AMX, or treated with AMX and DXM, infected non-treated, infected but treated with AMX or treated with AMX and DXM). After 6 hours of infection with 24198LUX strain in transgenic mice expressing the human transferrin, a large number DETs was observed. Among those DETs, 231 were significantly up-regulated and 61 were significantly down-regulated ($p<0.002$) in infected mice compared to non-infected mice. Using PANTHER and DAVID databases (See the Methods section), we were able to cluster the DETs into several ontology pathways related to the immune process (innate immunity response, neutrophil chemotaxis, monocyte chemotaxis, monocyte and neutrophil chemotaxis and cytokine response (Supplementary Tables 1 and 2). We next focused on the comparison of infected mice that were treated with AMX alone or with AMX and DXM as these conditions are relevant to the medical management of IMD (but all combinations of comparison of DETs are available on NCBI under the accession number PRJNA412849). Transcriptomic analysis in the blood of mice treated with both AMX and DXM showed that 14 DETs showed significant changes (Table 1). Four of those genes were up-regulated and 10 were down-regulated. Two were involved in the immune system process: the up-regulated Schlafen 8 protein that is implicated in regulation of LPS- and IFN-γ activated macrophages and in the differentiation of monocytes and macrophages [32, 33] as well as the down-regulated Bpifa6 (of the family PLUNC that is expressed in the respiratory pathway and that shows similarity to LPS- binding protein which is involved in the response of monocytes to LPS [34-36]). These results may

suggest that Monocytes-Marophages have an important role in the meningococcal infection and are modified by the treatment with DXM and AMX.

3.2. Monocytes count in pediatric patients with IMD.

In order to explore the relevance of the findings on the possible role of Monocytes-Marophages in the severity of IMD, we scored differences in the monocyte counts between patients with PF and those with meningitis. The characteristics of these patients (n=74) are presented in Table 2. Patients with PF had worse outcomes than those with meningitis. They had a mortality of 29% (n=7, representing serogroups B, C, Y and W) and 13% of them experienced sequelae (n=3) such as loss of a limb. At the opposite, only one fatal case was reported among the meningitis cases (2%) and four patients had sequelae (8%). When focusing on laboratory findings, patients with PF had a significant lower count of monocytes in the blood compared to patients with meningitis: 361 cells/mm3 versus 1,256 cells/mm3 ($p<0.0001$). These findings are also in favor of an important role of monocytes in the outcome of IMD with excessive inflammatory reaction.

3.3. Impact of Monocytes-Macrophages depletion on experimental meningococcal sepsis

We next further explored the role of monocytes-macrophages during experimental infection in our model of transgenic mice expressing the human transferrin. We performed specific depletions of Monocytes-Macrophages in mice 12 hours before infection (MONO- mice) and compared those to non-depleted mice but infected (MONO+ mice). The depletion of monocytes was controlled by flow cytometry in peritoneal lavage (Supplementary Fig. 1). One mouse died in the infected MONO- group while all mice survived in the infected MONO+ group. The clinical data of mice are presented in (Supplementary Fig. 2). Hypothermia at 24 h post-infection, marker of sever infection in our model [20], was significantly more pronounced in the MONO- group (24.5 ±1.1] vs 25.6 ±1.1, p= 0.0459).

MONO- mice also showed lower vitality scores (2.3 ±1.4 vs 3.6 ± 0.7, p = 0.0117) and higher mouse sepsis score (14 ± 5.6 vs 10 ±4.1, p=0.0463) after 24 hours of infection.

The intensity of bacterial infection was followed by dynamic bioluminescence imaging. After 6h of infection, there was no difference between the two groups of infected mice (both MONO- and MONO+) (Fig. 1). However, after 24 hours of infection, bacterial bioluminescent signals (all the body of infected mice) were stronger in MONO– mice compared with the MONO+ mice but the difference was not significant (p=0.30). Similar stronger although non-significant bioluminescence was also observed in MONO– mice compared with MONO+ mice when measuring only the bacterial signals from the skulls of infected mice (p=0.15). Histological examination of brain section also showed more intense meningeal cellular reaction in MONO- mice than MONO+ mice (Fig. 2).

We next explored the inflammatory response in both groups. MONO- mice had a significant higher level of Lcn2 compared to MONO+ mice; 211.3 ng/ml ±57.9 versus 132.5 ng/ml ± 61.5, p=0.0297 (Supplementary Fig. 3).

Pro-inflammatory cytokines were also more expressed in MONO– mice (Fig. 3) with an increased level of IL-6 (p=0.0251), KC (p=0.0353) and TNF-α (p=0.0237). Likewise, there was more IL-10 in MONO– mice (p=0.0120).

We also assessed the impact of Monocytes-Macrophages depletion after three hours of infection. No mice died within the 24 hours and clinical data of mice are presented in (Supplementary Fig. 4). There was no significant difference regarding weight loss between the two groups of mice (-2.80 ± 0.472 vs -2.64 [0.49]). However, MONO– mice experienced more pronounced hypothermia at 24 h post-infection (22.9 ± 1.0 vs 24.0 ± 1.2, p= 0.0537). MONO- mice also had lower vitality scores and higher MSS at 24 hours of infection but the difference was not significant (respectively 3.1 ± 1.3 vs 3.9 ±1.4, p=0.2287 and 13 ± 2.3 vs 11 ±1.5, p=0.0537).

The bacterial infection was followed by dynamic bioluminescence imaging and showed no difference between the two groups of infected mice at six hours of infection, three hours after monocyte depletion (Fig. 4). However, after 24 hours of infection, there were significantly higher bioluminescent signals in MONO– mice compared to the MONO+ mice; $3.743x10^{8}$ photons/sec ± $2.610x10^{8}$ emitted by the entire body in the MONO – mice compared to $6.421x10^{7}$± $5.064x10^{7}$ photons/sec in the MONO + mice (p=0.0024). When focusing only on the area of the skull, there were also significant higher bacterial signals in MONO– mice compared with MONO+ mice (p=0.0230).

Pro-inflammatory and anti-inflammatory cytokines were more expressed in MONO– mice (Fig. 5) with increased levels of IL 6 pg/ml (p=0.0426), KC (p=0.0813), TNF (p=0.0442) and IL-10 (p=0.0318).

4. DISCUSSION

Invasive meningococcal infections are usually associated with intense inflammatory response and an uncontrolled inflammatory response has been suggested to contribute to the severity of the disease. The balance of both pro- and anti-inflammatory cytokines is also suggested to impact on the clinical outcomes during meningococcal sepsis [37]. An uncontrolled compartmentalized immune response may be associated with complications of IMD [38] similarly to the observations made in pneumococcal meningitis [39]. It is noteworthy that meningococcal isolates that are most frequently associated with PF are members of the hyperinvasive cc11 lineage [13]. These isolates were reported to induce TNF-mediated apoptosis in infected cells [14]. Innate immune response is triggered by meningococcal components such as LOS and complement activation [40, 41]. Recently, CD46, a membrane cofactor expressed on human cells and that acts as a negative regulator of complement pathway, was shown to accelerate macrophage-mediated host susceptibility to meningococcal sepsis in a murine model of human transgenic CD46 mice (CD46(+/+). Indeed, macrophages rapidly undergo apoptosis upon LPS challenge or meningococcal infection [42]. Our Monocytes-Macrophages depletion data provide additional evidence on the involvement of both humoral and cellular innate immunity in the control of meningococcal sepsis. Further support came from our data in children with severe meningococcal sepsis with septic shock (PF) who showed significant lower monocytes counts compared to patients with meningococcal meningitis.

The added value of DXM to AMX in treating meningococcal sepsis may be due to a modulation of Monocytes-Macrophages in line with corticosteroids function [43-45]. Indeed, gene expression in Monocytes-Macrophages is modified upon stimuli by bacterial components to control the balance between a pro-inflammatory M1 or an anti-inflammatory M2 phenotype [46]. Our Transcriptomic analysis suggest down regulation of genes that may

be involved in LOS signaling such as Bpifa6 (of the family PLUNC). Reducing Monocytes-Macrophages in mice (and also in PF patients) may therefore prevent the M1/M2 polarization of Monocytes-Macrophages during infection [46]. Corporately, the human transgenic CD46(+/+) mice that are prone to lethal meningococcal produce high numbers of M1 type macrophages with enhanced production of pro-inflammatory mediators [42] in addition to reactive oxygen and nitrogen intermediates that can damage neighboring tissues [47]. The severity of meningococcal infections were reduced in CD46 (+/+) mice upon depletion of macrophages [42].

In agreement with this hypothesis is our observation on the concomitant increase of both pro-inflammatory and anti-inflammatory cytokines in Monocytes-Macrophages depleted mice. As we previously reported [20], DXM-mediated early induction of IL-10 may enhance M1 to M2 polarization as IL-10 down regulates pro-inflammatory cytokines release by monocytes [48]. Corticosteroids can interfere with these cells to promote their activities for maintaining homeostasis during IMD [44]. However, further studies are required to analyze Monocytes-Macrophages phenotypes under DXM adjuvant treatment in IMD. Moreover, the link between the genes of the PLUNC family and the LPS signaling in Monocytes-Macrophages as suggested by our transcriptomic analysis, requires additional works.

5. CONCLUIONS

We found that adding corticosteroids to appropriate antibiotics could have a beneficial effect in our experimental model. This effect may be mediated by an impact on Monocytes-Macrophage regulation by the enhancement of a polarization from a pro-inflammatory M1 to an anti-inflammatory M2 phenotype.

ACKNOWLEDGMENTS

We would like to acknowledge the IMAGOPOLE platform at the Institut Pasteur for the use of equipments of dynamic imaging. We also thank Patricia Flamant (Unite d'Histopathologie humaine et modeles animaux) for technical advises in histology.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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Legends of figures

Figure 1

Dissemination of *N. meningitidis* in BALB/c hTf transgenic mice. Mice were infected by intra-peritoneal injection of 1.5 x 10^7 CFU of the strain 24198LUX. Mice were either monocyte-depleted (MONO-) by intra-peritoneal injection of anti-CSF1R– CD115 12 hours before infection or monocyte-nondepleted (MONO +). Mice were analyzed for bioluminescence at the indicated times using an IVIS 100 system (Xenogen Corp., Alameda, CA) that has the maximal capacity to analyze five mice at the same time. Images depict photographs overlaid with color representations of luminescence intensity, measured in total photons/sec and indicated on the scales, where red is most intense and blue is least intense. (A) Dorsal view of 5 mice MONO + and 5 mice MONO- infected with 24198LUX (cc11 strain) at different times post-infection (30 min, 3 hours, 6 hours and 24 hours). (B) Brain luminescence quantification. The luminescence was quantified and expressed as means ± SD from each category at the indicated times by defining specific representative region of interest encompassing the brain area of the mouse. (C) Total body luminescence quantification. The luminescence was quantified and expressed as means ± SD from each category at the indicated times by defining specific representative region of interest encompassing the entire animal. NS= non significant. One representative experiment is shown. The experiment was repeated twice with 5 mice in each group and each experiment.

Figure 2

Brain histopathology induced by *N. meningitidis* 24198LUX strain. Analysis in mice proficient for monocytes (MONO+, A) or depleted for monocytes (MONO-, B). Representative sections are shown. Monocyte-were depleted 12 hours before infection as indicated in the (Materials and Methods) section. Hematoxylin and Eosin staining (H&E) was

performed on paraffin 3µm brain sections and microscope-examined at 100x, 200x and 400x as indicated on the left. In MONO+ group no lesions were observed. H&E stained paraffin 3µm brain sections of MONO- group showed intense meningitis with thickening of the meninges (Arrow*)* and afflux of immune cells.

Figure 3

Cytokines production in BALB/c hTf transgenic mice infected by intra-peritoneal injection of 1.5 x 10^7 CFU 24198LUX strain. ELISA assays were used to quantify (A) IL-6, (B) KC, (C) IL-10 and (D) TNFalpha (TNF) in blood samples from mice MONO + and MONO – at 24 h post-infection. The experiment was repeated twice with 5 mice in each group and each experiment. Data are pooled for all mice in both experiments.

Figure 4

Dissemination of *N. meningitidis* in BALB/c hTf transgenic mice. Mice were infected by intra-peritoneal injection of 1.5 x 10^7 CFU of the strain 24198LUX. Mice were either monocyte-depleted (MONO-) by intra-peritoneal injection of anti-CSF1R– CD115 3 hours after infection or monocyte-nondepleted (MONO +). Mice were analyzed for bioluminescence at the indicated times using an IVIS 100 system (Xenogen Corp., Alameda, CA) that has the maximal capacity to analyze five mice at the same time. Images depict photographs overlaid with color representations of luminescence intensity, measured in total photons/sec and indicated on the scales, where red is most intense and blue is least intense. (A) Dorsal view of 4 mice MONO + and 4 mice MONO- infected with 24198LUX (cc11 strain) at different times post-infection (30 min, 3 hours, 6 hours and 24 hours).
(B) Brain luminescence quantification. The luminescence was quantified and expressed as means ± SD from each category at the indicated times by defining specific representative

region of interest encompassing the brain area of the animal. (C) Total body luminescence quantification. The luminescence was quantified and expressed as means ± SD from each category at the indicated times by defining specific representative region of interest encompassing the entire animal. One representative experiment is shown. The experiment was repeated twice.

Figure 5

Cytokines production in BALB/c hTf transgenic mice infected by intra-peritoneal injection of 1.5 x 10^7 CFU 24198LUX strain. Mice were either monocyte-depleted (MONO-) by intra-peritoneal injection of anti-CSF1R– CD115 3 hours after infection or monocyte-nondepleted (MONO+). (A) Lipocalin 2 detection in blood of infected mice. Western blotting analysis using antibodies specific to mouse Lcn2. Plasma samples were collected at 24 h post-infection from 7 mice of each tested group (MONO+ and MONO-). The same positive control with a known concentration was used for each Western Blot. ELISA assays were used to quantify (B) IL-6, (C) KC, (D) IL-10 and (E) TNF-alpha (TNF) in blood samples from mice MONO + and MONO – at 24 h post-infection. The experiment was repeated twice with 4 mice in each group and each experiment. Data are pooled for all mice in both experiments.

Figure1

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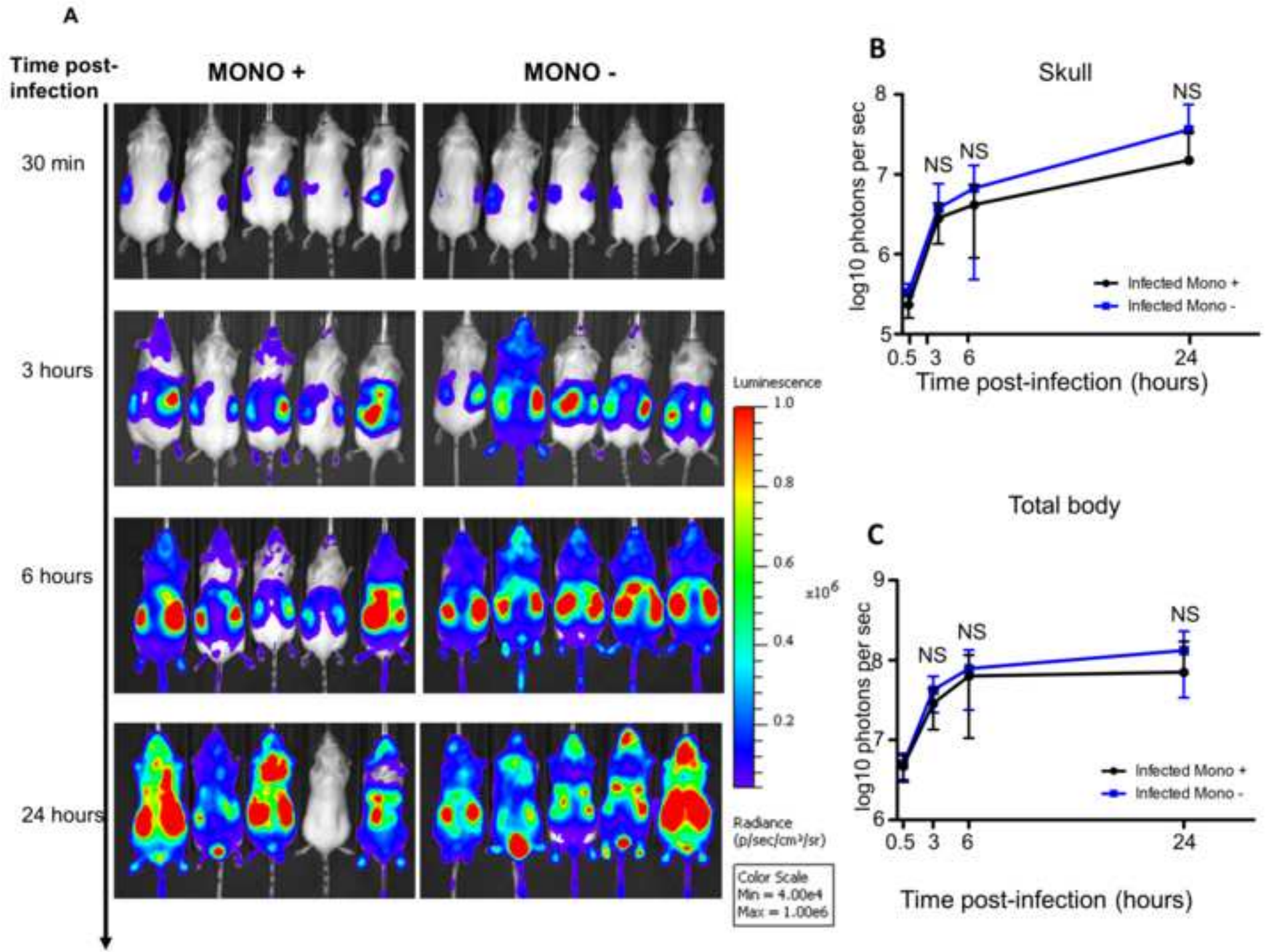

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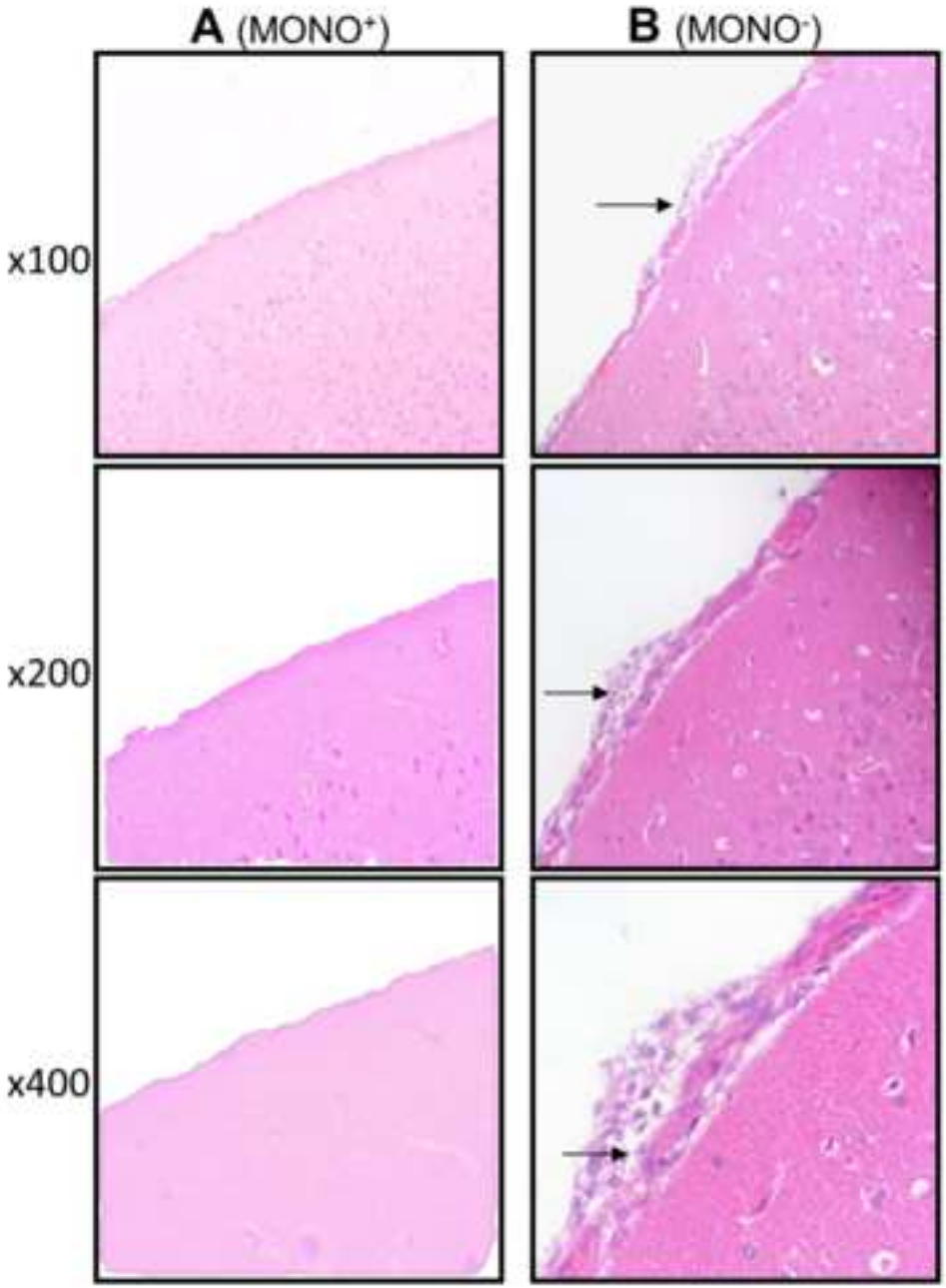

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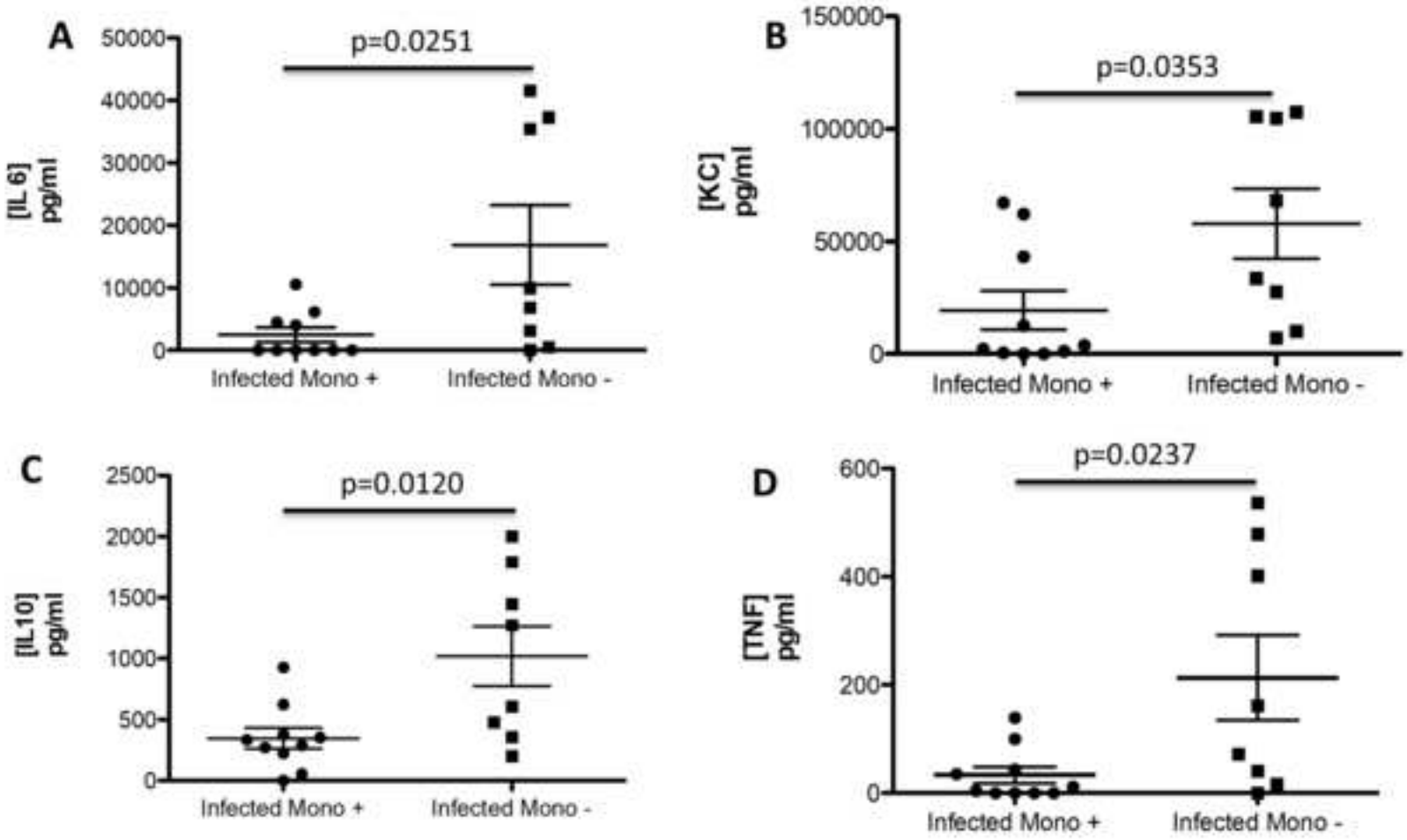

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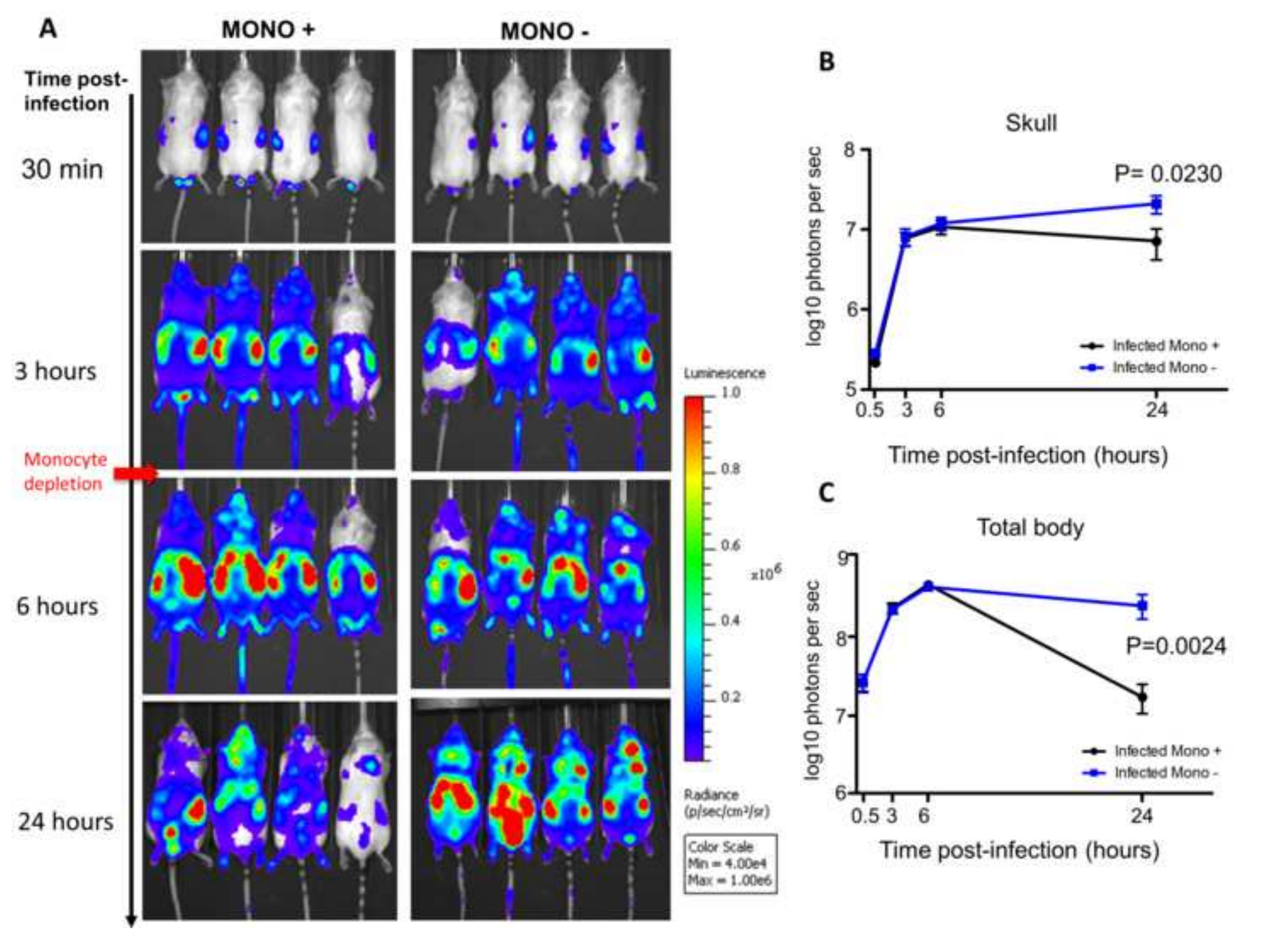

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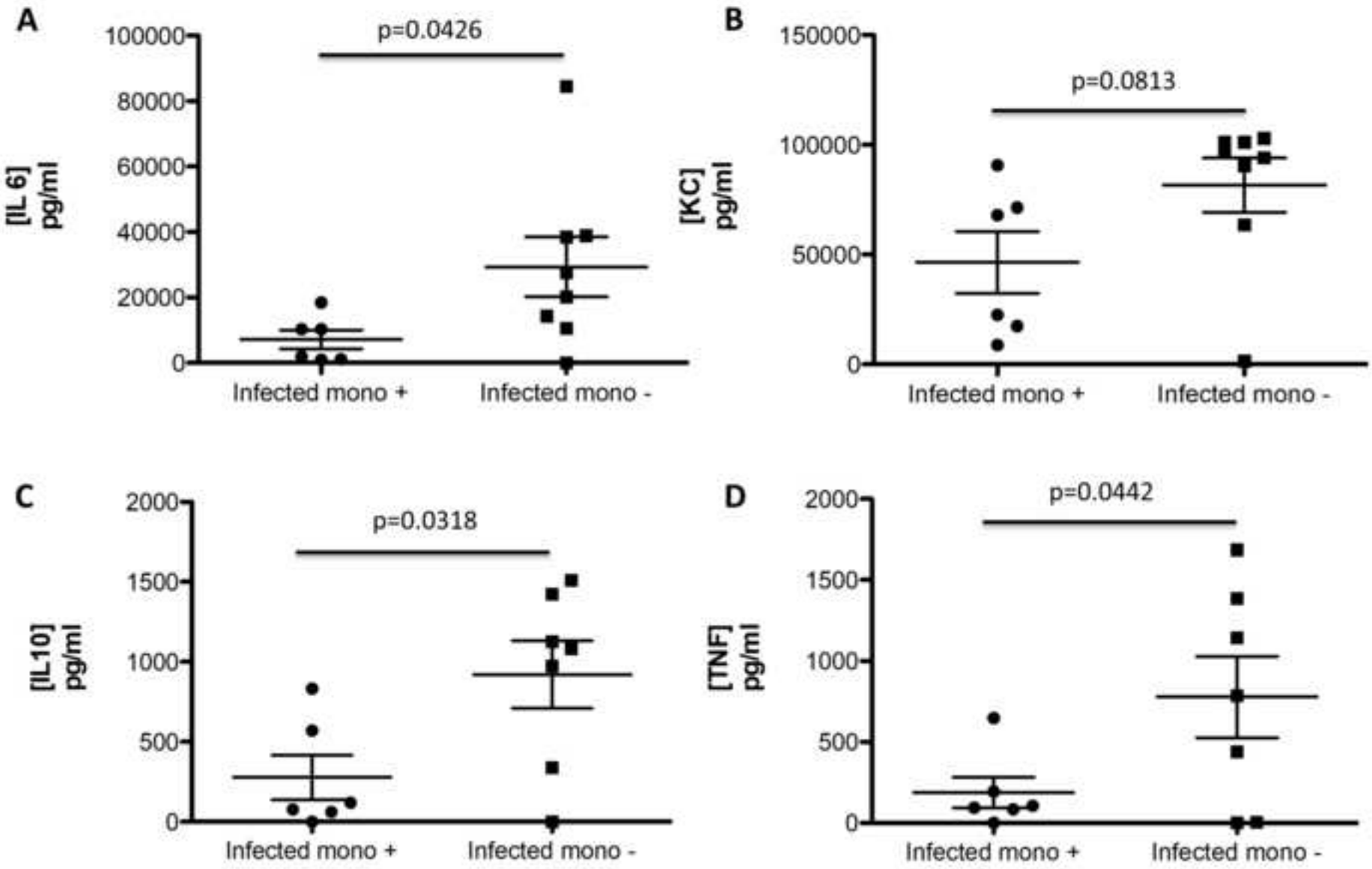

Table 1. Significant differentially expressed transcripts between AMX and DXM infected mice

DET	DET	FPKM AMX	FPKM DMX	P value	Implicated pathway
Upregulated					
Fancl	NM_001277273	0	37,909	$5x10^{-5}$	Metabolic process
Slfn8	NM_001167743	0	4,3998	0.0001	Immune system process; cell cycle; cellular defense response
Pbk	NM_023209	0	40,444	$5x10^{-5}$	Phosphate-containing compound metabolic process; protein phosphorylation; cellular process
Mnd1	NM_029797	0	23,0644	$5x10^{-5}$	Cell Cycle, Mitotic and Meiosis
Downregulated					
Mogat1	NM_026713	15,6835	0	$5x10^{-5}$	Acyl-CoA metabolic process /acyl-CoA metabolic process
Sqle	NM_009270	17,1772	0	$5x10^{-5}$	Metabolic process
Elovl3	NM_007703	10,7793	0	$5x10^{-5}$	Fatty acid metabolic process
Sptlc3	NM_175467	13,9263	0	$5x10^{-5}$	Lipid metabolic process
Bpifa6	NM_001080811	132,434	2,41168	$5x10^{-5}$	Immune system process; cholesterol metabolic process; response to stress; defense response to bacterium; lipid transport
Mgp	NM_008597	23,3963	0	$5x10^{-5}$	Cell-cell signaling; mesoderm development; skeletal system development
Hps5	NM_001005247	12,6703	0	$5x10^{-5}$	Biogenesis of lysosomal organelles
Mapk1ip1	NM_027115	10,9543	0	0,0001	Cell Cycle and Meiosis
Tmem184c	NM_145599	33,73	0	$5x10^{-5}$	Transport
Elovl5	NM_134255	30,8415	7,26601	$5x10^{-5}$	Fatty acid metabolic process

DET=Differentially expressed transcripts, FKM = fragment per kilobase per million mapped reads, AMX = amoxicillin, DXM = dexamethasone

Table2

Table 2. Characteristics of patients with meningitis and purpura fulminans in Robert-Debré University Hospital (Paris, France), 1999-2016.

	Meningitis	Purpura fulminans	p value
NUMBER OF CASES	50	24	
AGE	4 (4.2)	4.5 (5.3)	0.704
SEX			
Male	27 (54%)	17 (71%)	0.379
Female	23 (46%)	7 (29%)	0.225
SEROGROUP			
A	1 (2%)	0 (0%)	
B	28 (56%)	15 (63%)	0.731
C	7 (14%)	5 (21%)	0.494
W	11 (22%)	1 (4%)	0.074
Y	1 (2%)	1 (4%)	0.059
ND	2 (4%)	2 (8%)	0.452
BIOLOGY			
CRP (mg/l)	199 (107)	97 (66)	< 0.0001
PCT (µg/l)	46 (50)	154 (181)	0.11
FG (g/l)	7,3 (2,3)	3.7 (2.2)	< 0.0001
Leucocytes (cells/mm3)	18,826 (8418)	10,589 (9,771)	0.0015
PMN (cells/mm3)	15,783 (7,885)	7,026 (9,007)	0.00042
Lymphocytes (cells/mm3)	3,371 (2,835)	2,775 (2,948)	0.45
Monocytes (cells/mm3)	1,256 (920)	361 (330)	< 0.0001
Platelets (cells/mm3)	280,848 (125,904)	130,295 (91,040)	< 0.0001
Hemoglobin (cells/mm3)	10.8 (1.4)	9.9 (1.2)	0.0196
OUTCOME			
Death	4 (8%)	7 (29%)	0.027
Sequelae	1 (2%)	3 (13%)	0.068

CRP=C-reactive protein, PCT=procalcitonin, FG=fibrinogen, PMN=polymorphonuclear cells

Supplementary Material

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