Transcriptomes of microglia in experimental cerebral malaria in mice in the presence and absence of Type I Interferon signaling

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Abstract

Objectives: Plasmodium berghei ANKA infection in mice is a model for human cerebral malaria, the most severe complication of Plasmodium falciparum infection. Responses of brain microglia have been little investigated, and may contribute to the pathogenesis of cerebral malaria. We showed previously that microglia are activated in P. berghei infections, and that Type 1-Interferon signaling is important for activation. This dataset compares transcriptomic profiles of brain microglia of infected mice in the presence and absence of Type 1 interferon signaling, with the aim of identifying genes in microglia in this pathway during experimental cerebral malaria.

Data description: We documented global gene expression from microglial RNA from uninfected and P berghei-infected wild-type C57BL/6 and IFNA Receptor Knock-out mice using Illumina Beadarrays. Principal component analysis showed 4 groups of samples corresponding to naïve wild-type, naïve IFNA Receptor knock-out, infected wild-type, and IFNA Receptor knock-out mice. Differentially-expressed genes of microglia from the two groups of infected mice are documented. Gene set enrichment analysis showing the top 500 genes assigned to Reactome pathways from infected IFNA Receptor knock-out versus naïve, and infected WT versus naïve has been generated. These data will be useful for those interested in microglia cells, and in experimental cerebral malaria.

Keywords: Malaria, Microglia, Plasmodium berghei, Experimental cerebral malaria, Type I Interferons, Microarray
IFN-1 receptor. In the analysis shown in this Data Note, we compared the transcriptome of purified microglia from *P. berghei*-infected IFNA Receptor knock-out mice with that of infected wild-type C57BL/6 mice using Illumina Beadarrays.

An analysis of gene expression microglia in wild-type C57BL/6 mice has been published [2], but a comparative study with microglia from IFNA Receptor knock-out mice has not been documented previously. These microarray results have not been incorporated into a research publication as we could not continue this study in this form as the Illumina Beadarrays have been discontinued. Nevertheless, we believe these data may be a useful for those research groups interested in activation of microglia in various settings and for malaria immunologists interested in the mechanisms of ECM in this rodent model.

Data description

We collected gene expression data from microglia isolated from wild-type C57BL/6 mice and IFNA Receptor knock-out mice infected with *Plasmodium berghei* ANKA using Illumina Beadarrays (Table 1, data file 1).

Female C57BL/6 mice and mice lacking the IFNA Receptor (IFNARKO) (aged 6–9 weeks of age) were obtained from the SPF breeding unit of the Mill Hill Laboratory, Francis Crick Institute, and for experimental work were conventionally housed with sterile bedding, food and water were provided ad libitum. Mice were injected with 10⁵ *P. berghei* ANKA infected erythrocytes intraperitoneally. Mortality, parasitemia and clinical scores indicative of ECM were monitored daily. Naïve and day 7-infected (d7) infected WT and IFNARKO mice were euthanized with pentobarbital, injected intraperitoneally (600 mg/kg body weight). Isolation of microglia was described in detail in [2]. Briefly, microglia were isolated from the brains of uninfected C57BL/6 and IFNARKO mice and from both groups of infected mice. Microglia (CD45<sup>lo</sup> and CD11b<sup>+</sup>) were purified from other brain cells by flow cytometry (MoFlo XPD, Beckman Coulter) using a combination of fluorophore conjugated antibodies: APC-anti-CD11b, PE-CD45, APCCy7-Ly6C, pacific blue- H-2 Kb (Biologend). Cells were washed and resuspended in PBS containing 2% FCS. Analysis was carried out using FlowJo-X software (Treestar). The sorted cells were confirmed as microglia based on the lack of cell surface marker Ly6C.

Total RNA was extracted immediately after sorting from approximately 10⁵ microglial cells using Ribopure kit (Ambion), and concentrations determined by Qubit quantitation using the HS assay kit (ThermoFisher Scientific). Quality was assessed by the Agilent 2100 Bioanalyzer; samples with a RIN score above 8.5 were used. Total RNA (300 ng) of each sample was amplified using the Total prep RNA amplification kit (Illumina) and Amplified cDNA (1500 ng) were then hybridized to Illumina MOUSE WG-6 V2.0 Beadarrays at 58 °C for 14–20 h at the High Throughput Screening facility of the Francis Crick institute RNA and cDNA were quantified by Qubit fluorometric quantitation and the quality were analysed using Agilent 2100 Bioanalyzer at each step [2] and Table 1, Data file 1.

Data analysis was conducted using the limma package [3] within R v3.5.1 running Bioconductor v3.7. Illumina idat files were read using "read.idat" function together with manifest file MouseWG-6_V2.0.R3.11278593_A.bgx downloaded from the Illumina website. Detection p-values were calculated using the "detectionPValues" function with default settings. Background correction was performed using negative control probes followed by quantile normalization using negative and positive control probes via the "neqc" function. Normalised expression values are reported in a log, scale.

Principal Component Analysis was performed on the 500 genes showing greatest variance across samples (Table 1, Data file 2). Differential gene expression was assessed between infected and naïve cell states within KO and WT cells separately using a linear model (Table 1, Data files 3 and 4). Significance was determined using a threshold

| Table 1 Overview of data files/data sets |
|----------------------------------------|
| **Label**  | **Name of data file/data set** | **File types (file extension)** | **Data repository and identifier (DOI or accession number)** |
| Data file 1 | Microarray data C57BL/6 mice infected with *P. berghei* | Illumina idat | [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE119650](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE119650) |
| Data file 2 | PCA.pdf | pdf | [https://doi.org/10.6084/m9.figshare.7171952](https://doi.org/10.6084/m9.figshare.7171952) |
| Data file 3 | wt.inf_vs_wt.naive-p01.fc2.results.txt | txt | [https://doi.org/10.6084/m9.figshare.7171952](https://doi.org/10.6084/m9.figshare.7171952) |
| Data file 4 | ko.inf_vs_ko.naive-p01.fc2.results.txt | txt | [https://doi.org/10.6084/m9.figshare.7171952](https://doi.org/10.6084/m9.figshare.7171952) |
| Data file 5 | ToppGene.enrichment.barplot.pdf | pdf | [https://doi.org/10.6084/m9.figshare.7171952](https://doi.org/10.6084/m9.figshare.7171952) |
| Data file 6 | ko_specific_treatment_effect.heatmap.pdf | pdf | [https://doi.org/10.6084/m9.figshare.7171952](https://doi.org/10.6084/m9.figshare.7171952) |
based on a FDR $\leq 0.01$ together with an absolute fold change $\geq 2$.

The two resulting lists were ordered by absolute fold change and the top 500 unique Entrez gene identifiers from each were put forward for gene list enrichment analysis using the ToppGene Suite [4]. Hits to the Reactome [5] pathway (FDR $\leq 0.01$) are presented in the bar-plot (Table 1, Data file 5).

A nested interaction formula was used to select genes responding differently to infection between KO and WT cells. Genes showing a KO specific response but remain unchanged in WT cells were selected for visualisation in a heatmap (Data file 6). Each gene’s expression across samples was converted to a z-score to aid visualisation. Clustering of row and columns was conducted using complete linkage on a Euclidean distance matrix.

**Limitations**

The sample size for each group is relatively small, 5 mice per group. There is only one time-point in the infection.

It was intended to continue this study; however, Illumina ceased production of these arrays during the study, and therefore the experiment could not be extended using this microarray format.

**Abbreviations**

ECM: experimental cerebral malaria; IFNARKO: Interferon Alfa Receptor 1 knock-out; IRBC: infected red blood cells; KO: knock out; P. berghei: Plasmodium berghei; WT: wild-type.

**Authors’ contributions**

BC designed the study, carried out the experimental work with mice, isolated the microglia, extracted RNA and prepared material for microarray hybridisation. JWL assisted with the experimental work. CT and RM carried out the analysis of the microarray data, and wrote the paper. JL conceived the project, and wrote the paper. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data materials**

The datasets generated and analysed (data file 1, data file 2; data file 3; data file 4, data file 5) during the current study are available on: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE119650 and the figshare repository: Talavera-López, Carlos; Capuccini, Varbara; Lin, Jing-wen; Mitter, Richard; Langhorne, Jean; (2018). figshare https://figshare.com/s/37fc3835ea53be4bf94f.

**Consent for publication**

There are no data from individual humans.

**Ethics approval and consent to participate**

The study was carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 (Home Office licence 80/2538 and 70/8326), was approved by the MRC National Institute for Medical Research Ethical Committee and by The Francis Crick Institute Ethical Committee.

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**References**

1. de Oca MM, Engwerda C, Haque A. Plasmodium berghei ANKA (PbA) infection of C57BL/6J mice: a model of severe malaria. Methods Mol Biol. 2013;1031:203–13. https://doi.org/10.1007/978-1-62703-481-4_23.

2. Capuccini B, Lin J, Talavera-López C, Khan SM, Sodenkamp J, Spaccapelo R, Langhome J. Transcriptomic profiling of microglia reveals signatures of cell activation and immune response, during experimental cerebral malaria. Sci Rep. 2016;6:39258. https://doi.org/10.1039/nar/gkv007.

3. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research. 2015;43(7):e47. https://doi.org/10.1093/ nar/gkv007.

4. Chen J, Bardes EE, Aronov BJ, Jegga AG. ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res. 2013;41:203–13. https://doi.org/10.1093/nar/gkt427.

5. Fabregat A, Jupe S, Matthews L, Sidiropoulos K, Gillespie M, Garapati P, Haw R, Jassal B, Körninger F, May B, Milacic M, Roca CD, Rothfels K, Sevilla C, Shamsky H, D’Eustachio P. The reactome pathway knowledgebase. Nucleic Acids Res. 2018;46(D1):D649–55. https://doi.org/10.1093/nar/gkx1132.