

## Supporting information

### **The biosynthetic monosphoryl lipid A enhances the therapeutic outcome of antibiotic therapy in pneumococcal pneumonia**

Fiordiliegia Casilag<sup>1</sup>, Laura Matarazzo<sup>1</sup>, Sebastian Franck<sup>2</sup>, Martin Figeac<sup>3</sup>, Robin Michelet<sup>2</sup>, Charlotte Kloft<sup>2</sup>, Christophe Carnoy<sup>1,\*</sup>, and Jean-Claude Sirard<sup>1,\*</sup>

<sup>1</sup> Univ. Lille, CNRS, INSERM, CHU Lille, Institut Pasteur de Lille, U1019 – UMR9017 - CIIL - Center for Infection and Immunity of Lille, F-59000 Lille, France

<sup>2</sup> Freie Universitaet Berlin, Institute of Pharmacy, Dept. of Clinical Pharmacy & Biochemistry, D-12169 Berlin, Germany

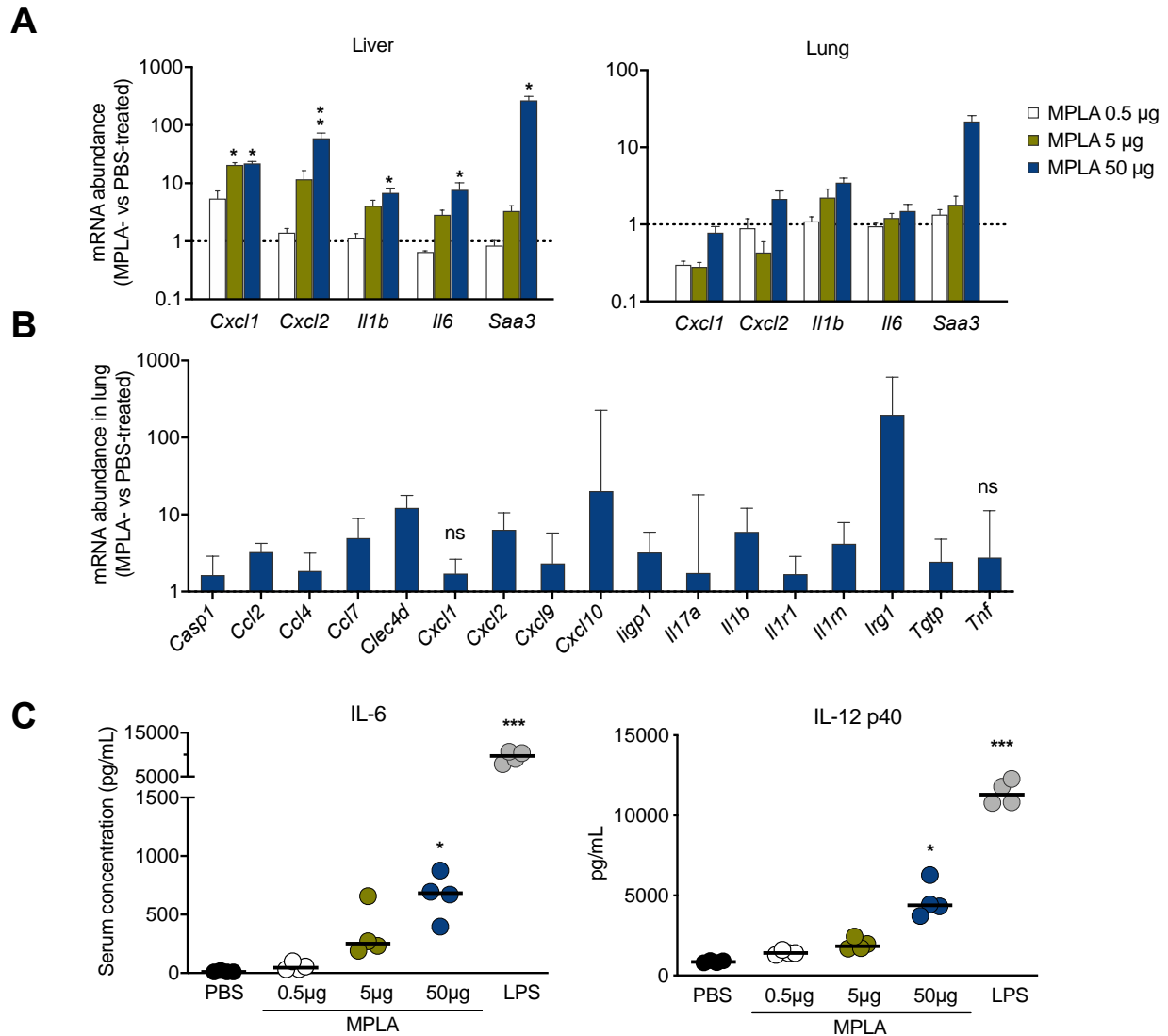
<sup>3</sup> Univ. Lille, Plateforme de Génomique Fonctionnelle et Structurale, F-59000 Lille, France

#### **\*Corresponding authors**

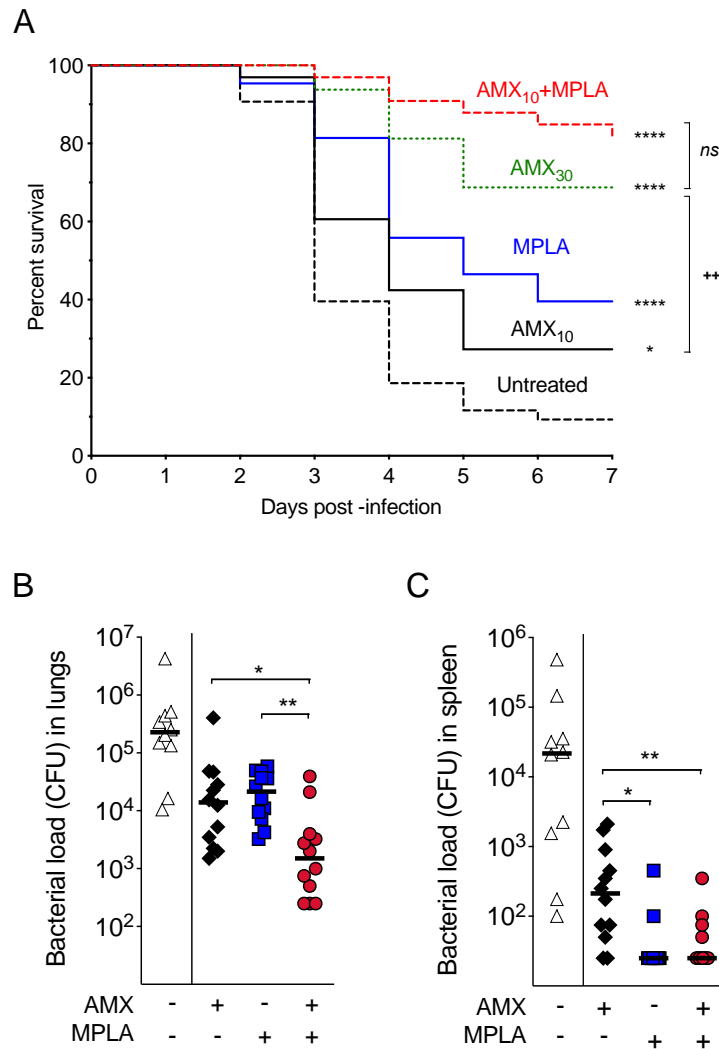
**Christophe Carnoy and Jean-Claude Sirard.** Institut Pasteur de Lille, Center for Infection and Immunity of Lille, Team Bacteria Antibiotics & Immunity, 1 rue du Pr Calmette, 59019 Lille, France

Orcid ID: <https://orcid.org/0000-0003-2047-5153>; Email: [christophe.carnoy@univ-lille.fr](mailto:christophe.carnoy@univ-lille.fr)

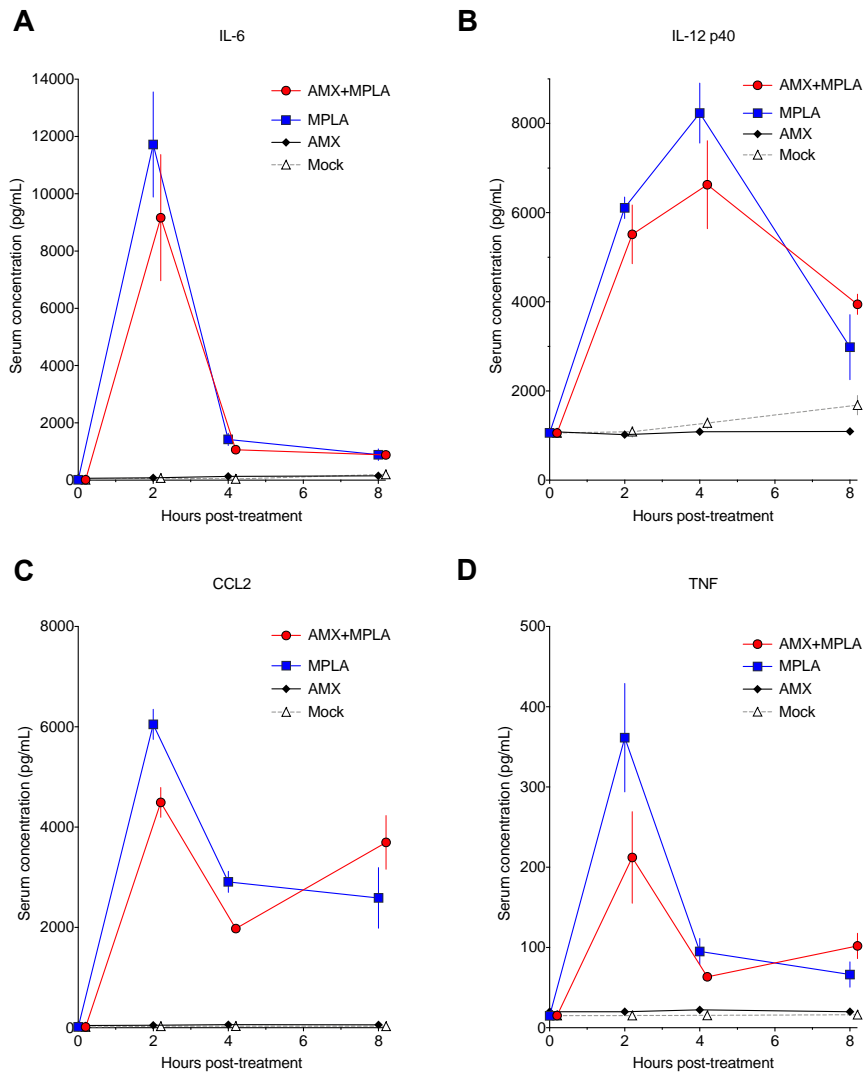
Orcid ID: <https://orcid.org/0000-0001-9854-4652>; Email: [jean-claude.sirard@inserm.fr](mailto:jean-claude.sirard@inserm.fr)



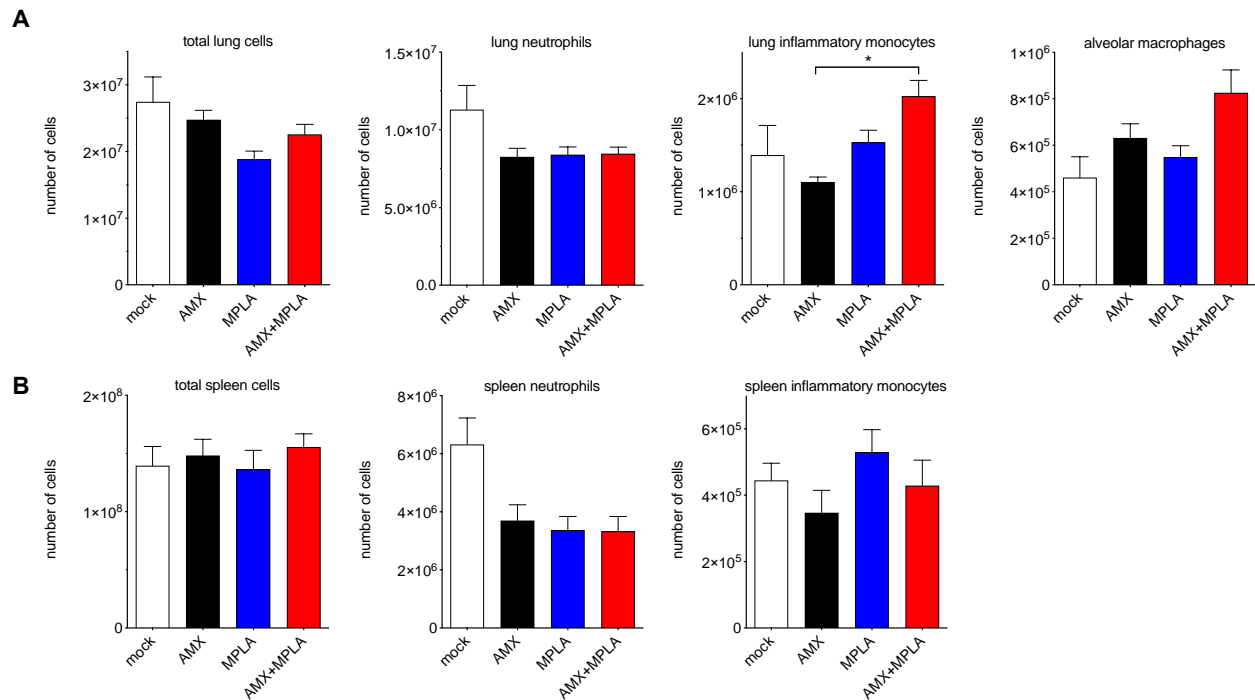
**Figure S1. Systemic MPLA administration leads to dose-dependent immune activation.** C57BL/6 mice (n=4/group) were injected intraperitoneally with MPLA (0.5, 5 or 50 µg), LPS (10 µg) or PBS. Three hours later, lungs, liver and sera were collected for analysis. (A, B) Relative gene expression is represented by the mRNA abundance of selected pro-inflammatory markers, compared with PBS-injected animals (normalized to the arbitrary value of 1). (A) Proinflammatory gene expression of selected markers in the lungs and liver was assessed by RT-qPCR. The bars correspond to the mean  $\pm$  SEM values. A one-way ANOVA (the Kruskal-Wallis test with Dunn's post-test for multiple comparisons) was applied. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ . (B) Gene expression in the lungs of mice treated with 50 µg MPLA, analyzed using a specific TaqMan Low Density Array and Thermo Fisher RQ software for biogroup analysis and expressed by mean  $\pm$  RQ<sub>min</sub>/RQ<sub>max</sub> values. (C) Serum cytokine concentrations were measured using an ELISA. Lines represent medians. A one-way ANOVA (the Kruskal-Wallis test with Dunn's post-test for multiple comparisons) was applied. \* =  $p < 0.05$ , and \*\* =  $p < 0.01$  vs. PBS-injected controls.



**Figure S2. MPLA treatment augments the therapeutic effect of low-dose amoxicillin.** (A) Survival curves of *S. pneumoniae*-infected Swiss mice receiving at 12 h post-infection different oral doses of amoxicillin (10  $\mu$ g and 30  $\mu$ g; AMX<sub>10</sub> and AMX<sub>30</sub>, respectively) as a stand-alone treatment or AMX<sub>10</sub> in combination with intraperitoneally administered 50  $\mu$ g MPLA (AMX<sub>10</sub>+MPLA) or untreated (n=16-43/group). Log-rank (Mantel-Cox) test was applied as follows: \*= $p$ <0.05, \*\*\*\*= $p$ <0.0001 vs. the infected and mock-treated control group, ++= $p$ <0.01 vs. the indicated comparator groups, ns=not significant. (B-C) Combination treatment is effective in BALB/c mice. BALB/cJRj mice (n=10-12/group) were infected intranasally with  $1 \times 10^6$  *S. pneumoniae* and then given either 5  $\mu$ g of AMX intragastrically, 50  $\mu$ g MPLA intraperitoneally, a combination of the two treatments (AMX+MPLA), or water and saline mock treatments 12 h post-infection. Lungs (B) and spleens (C) were collected at 12 h post-treatment (i.e., 24 h post-infection) for quantification of the bacterial load using standard plate counting methods. The solid lines indicate the median values. A one-way ANOVA (the Kruskal-Wallis test with Dunn's post-test for multiple comparisons) was applied. \*= $p$ <0.05, \*\*= $p$ <0.01 vs. the indicated comparator groups. Data from the infected and mock-treated control group were excluded from statistical analyses of the treatment groups.



**Figure S3. MPLA treatment induces a cytokine response independently of mouse genotype.** BALB/cJRj mice (n=3-4/group) were infected intranasally with  $10^6$  *S. pneumoniae* and then treated 12 h later with either AMX (10  $\mu$ g, intragastric administration), MPLA (50  $\mu$ g intraperitoneal administration), a combination of AMX and MPLA, or left untreated. Blood samples were collected at 2 h post-treatment. Serum levels of pro-inflammatory mediators were determined using ELISAs. (A) IL-6, (B) IL-12 p40, (C) CCL2, and (D) TNF. The mean  $\pm$  SEM values are shown.



**Figure S4. MPLA treatment influences the number of monocytes but not the number of neutrophils in lung.**

BALB/cJRj mice (n=4/group) were infected with *S. pneumoniae* and treated 12 h post-infection with either 10  $\mu$ g of AMX intragastrically, 50  $\mu$ g MPLA intraperitoneally, a combination of the two treatments (AMX+MPLA), or left untreated. Lung and spleen were sampled at 24 h post-infection and perfused with PBS, excised and finely minced then digested in a solution of RPMI 1640 medium (Gibco) containing 1 mg/ml collagenase VIII (Sigma-Aldrich) and 80  $\mu$ g/ml DNase I (Sigma-Aldrich) for 15 min at 37°C. After washes, red blood cells were removed using a lysis solution (Pharmlyse, BD Bioscience). Lung cell homogenates were then suspended in a 20% Percoll gradient and centrifuged at 2000 rpm without brake at room temperature for 10 min. Spleens were digested by injection of 1ml of the collagenase / DNase solution and incubated 15 min à 37°C, then crushed in a petri dish and filtered. After washes, red blood cells were removed using a lysis solution (Pharmlyse, BD Bioscience). The cell pellets from lungs and spleens were washed with PBS supplemented with 2% FCS and cells were filtrated before antibody labeling. Lung and spleen cells were stained with anti-CD45-allophycocyanin-cyanine 7 (clone 30F11), anti-CD11b-Brilliant Violet 785 (clone M1.70), anti-SiglecF-AlexaFluor 647 (clone E50-2440), anti-Ly6C-FITC (clone HK1.4), anti- Ly6G-phycoerythrin (clone 1A8), anti-CD11c-phycoerythrin-cyanine 7 (clone HL3) and CCR2-Brilliant Violet 421 (clone SA203G11) antibodies. Dead cells were excluded from the analysis using propidium iodide. The antibodies were purchased from BD Biosciences (San Jose, CA) or BioLegend (San Diego,

CA). Data were collected on a BD LSR Fortessa and analyzed with the BD FACSDiva software. Neutrophils were defined as CD45<sup>+</sup>CD11b<sup>+</sup>Ly6G<sup>+</sup> cells, inflammatory monocytes as CD45<sup>+</sup>CCR2<sup>+</sup>Ly6C<sup>+</sup>, and alveolar macrophages as CD45<sup>+</sup>SiglecF<sup>+</sup>CD11c<sup>+</sup> cells after exclusion of dead cells from the analysis. Numbers of cells in the lung parenchyma (A) and spleen (B) are shown as mean ± SEM values. Data from AMX+MPLA group were compared to those of AMX+PBS group in a Mann-Whitney test. Statistical significance is indicated as follows: \*  $p < 0.05$ .

**Table S1. List of primers used in this study**

<b>Gene</b>	<b>Forward primer</b>	<b>Reverse primer</b>
<i>Camp</i>	GCC GCT GAT TCT TTT GAC AT	AAT CTT CTC CCC ACC TTT GC
<i>Ccl12</i>	CAGTCACGTGCTGTTATAATGTTGTT	TGCTTGTGATTCTCCTGTAGCTCTT
<i>Ccl20</i>	TTTTGGGATGGAATTGGACAC	TGCAGGTGAAGCCTTCAACC
<i>Cxcl1</i>	CTTGTTTCAGAAAATTGTCCAAAA	CAGGTGCCATCAGAGCAGTCT
<i>Cxcl2</i>	CCCTCAACGGAAGAACCAAA	CACATCAGGTACGATCCAGGC
<i>Fkbp5</i>	TGTTCAAGAAGTTCGCAGAGC	CCTTCTTGCTCCAGCTTT
<i>Ifitm6</i>	CCGGATCACATTACCTGGTC	CATGTCGCCCACCATCTT
<i>Il12b</i>	GCAAAGAAACATGGACTTGAAGTTC	CACATGTCACTGCCCCGAGAGT
<i>Il1b</i>	AATCTATACCTGTCCTGTGTAATGAAAGAC	TGGGTATTGCTTGGGATCCA
<i>Il4i1</i>	GCTCTGTGGACATGGATGC	AGCCAGCCTGGGAACATAG
<i>Il6</i>	GTTCTCTGGGAAATCGTGGAAA	AAGTGCATCATCGTTGTTTCATACA
<i>Itgb2l</i>	CCTCCAGGGAGCAAAGTG	CCCAGCTAGGACAGGTAGCA
<i>Mmp8</i>	AACGGGAAGACATACTTCTTCATAA	GGGTCCATGGATCTTCTTTG
<i>Ngp</i>	GCCTAAAGACTGCGACTTCC	TGAAGAATTTCCCTGTGCAA
<i>Prok2</i>	CCC CCT GAC TCG GAA AAG	TCT TTC TTC TCT TCG CCC TTC
<i>S100a9</i>	CAC CCT GAG CAA GAA GGA AT	TGT CAT TTA TGA GGG CTT CAT TT
<i>Saa3</i>	GCCTGGGCTGCTAAAGTCAT	TGCTCCATGTCCCCTGAA
<i>Zbtb16</i>	AATGCATTTACTGGCTCATTC	CAGGGCATCCTCCTTTGAG