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Impact of corticosteroids on experimental meningococcal sepsis in mice.

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7 **Impact of corticosteroids on experimental meningococcal**
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10 **sepsis in mice**
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HIGHLIGHTS

- Meningococcal sepsis is associated with strong inflammation and high fatality.
- Corticosteroids adjuvant treatment can be used to reduce inflammatory response.
- Clinical trials are difficult to conduct but animal models can substitute.
- Corticosteroids-treated mice showed better clinical and inflammatory scores.
- Interleukin 10 was early induced and could be responsible for this positive impact.

ABSTRACT:

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2 *Neisseria meningitidis* is responsible for septicemia and meningitis with high fatality that is
3
4 associated with an excessive inflammatory reaction particularly with hyperinvasive isolates of
5
6 the clonal complex ST-11 (cc11). However, anti-inflammatory adjuvant treatment remains
7
8 controversial and difficult to assess in patients. We addressed this topic in a well-defined
9
10 experimental meningococcal infection in transgenic mice expressing the human transferrin.
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12 Mice were infected by intra-peritoneal challenge with bioluminescent serogroup C/cc11 strain.
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14 After 3 hours of infection mice were differentially treated every 6 hours by saline, amoxicillin
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16 alone or amoxicillin and dexamethasone (DXM). Infected mice were scored for clinical status,
17
18 temperature and weight. Biological markers of inflammation were also quantified.
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21 Significant clinical improvement was observed in mice treated with amoxicillin and DXM
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23 compared to the two other groups. A significant reduction of the inflammatory reaction
24
25 assessed by CRP and Lipocalin 2 (two acute phase proteins) was also observed with this
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27 treatment. DXM significantly increased blood levels of IL-10 at six hours post-infection.
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29 DXM/amoxicillin treated mice, compared to the two other groups, also showed lower levels
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31 of TNF- α and lower bacterial blood load assessed by serial dilutions of blood and
32
33 bioluminescence dynamic imaging. Our results suggest that DXM, added to an appropriate
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35 antibiotic therapy, has a beneficial effect on experimental sepsis with a hyperinvasive
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37 meningococcal strain in transgenic mice expressing human transferrin. This is most likely due
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39 to the reduction of inflammatory response by an early induction of IL-10 cytokine. These data
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41 may allow better decision-making to use or not corticotherapy during meningococcal sepsis.
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56 **Keywords:** Meningococcus, corticosteroids, sepsis, inflammation, cytokine, mice
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1. INTRODUCTION

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2 *Neisseria meningitidis* (Nm) is frequently encountered in the nasopharynx of asymptomatic
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4 carriers but is also responsible for severe invasive infections that are a major cause of
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6 morbidity and mortality worldwide. Meningococcal isolates are variable, belong to several
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8 genotypes and express diverse phenotypes [1]. Genetic analyses of meningococcal
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10 populations by multilocus sequence typing (MLST) clusters meningococcal isolates in clonal
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12 complexes (cc). Invasive isolates belong to few clonal complexes called “hyperinvasive
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14 genotypes (in Europe cc8, cc11, cc32, cc41/44 and cc269) [1]. However, no specific sets of
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16 genes were identified so far to be directly responsible for the pathogenesis of hyper-invasive
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18 isolates of Nm. Nevertheless, several bacterial factors were shown to be involved in
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20 meningococcal virulence such as the pili, the polysaccharidic capsule as well iron acquisition
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22 [2]. Genotypes rather than the capsule group of meningococcal isolates are suggested to be,
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24 at least partially, involved in determining the outcome of host-bacteria interaction. Indeed,
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26 isolates of the cc11 are significantly associated with the fatal outcome of the disease [3, 4].
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28 cc11 isolates were also reported to induce more apoptosis than carriage isolates or invasive
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30 isolates belonging to other cc [4, 5].
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38 During infection, Nm releases microbial associated molecular patterns (MAMPs) such as
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40 lipooligosaccharide (LOS) and peptidoglycan that induce up-regulation of pro-inflammatory
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42 cytokines (like tumor necrosis factor α (TNF- α) and interleukin 1 and 6 (IL-1, IL-6)) as well
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44 as a down-regulation of anti-inflammatory cytokines (like IL-1 β , IL-10 or soluble receptor to
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46 TNF- α) and this has been shown to be essential in the evolution of meningococemia [6].
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51 Corticosteroids are strong anti-inflammatory drugs whose action pass through the modulation
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53 of the expression of genes involved in the inflammatory reaction and can thus be used to
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55 reduce the uncontrolled inflammatory response [7, 8]. The prescription of corticosteroids in
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57 IMI is controversial and this is mostly due to the fact that most of the studies include patients
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1 infected with different bacterial species and do not focus on genotypes of the bacterial isolates
2 [9, 10]. Interestingly, Madhi et al. [11] showed that in the absence of corticosteroids, there
3 was a significant higher death rate in IMI in children infected with cc11 isolates compared to
4 cases due to other cc. This difference disappeared in children who received corticosteroids.
5
6 However, the timing of the treatment and the diverse genetic background of the patients may
7 hinder definitive conclusions. [A relevant animal model of meningococcal sepsis is available](#)
8 [in transgenic mice expressing the human transferrin. Meningococci acquire iron for their](#)
9 [growth during infection specifically from human sources such as the human transferrin \[12\].](#)
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11 [This](#) model allowed us to evaluate the impact of corticoids during meningococcal
12 experimental infection in this defined model.
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2. EXPERIMENTAL

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 strains

The strain 24198LUX is derived from the strain 24198 (serogroup C strain that belongs to ST-11 clonal complex) [13, 14]. The strain 2C4-3LUX is derived from the strain 2C4-3 (serogroup C that belongs to the cc18) [15]. Both strains have been modified to be bioluminescent by the adjunction on the chromosome of the operon coding for the luciferase (*luxCDABE*) under the control of the promoter of *porB* (PrpoB) meningococcal gene as previously described [14]. We have previously shown that the insertion of the *lux* operon did not modify bacterial invasion in this animal model nor it modifies MIC of β -lactam antibiotics [12, 16].

2.3. Mice, experimental infection and assessment

Congenic BALB/c transgenic mice expressing human transferrin were previously described [12]. Experimental meningococcal infections were performed in 8 week-old female BALB/c transgenic mice expressing human transferrin (hTf) by intra-peritoneal injection of 1 to $2 \cdot 10^7$

1 CFU/mouse diluted in 0.5ml of saline. Mice were separated into three groups and were treated
2 after three hours of infection: one group infected not treated, one group infected treated with
3 amoxicillin (AMX) and one group infected treated with AMX and dexamethasone (DXM).
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5 The mice from the non-treated group received a 0.1 ml intramuscular injection of saline in
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7 each thigh every six hours. Mice treated with AMX received a 0.1 ml intramuscular injection
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9 of amoxicillin diluted in saline (Clamoxyl® forme injectable IM/IV 500mg) for a 50mg/kg
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11 dose in one thigh and 0.1ml of saline in the other thigh. Mice treated with AMX and DXM
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13 received a 0.1 ml intramuscular injection of DXM (dexamethasone sodium phosphate Mylan
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15 4mg/ml) for a 0.15 mg/kg dose in one thigh and a 0.1 ml injection of amoxicillin diluted in
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17 saline (Clamoxyl® forme injectable IM/IV 500mg) for a 50mg/kg dose in the other thigh. The
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19 three groups were injected every six hours for 48 hours after the first injection at three hours
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21 post-infection. One group of non-infected mice was also followed and received saline
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23 injection the same way as the non-treated group.
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31 Clinical assessment was performed before bacteria inoculation, 3 hours, 6 hours, 12 hours, 24
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33 hours, 36 hours and 48 hours post-inoculation. Mice weight was recorded and body
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35 temperature was measured transcutaneously using an infrared thermometer (BIOSEB® IR
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37 Rodent Thermometer, Vitrolles, France). A clinical score (ranging from 0 to 5 and adapted
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39 from those previously published [17, 18] was used to assess disease severity including fur
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41 aspect that ranged from 0 to 2 (0=Erection of the hair, 1=Hair coat appears soiled, 2=Hair
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43 coat appears glossy and smooth) and activity score of living mice that ranged from 0 to 3
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45 (0=No resistance to traction, 1=Weak resistance to traction, 2=Strong resistance to traction,
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47 3=Spontaneous forward propulsion). Blood was withdrawn from the retro-orbital plexus into
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49 heparinized tubes [19]. Bacteria load in blood was then evaluated by plating serial dilutions
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51 on GCB medium (Difco) supplemented with Kellogg supplements [20] and results were
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53 expressed as colony forming units (CFU) per milliliter. In other sets of experiments, no blood
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1 samples were taken and meningococcal infection was followed by dynamic imaging as
2 previously described [12]. Quantification was performed using total photons per second
3 emitted by each mouse after 30 min, 3 hours, 6 hours and 24 hours post-infection by defining
4 regions of interest.
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10 11 **2.4. Cytokine immunoassays, CRP and full blood count**

12 Immunoreactive CRP, IL-6, KC, IL-10 and TNF- α were performed on blood samples using
13 ELISA kit (R&D Systems Europe) according to the manufacturer's recommendations.
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15 Total blood count was performed on 50 μ l of heparinized blood using Heska hematometer
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22 (Vet ABC Animal Blood Counter, Florida, USA).
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26 27 **2.5. Detection of mouse Lipocalin 2 by Western Blot**

28 Heparinized plasmas were analyzed using Western Blot to detect the acute phase reactant
29 protein, the Lipocalin 2 (Lcn2) as previously described [14]. The same positive control with a
30 known concentration of Lcn2 was added to each Western blotting and used to quantify the
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66 67 **2.6. Histological analysis**

68 Mice were euthanized by intra-peritoneal 300 mg/kg injection of sodium pentobarbital.
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70 At necropsy, brain, spleen, liver, kidneys and adrenal glands were sampled, fixed in 4%
71 buffered formalin, then routinely embedded in paraffin. 4 μ m sections were performed and
72 stained with hematoxylin and eosin (H&E). Histological findings were microscopically
73 evaluated using a semi-quantitative score (1: minimal, 2: mild; 3: moderate, 4: marked, 5:
74 severe).
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2.7. Statistical analysis

Results were expressed as means +/- standard deviations for the quantitative values and reproducibility was confirmed on two separated experiments. Statistical analyses were made using Prism 6® software (GraphPad Inc. San Diego, CA, USA). Comparison of data between 24198LUX strain and 2C4-3LUX strain were made using unpaired student's t-test. Variance analysis (two-way Annova) was used to compare the data of the three groups of treatment and a p-value < 0.05 was considered as significant (taking into account the multiple comparisons).

3. RESULTS

3.1. Characteristics of the experimental infection in hTf transgenic mice using different meningococcal genotypes

We first tested whether the characteristics of experimental sepsis in transgenic mice expressing the human transferrin show different kinetics according to the genotypes of the isolates. We therefore infected 8-week-old female BALB/c mice expressing hTf by 2×10^7 CFU intra-peritoneal injection of either strain 24198LUX (that belongs to the hyperinvasive genotype cc11) or 2C4-3LUX (that belongs to the non-hyperinvasive genotype cc18). Infection was monitored for 24 h. Bioluminescent signals were similar for the two strains after 30 minutes and 3 h of infection. Dynamic bioluminescence imaging approach showed that after the intraperitoneal injection of the bacterial suspension, bacteria were spread to other organs such as the brain as confirmed by three-dimensional imaging (Supplementary Fig.S1 and video materials). Total body signals and signals of the skull (dorsal view) were higher for the 24198LUX strain 6 h after infection than for the 2C4-3LUX strain ($p=0.02$ and $p=0.047$ respectively) (Fig. S1). Mice infected by the strain of the cc11 were more severely ill with significantly higher loss weight than mice infected by the cc18 isolate (data not shown). The levels of three cytokines (IL-6, KC and IL-10) were assessed on blood withdrawn 24 h after infection (Supplementary Table S1). Levels of IL-6, KC and IL-10 were significantly higher in mice infected with 24198LUX than in mice infect with 2C4-3LUX ($p<0.0001$, $p<0.0001$ and $p=0.026$ respectively). These results strongly suggest that 24198LUX strain belonging to cc11 is responsible for a more severe systemic infection than the strain belonging to the cc18. These results indicate that the cc11 strain was able to induce an intense inflammatory response. All these data strongly suggest that the experimental infection in transgenic mice expressing human transferrin reflects different pathogenic properties of Nm.

3.2. Impact of dexamethasone adjuvant treatment on the clinical evolution of experimental infection in hTf transgenic mice

As the cc11 isolate provoked severe infection in mice with important inflammatory response, we next evaluated the effect of using corticosteroids as an adjuvant treatment of experimental meningococcal infection in mice. Three groups of mice (n=12 per group) were infected by intra-peritoneal injection of 1.2×10^7 CFU of 24198LUX strain. At 3 h, mice of the three groups showed similar development of the infection as viewed by dynamic imaging (Fig. 1). After 3 h post-infection, one group of mice received intramuscular antibiotics (AMX), the second group received both AMX and DXM while the third group received saline and was used as untreated control (See Methods). Untreated mice showed reduced survival and only 42% of mice infected with 24198LUX strain (cc11) survived when not treated while all mice that were treated by AMX and DXM survived (100% versus 42%, $p=0.002$). Mice that were only treated by AMX also showed significantly higher survival when compared to untreated mice but did not differ significantly from those treated by both AMX and DXM (respectively, 91% versus 100%, $p=0.31$) (Fig. 2A). AMX-treated mice and AMX/DXM-treated mice showed better clinical scores when compared to the untreated group (Fig. 2B). Moreover, AMX/DXM-treated mice showed significantly better clinical score compared to AMX-treated mice as assessed by the vitality score after 24 h and 36 h post-infection ($p=0.02$, $p<0.0001$ respectively). Hypothermia at 24 h post-infection was significantly more severe in the non-treated group compared to the AMX/DXM-treated or AMX-treated groups. However, it was not significantly different between the AMX/DXM-treated mice and the AMX-treated mice ($p=0.97$) (Fig. 2C). The better clinical conditions observed for AMX/DXM-treated mice was not due to lower blood bacterial loads since it did not differ significantly ($p=0.98$) between the two groups (AMX and AMX/DXM), at 6 h post-infection (3 hours after the first dose of treatment) (Supplementary Fig.S2). Moreover bacteria were rapidly cleared from the blood in

1 the two groups of treated mice (before 24 h) whereas bacteremia was still detectable in the
2 non-treated group at 36 h of infection (Supplementary Fig.S2).
3

4 After 6 h of infection (3 h of treatment), dynamic bioluminescence imaging approach showed
5 that the two groups of treated mice (AMX/DXM- or AMX-treated) showed similar total
6 signals all over the body that were significantly lower than signals of untreated mice (Fig 1A
7 and 1B).
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14 **3.3. Impact of dexamethasone adjuvant treatment on acute phase markers during** 15 **experimental infection** 16

17 We next explored the immediate modifications in blood formula and acute phase markers.
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19 All infected mice developed leucopenia and neutropenia with a nadir at 6 h of infection
20 (Table 1). Profound neutropenia persisted in the untreated group at 24 h whereas neutrophils
21 rate had increased in the two other treated groups. Neutrophils were not significantly higher at
22 24 h in AMX/DXM-treated mice compared to AMX-treated mice ($p=0.23$).
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33 Inflammatory response was monitored using two acute phase proteins: Crp and Lcn2. Crp
34 levels in blood samples were determined by ELISA. Although Crp levels increased after 6
35 hours post-infection, its values were significantly lower in the AMX/DXM group compared to
36 the non-treated group ($p<0.0001$) and to the AMX-treated group ($p<0.0001$) (Table 1).
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43 The levels of Lcn2 were also significantly lower in the AMX/DXM group at 6 hours post-
44 infection when compared to the untreated group ($p<0.0001$) and to the AMX group
45 ($p=0.0276$) (Table 1 and Fig.3) following the same observed trend for Crp. Lcn2 levels
46 decreased thereafter in all groups and remained lower in AMX/DXM-treated mice compared
47 to untreated and AMX-treated mice at 48 hours post-infection (data not shown). The high
48 level of inflammatory response in untreated mice infected by the cc11 isolate was also
49 confirmed by the histological findings in the liver of cc11-infected mice. At 6 h post
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1 inoculation, hepatic lesions were important but similar in the three groups (non treated, AMX-
2 treated and AMX/DXM-treated) and consisted of multifocal diapedesis and infiltration of
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4 neutrophils associated with minimal necrosis of hepatocytes. At 24 h post inoculation,
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6 hepatocellular necrosis and neutrophilic infiltration were more severe and were occasionally
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8 associated with mild thrombosis (Supplementary Fig. S3). Overall score of microscopic
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10 findings was slightly higher in non-treated mice as compared to mice treated with either
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12 AMX or AMX and DXM.
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19 **3.4. Impact of dexamethasone adjuvant treatment on cytokine expression during** 20 21 **experimental infection** 22

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24 To further have more insights on the evolution of the infection with dexamethasone treatment,
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26 we explored the levels of pro-inflammatory and anti-inflammatory cytokines in blood samples
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28 of mice from the three groups at different time-points of the infection. IL-6 and KC were non-
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30 significantly different in all the three groups at all time-points (Fig. 4 A and B). However,
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32 AMX/DXM-treated mice showed lower concentration of TNF- α at 6 h of infection compared
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34 to AMX-treated mice ($p=0.34$) and significant lower concentration compared to the untreated
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36 group ($p= 0.04$) (Fig. 4 C). Inversely, the anti-inflammatory cytokine, IL-10 appeared early in
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38 AMX/DXM-treated mice and was detected at significantly higher levels at 6 h of infection in
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40 AMX/DXM-treated mice compared to AMX-treated mice ($p=0.004$) (Fig. 4 D). IL-10
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42 decreased thereafter in the AMX/DXM-treated mice and was no longer detected after 24 h
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44 post-infection. IL-10 levels were very low in the AMX-treated mice at all time-points of the
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46 infection. In non-treated mice, IL-10 was also detectable after 6 h of infection but continued
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48 to increase and unlike treated mice, high levels of IL-10 were observed during all time-points
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50 of the infection.
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4. DISCUSSION

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2 The controversial question of the use of corticosteroids in meningococcal invasive infections
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4 remains open and the availability of a reliable animal model may offer an experimental
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6 approach to address this point in a defined system. Not only this model of transgenic mice
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8 expressing the human transferrin allows meningococci to grow but it also mimics the
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10 pathogenic potential of meningococcal isolates. *Indeed, without transferrin, meningococci are*
11
12 *unable to grow in the blood of infected mice and are rapidly cleared out [21].* A more severe
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14 infection was observed when cc11, a hyperinvasive genotype, was used. These isolates induce
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16 a strong inflammation and apoptosis during meningococcal sepsis (9). We confirmed the
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18 ability of these isolates to induce strong inflammation in mice that was reflected by strong
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20 production of pro-inflammatory cytokines (KC and IL-6) concomitant to tissue damages as
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22 shown by histological analysis. Our data suggest that hyperinvasive isolates like the isolate
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24 24198 (cc11) used in this study are responsible for bad prognosis because of the important
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26 and uncontrolled inflammatory reaction they trigger. This excessive inflammatory reaction
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28 responsible of fatal outcome of meningococcal sepsis has been known for a long time and
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30 especially concerning the TNF- α secretion [22]. Meningococcal isolates belonging to
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32 different genetic lineages may therefore provoke invasive infections with variable severity.
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34 Similar observations were also reported in other bacterial species as in *Streptococcus*
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36 *pneumonia* where different genotypes and capsular types determine the pathogenic behavior
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38 of pneumococci [23].
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48 Dexamethasone as an adjuvant treatment is associated with higher survival and seems to
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50 improve the clinical manifestations of the experimental infection compared to mice only
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52 treated with AMX with a significant reduction of the acute phase proteins assessed by Crp
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54 and Lcn 2 analysis. This improvement does not seem to be due to faster bacterial clearance as
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56 no significant difference in meningococcal bacteremia was observed. However, this
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1 improvement could be due to the ability of corticosteroids to induce anti-inflammatory
2 cytokines such as IL-10. In fact, the early increase of this cytokine (6 h after the infection and
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4 3 h after the first dose of combined treatment) is in accordance with this explanation.
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7 The interference of different treatments (saline, AMX and AMX/DXM) with the renin-
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9 angiotensin-aldosterone system (RAAS) for the induction of the inflammatory response also
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11 require to be investigated [24]. Indeed, these treatments as well as the early infection in
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13 different organ (kidneys and liver) may impact on the RAAS (Supplementary Fig. S1).
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16 The induction of these pro-inflammatory cytokines is required for optimal clearance of
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18 meningococci. However, excessive and/or uncontrolled inflammatory reaction may also lead
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20 to tissue damage as we observed in histological hepatocytes lesions.
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23 The impact of IL-10 is clearly linked to the timing of its production. Indeed, in untreated mice
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25 late production of IL-10 may correspond to a negative feedback mechanism to limit the
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27 potential damage of inflammatory cytokines. This late production occurs upon the control of
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29 infection and therefore is not expected to impede pathogen clearance but rather improve
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31 immunopathology [25]. An earlier release of IL-10 may worsen the infection as it may
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33 preclude pathogen clearance. In the AMX/DXM-treated mice, the early production of IL-10
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35 was advantageous because antibiotic treatment allowed rapid bacterial clearance and therefore
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37 permitted clinical benefit from the early release of IL-10. These data corroborate previous
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39 results from our laboratory that suggested that the use of corticosteroids as adjuvant treatment
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41 of meningococcal sepsis may reduce mortality and severe forms of the disease [11]. High
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43 levels of IL-10 were reported in patients with meningococcal sepsis when they reach the
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45 hospital 8 h to 24 h after the first symptoms are recognized and that it is correlated with high
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47 plasma levels of meningococcal endotoxin [26]. Nm-infected human monocytes produce high
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49 levels of pro-inflammatory cytokines (TNF- α , IL-6 and IL-8). These levels were reduced by
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51 36% to 58% when IL-10 was given with infecting meningococci [27]. Beneficial effect of IL-
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10 on sepsis mortality in mice have already been described [28-30] but these early beneficial effects are counterbalanced by secondary pejorative effects of those anti-inflammatory cytokines in sepsis. In fact, a phase of “immunoparalysis” might be responsible of secondary deaths in sepsis [31, 32].

5. CONCLUIONS

Corticosteroids administration might thus be particularly interesting by inducing early temporary secretion of IL-10 without secondary immune suppression providing that the bacterial infection is controlled by appropriate antibiotic treatment. DXM when early administered with antibiotic may have a crucial impact in early inducing IL-10 that may then protect mice from rapid overstimulation of pro-inflammatory cytokines.

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1 **Conflict of Interest:**
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3 The authors declare that they have no conflict of interest.
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REFERENCES

- 1 [1] Bratcher HB, Bennett JS, Maiden MC. Evolutionary and genomic insights into
2 meningococcal biology. *Future Microbiol.* 2012;7:873-85.
- 3 [2] Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease.
4 *N Engl J Med.* 2001;344:1378-88.
- 5 [3] Trotter CL, Fox AJ, Ramsay ME, Sadler F, Gray SJ, Mallard R, et al. Fatal outcome from
6 meningococcal disease--an association with meningococcal phenotype but not with reduced
7 susceptibility to benzylpenicillin. *J Med Microbiol.* 2002;51:855-60.
- 8 [4] Zarantonelli ML, Lancellotti M, Deghmane AE, Giorgini D, Hong E, Ruckly C, et al.
9 Hyperinvasive genotypes of *Neisseria meningitidis* in France. *Clin Microbiol Infect.*
10 2008;14:467-72.
- 11 [5] Deghmane AE, Veckerle C, Giorgini D, Hong E, Ruckly C, Taha MK. Differential
12 modulation of TNF-alpha-induced apoptosis by *Neisseria meningitidis*. *PLoS Pathog.*
13 2009;5:e1000405.
- 14 [6] van Deuren M, van der Ven-Jongekrijg J, Bartelink AK, van Dalen R, Sauerwein RW, van
15 der Meer JW. Correlation between proinflammatory cytokines and antiinflammatory
16 mediators and the severity of disease in meningococcal infections. *J Infect Dis.* 1995;172:433-
17 9.
- 18 [7] Prigent H, Maxime V, Annane D. Clinical review: corticotherapy in sepsis. *Crit Care.*
19 2004;8:122-9.
- 20 [8] Annane D. Corticosteroids for severe sepsis: an evidence-based guide for physicians. *Ann*
21 *Intensive Care.* 2011;1:7.
- 22 [9] Nguyen TH, Tran TH, Thwaites G, Ly VC, Dinh XS, Ho Dang TN, et al. Dexamethasone
23 in Vietnamese adolescents and adults with bacterial meningitis. *N Engl J Med.*
24 2007;357:2431-40.
- 25 [10] Scarborough M, Gordon SB, Whitty CJ, French N, Njalale Y, Chitani A, et al.
26 Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. *N Engl J Med.*
27 2007;357:2441-50.
- 28 [11] Madhi F, Levy C, Deghmane AE, Bechet S, Cohen R, Taha MK. Corticosteroid Therapy
29 in Genotype ST-11 Meningococcal Infections. *Pediatr Infect Dis J.* 2013;32:291-3.
- 30 [12] Szatanik M, Hong E, Ruckly C, Ledroit M, Giorgini D, Jopek K, et al. Experimental
31 meningococcal sepsis in congenic transgenic mice expressing human transferrin. *PLoS One.*
32 2011;6:e22210.
- 33 [13] Deghmane AE, Parent du Chatelet I, Szatanik M, Hong E, Ruckly C, Giorgini D, et al.
34 Emergence of new virulent *Neisseria meningitidis* serogroup C sequence type 11 isolates in
35 France. *J Infect Dis.* 2010;202:247-50.
- 36 [14] Guiddir T, Deghmane AE, Giorgini D, Taha MK. Lipocalin 2 in cerebrospinal fluid as a
37 marker of acute bacterial meningitis. *BMC Infect Dis.* 2014;14:276.
- 38 [15] Taha MK, Giorgini D, Nassif X. The *pilA* regulatory gene modulates the pilus-mediated
39 adhesion of *Neisseria meningitidis* by controlling the transcription of *pilC1*. *Mol Microbiol.*
40 1996;19:1073-84.
- 41 [16] Zarantonelli ML, Skoczynska A, Antignac A, El Ghachi M, Deghmane AE, Szatanik M,
42 et al. Penicillin resistance compromises Nod1-dependent proinflammatory activity and
43 virulence fitness of *Neisseria meningitidis*. *Cell Host Microbe.* 2013;13:735-45.
- 44 [17] Huet O, Ramsey D, Miljavec S, Jenney A, Aubron C, Aprico A, et al. Ensuring animal
45 welfare while meeting scientific aims using a murine pneumonia model of septic shock.
46 *Shock.* 2013;39:488-94.

- 1 [18] Shrum B, Anantha RV, Xu SX, Donnelly M, Haeryfar SM, McCormick JK, et al. A
2 robust scoring system to evaluate sepsis severity in an animal model. BMC Res Notes.
3 2014;7:233.
- 4 [19] Angelov O, Schroer RA, Heft S, James VC, Noble J. A comparison of two methods of
5 bleeding rats: the venous plexus of the eye versus the vena sublingualis. J Appl Toxicol.
6 1984;4:258-60.
- 7 [20] Kellogg DS, Jr., Peacock WL, Jr., Deacon WE, Brown L, Pirkle DI. *Neisseria*
8 *gonorrhoeae*. I. Virulence Genetically Linked to Clonal Variation. J Bacteriol. 1963;85:1274-
9 9.
- 10 [21] Zarantonelli ML, Szatanik M, Giorgini D, Hong E, Huerre M, Guillou F, et al.
11 Transgenic mice expressing human transferrin as a model for meningococcal infection. Infect
12 Immun. 2007;75:5609-14.
- 13 [22] Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in
14 serum and fatal outcome in patients with meningococcal disease. Lancet. 1987;1:355-7.
- 15 [23] Sjöstrom K, Spindler C, Ortqvist A, Kalin M, Sandgren A, Kuhlmann-Berenzon S, et al.
16 Clonal and capsular types decide whether pneumococci will act as a primary or opportunistic
17 pathogen. Clin Infect Dis. 2006;42:451-9.
- 18 [24] Salgado DR, Rocco JR, Silva E, Vincent JL. Modulation of the renin-angiotensin-
19 aldosterone system in sepsis: a new therapeutic approach? Expert Opin Ther Targets.
20 2010;14:11-20.
- 21 [25] Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection.
22 J Immunol. 2008;180:5771-7.
- 23 [26] Brandtzaeg P, Bjerre A, Ovstebo R, Brusletto B, Joo GB, Kierulf P. *Neisseria*
24 *meningitidis* lipopolysaccharides in human pathology. J Endotoxin Res. 2001;7:401-20.
- 25 [27] Gopinathan U, Ovstebo R, Olstad OK, Brusletto B, Dalsbotten Aass HC, Kierulf P, et al.
26 Global effect of interleukin-10 on the transcriptional profile induced by *Neisseria*
27 *meningitidis* in human monocytes. Infect Immun. 2012;80:4046-54.
- 28 [28] Gerard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A, et al.
29 Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in
30 experimental endotoxemia. J Exp Med. 1993;177:547-50.
- 31 [29] Howard M, Muchamuel T, Andrade S, Menon S. Interleukin 10 protects mice from lethal
32 endotoxemia. J Exp Med. 1993;177:1205-8.
- 33 [30] Latifi SQ, O'Riordan MA, Levine AD. Interleukin-10 controls the onset of irreversible
34 septic shock. Infect Immun. 2002;70:4441-6.
- 35 [31] Germain RN. Maintaining system homeostasis: the third law of Newtonian immunology.
36 Nat Immunol. 2012;13:902-6.
- 37 [32] Volk HD, Reinke P, Docke WD. Clinical aspects: from systemic inflammation to
38 'immunoparalysis'. Chem Immunol. 2000;74:162-77.
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Figures legends

1
2 Figure 1 Dissemination of *N. meningitidis* in BALB/c hTf transgenic mice. Mice were
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4 infected by intraperitoneal injection of 1.2×10^7 CFU of the strain 24198LUX and treated
5
6 either with amoxicillin (AMX) alone, with amoxicillin and dexamethasone (AMX + DXM) or
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8 with saline (non-treated). Mice were analyzed for bioluminescence at the indicated times
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10 using an IVIS 100 system (Xenogen Corp., Alameda, CA) that has the maximal capacity to
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12 analyse five mice at the same time. Images depict photographs overlaid with color
13
14 representations of luminescence intensity, measured in total photons/sec and indicated on the
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16 scales, where red is most intense and blue is least intense. (A) Dorsal view of 5 mice infected
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18 with 24198LUX (cc11 strain) at different times post-infection and regarding three groups of
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20 treatment: one group non-treated (saline injection), one group treated with AMX and one
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22 group treated with AMX +DXM (B) Total body luminescence quantification. The
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24 luminescence was quantified and expressed as means \pm SD from each category at the
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26 indicated times by defining specific representative region of interest encompassing the entire
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28 animal. (C) Total luminescence delimited to the skull. The luminescence was quantified and
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30 expressed as means \pm SD from each category at the indicated times by defining specific
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32 representative region of interest encompassing only the skull of the animal. *p*-value expressed
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34 are those comparing the AMX + DXM (red) group to the non-treated group (black);
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36 ****= $p < 0.0001$. Note that after 24 hours of infection signals were not significantly different
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38 from the background (non infected mice with 10^5 photons/sec)
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51 Figure 2. Clinical evolution of BALB/c hTf transgenic mice infected with *N. meningitidis*
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53 24198LUX strain. Data from mice that were non-treated (black), treated with amoxicillin
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55 ((AMX) blue) or treated with amoxicillin and dexamethasone ((AMX + DXM) red) are
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57 displayed, with *N. meningitidis* 2C4-3LUX strain or non-infected. (A) Evolution of mice
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1 survival after infection. P-value expressed are those comparing the AMX + DXM group to the
2 non-treated group and to the AMX-treated group. (B) Evolution of mice vitality assessed by a
3 vitality score (ranging from 0 to 5) taking into accounts the fur aspect as well as the activity of
4 the mice. Fur aspect ranged from 0 to 2 (0=Erection of the hair, 1=Hair coat appears soiled,
5 2= Hair coat appears glossy and smooth) and activity score of live mice ranged from 0 to 3
6 (0=No resistance to traction, 1=Weak resistance to traction, 2=Strong resistance to traction,
7 3=Spontaneous forward propulsion). (C) Evolution of body temperature in °C. (D) Evolution
8 of weight loss in grams. *=p<0.05, ***=p<0.001, ****=p<0.0001.
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21 Figure 3. Lipocalin 2 detection in blood of infected mice. Western blotting analysis using
22 antibodies specific to mouse Lcn2. Plasma samples were collected at 6 h post-infection from
23 4 mice of each tested group. The same positive control with a known concentration was used
24 for each Western Blot.
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31 Figure 4. Kinetics of cytokines production in BALB/c hTf transgenic mice infected by intra-
32 peritoneal injection of 1.2×10^7 CFU 24198LUX strain. As in Fig.1, mice were treated either
33 with amoxicillin (AMX), with amoxicillin and dexamethasone (AMX + DXM) or with saline
34 (non-treated). ELISA assays were used to quantify (A) IL-6, (B) KC, (C) TNF-alpha and (D)
35 IL-10 in blood samples from mice at different time-points of infection. Data are expressed as
36 mean of concentration for each tested cytokine after 3, 6, 24 and 36 hours (IL-6, KC and IL-
37 10). P-value expressed are those comparing the AMX + DXM group (red) to the non-treated
38 group (black) and to the AMX-treated group (blue); *=p<0.05, **=p<0.01, ***=p<0.001,
39 ****=p<0.0001.
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Fig. 1

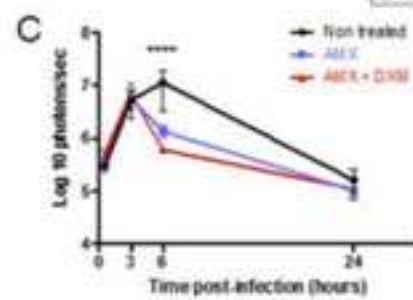
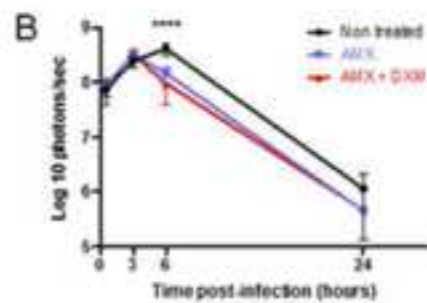
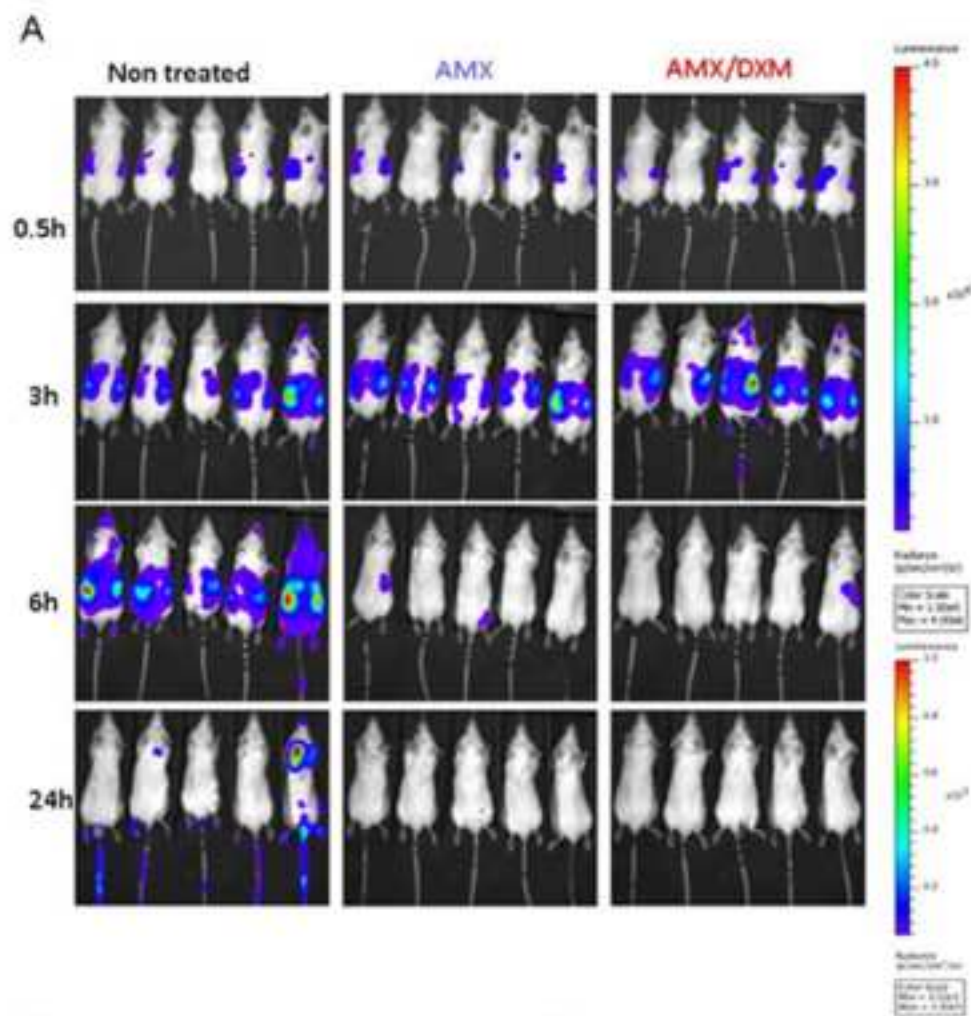


Figure2

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Fig.2

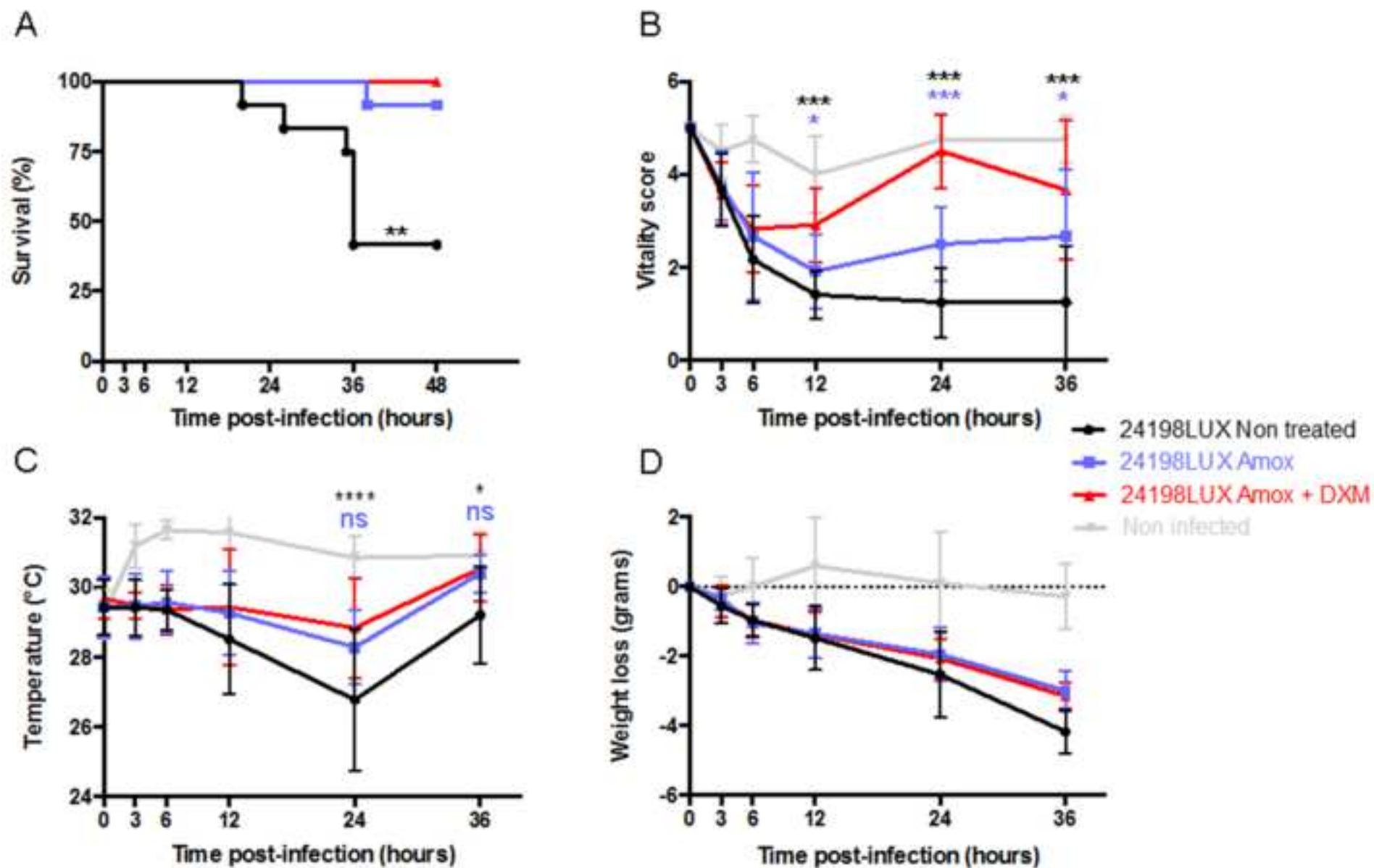


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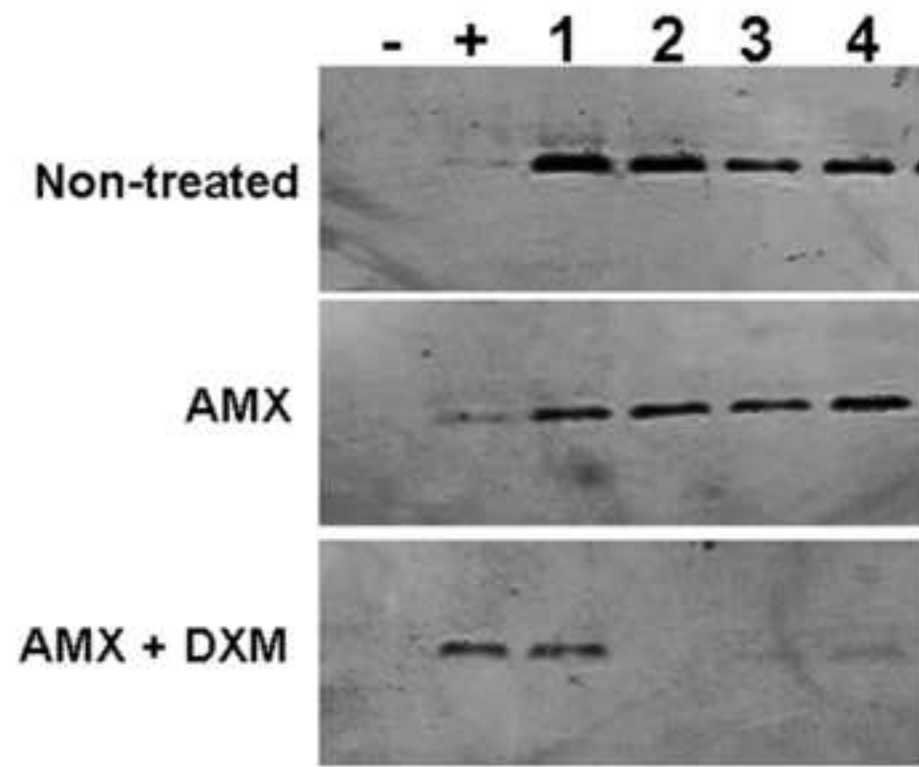


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Fig.4

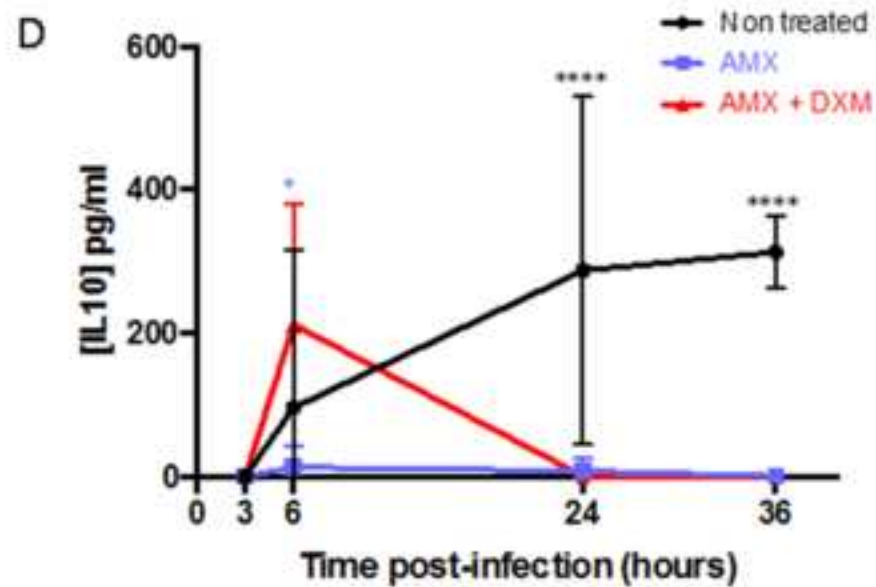
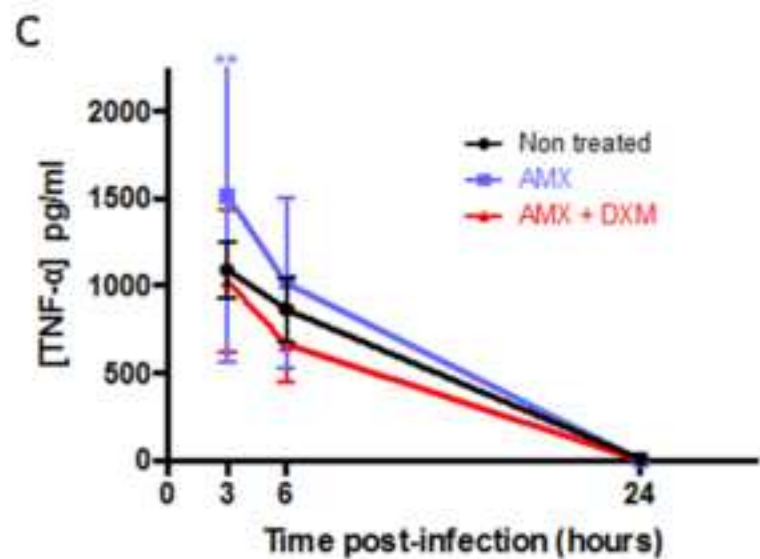
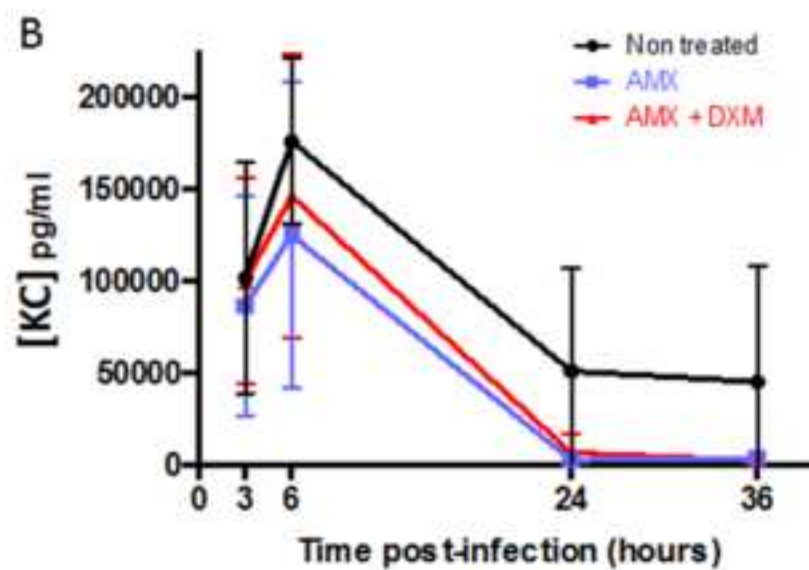
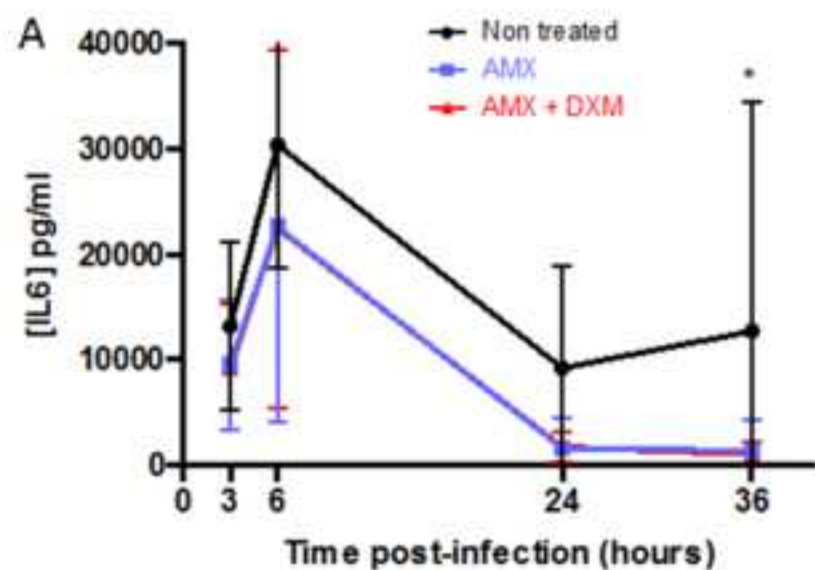


Table 1 White blood cell formulae, CRP and Lipocalin 2 levels in mice infected with *N. meningitidis* 24198LUX strain.

	Time	Non treated	AMX	AMX/DXM	p values
Polymorphonuclear cells	3h	616 ± 231	933 ± 504	1066 ± 750	ns
	6h	416 ± 132	300 ± 109	366 ± 51	ns
	24h	380 ± 83	1083 ± 511	1483 ± 604	ns
Total white blood cells	3h	2416 ± 685	3350 ± 1013	3816 ± 1866	ns
	6h	1683 ± 386	1050 ± 398	1633 ± 258	ns
	24h	2060 ± 230	2783 ± 1579	3400 ± 1454	ns
(CRP) µg/ml	6h	10475 ± 523	10019 ± 720	5527 ± 791	< 0.0001
(Lcn2) ng/ml	6h	610 ± 79	342 ± 104	104 ± 107	* < 0,0001 # 0.0276

(Time= time post-infection, AMX= amoxicillin, DXM= dexamethasone).

* p value for non treated group, # p value for AMX group (AMX + DXM group is the reference).