Systemic host inflammation induces stage-specific transcriptomic modification and slower maturation in malaria parasites

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Project / Study summary

Previous reports suggest that the maturation rate of malaria parasites within red blood cells (RBC) is not constant for a given species *in vivo*. For instance, maturation can be influenced by host nutrient status or circadian rhythm. Here we observed in mice that systemic host inflammation, induced by lipopolysaccharide (LPS) conditioning or ongoing acute malaria infection, slowed the progression of a single cohort of parasites from one generation of RBC to the next. LPS-conditioning and acute infection both triggered substantial changes to the metabolomic composition of plasma in which parasites circulated. This altered plasma directly slowed parasite maturation in a manner that could not be rescued by supplementation, consistent with the presence of inhibitory factors. Single-cell transcriptomic assessment of mixed parasite populations, exposed to a short period of systemic host inflammation *in vivo*, revealed specific impairment in the transcriptional activity and translational capacity of trophozoites compared to rings or schizonts. Thus, we provide *in vivo* evidence of transcriptomic and phenotypic plasticity of asexual blood-stage *Plasmodium* parasites when exposed to systemic host inflammation.

Study subject

C57BL/6J mice were purchased from the Animal Resource Centre (Perth, Australia). C57BL/6J.rag1−/− mice were bred at QIMR Berghofer Medical Research Institute. All mice were female, age-matched, between 6-8 weeks of age and were maintained under conventional conditions. This study was carried out in strict accordance with guidelines from The National Health and Medical Research Council of Australia. All animal procedures and protocols were approved (A02-633M and A1503-601M) and monitored by the QIMR Berghofer Medical Research Institute Animal Ethics Committee. Plasma was acquired from cardiac blood collected in lithium-heparin coated tubes.

Collection summary

Two independent experiments were conducted, each with 6 mice per treatment group (30 individuals in total). Mice were euthanized by CO_2 asphyxiation and their blood was taken by cardiac puncture into lithium-heparin coated tubes. Samples were spun for 5 min at 5000 rpm (approx. 7,043 × *g*) and plasma was immediately aliquoted into 1.5 mL tubes. In addition, a global sample pool containing equal volumes of each sample was prepared as quality control (QC) and four aliquots were transferred into 1.5 mL tubes. Finally, collection tube blank extractions were performed in triplicate by adding 1x PBS (same volume as blood collection) to lithium-heparin tubes and then transferring an aliquot into a 1.5 mL tube.

Treatment summary

Genotype	Treatment
C57BL/6J Naïve	**Control**, C57BL/6J mice were intraperitoneally injected (200 uL) with saline (0.9%) 9 hours prior to plasma acquisition.
C57BL/6J Acute	**Infected**, C57BL/6J mice were infected with 10^5 *Plasmodium berghei* ANKA parasitised red blood cells 5 days prior to plasma acquisition and intraperitoneally injected (200 uL) with saline (0.9%) 9 hours prior to plasma acquisition for analysis.
C57BL/6J LPS	**LPS treatment**, C57BL/6J mice were intraperitoneally injected (200 uL) with lipopolysaccharides (LPS) (0.75 mg/mL), from *E.coli* O127:B8, 9 hours prior to plasma acquisition.
rag1$^{-/-}$ Naïve	**Immune deficient control**, C57BL/6J.*rag1*$^{-/-}$ mice were intraperitoneally injected (200 uL) with saline (0.9%) 9 hours prior to plasma acquisition.
rag1$^{-/-}$ Acute	**Immune deficient infected control**, C57BL/6J.rag1-/- mice were infected with 10^5 *Plasmodium berghei* ANKA parasitised red blood cells 5 days prior to plasma acquisition and intraperitoneally injected (200 uL) with saline 9 hours prior to plasma acquisition for analysis.

Sample prep summary

Ten-times the sample volume of ice-cold butanol/methanol (1:1) containing 50 µg/mL antioxidant 2,6-di-*tert*-butyl-4-methylphenol (BHT) was added to each sample and vortexed for 10 s. Samples were snap frozen and transported on dry ice. Subsequently, samples were thawed on ice and labelled in a randomized order. Samples were sonicated for 15 min in an ice-cold water bath sonicator, stored for 2 hrs at -30^{o}C and then centrifuged for 15 min at 16,000 × *g* (4^{o}C). Lastly, samples were aliquoted, dried down using a vacuum concentrator and stored at -80^{o}C until LC/MS analysis.

MS acquisition

The LC/MS platform consisted of a 1290 Infinity II UHPLC coupled to a 6545 QTOF mass spectrometer via Dual AJS ESI source (Agilent, Santa Clara, USA) and was controlled using MassHunter data acquisition software (v.10.1). Assessment of MS instrument performance and usage of reference ions were also performed as described previously. Full scan MS data (m/z 50-1700) was acquired at a scan rate of 2.5 spectra/sec (equals 3224 transients/spectrum) with the following source conditions: Gas temperature 250°C, gas flow 13 L/min, sheath gas temperature and flow at 400°C and 12 L/min, respectively, nebulizer 30 psi, fragmentor 135, capillary voltage at +4500 V and -4000 V, nozzle voltage was zero.

Chromatography

Metabolite separation was performed on a Zorbax HILIC Plus RRHD (95Å, 1.8 µm, 2.1x100mm) analytical column connected to a 3 x 5 mm Zorbax HILIC Plus UHPLC guard column. The autosampler and column temperature were set to 4°C and 40°C, respectively. In positive and negative mode, eluent A was 10 mM ammonium acetate (pH neutral) in acetonitrile/milliQ water (95:5, v/v) and eluent B was 10 mM ammonium acetate (pH neutral) in acetonitrile/milliQ water (50:50, v/v). Total method runtime was 12 min with the following gradient for both modes: 0 min (1% eluent B) - 3.5 min (50% B) - 5.5 min (99%B) - 6.5 min (99% B) - 6.7 min (1% B) - 12 min (1% B). Flow rate was set to 0.5 mL/min.

For LC/MS analysis, a small volume of milliQ water was added to dried samples to reconstitute polar compounds by thoroughly vortexing and 10 min equilibration on ice. Following centrifugation, half of the reconstitution volume was transferred to a new tube and diluted with chilled acetonitrile (containing 1 µg/mL Val-Tyr-Val) to a final concentration of 80% acetonitrile. The Val-Tyr-Val standard was used to flag autosampler inaccuracies. All four QC samples were combined into a single 1.5 mL tube and QC dilutions (1:5, 1:10, 1:100) were prepared with chilled 80% acetonitrile (containing Val-Tyr-Val). Injection volumes were 2 µL for positive and 3 µL for negative mode.

The QC sample was used to assess instrument variation (retention time and signal stability) and to acquire MS/MS fragmentation data. Before a sample sequence, 6-12 replicate QC injections were performed to condition the column. Then, QCs were injected in duplicate at the beginning and end of each sequence and after every set of 10 samples. All samples were run in a randomized order and no blank injections were performed between samples. Following the sample sequence, acquisition of MS/MS data, QC dilutions and tube blank extractions was performed. The latter was being used in downstream data cleaning to identify and remove background noise and potential contaminants.

Data processing

Positive and negative mode data was analysed separately. Data files (30 sample files, 6 QC files and 3 tube blank extraction files) were loaded into MassHunter Profinder (v 10 SP1, Agilent) and assigned to sample groups. Spectral feature extraction was performed using the recursive feature extraction method employing default settings with minor adjustments: Peak extraction was restricted to retention time (Rt) range 0-6.5 min, compound binning and alignment tolerances were set to 1% + 0.3 min for Rt and 20 ppm + 2 mDa for mass, integrator Agile 2 was used for peak integration, peak filters were set to at least 2500 counts and features must have satisfied filter conditions in at least 75 % of files in at least one sample group.

Feature peak area was exported and data cleaning was performed using an in-house R script compiled of the following steps. Features were deleted if they: had a mean QC/tube blank area ratio of < 10; were absent across all QC samples; and had duplicates present. In addition, samples with a TIC scaling factor more than 50% above or below the median TIC were removed.