Samples used, extract preparation and labelling

Origin and characteristics of each biological sample:

Mice (Mus. musculus) were obtained from Charles River Canada Laboratories. Mice were male, 6-8 weeks old, and either BALB/c or C57BL/6.

P. berghei (ANKA clone) was obtained from the Malaria Reagent Repository (MR4, USA).

Manipulation of biological samples and protocols used:

All mouse experiments were approved by and conducted according to the University of Toronto animal ethics guidelines. Mice were housed under pathogen-free conditions. Cryopreserved *P. berghei* ANKA was thawed and passaged through naïve C57BL/6 donor mice.

On day 0, prior to infection, 5 mice from each strain were euthanized using carbon dioxide and served as a baseline control for all experimental mice. Whole blood was collected by cardiac puncture and intact brains, livers, spleens and lungs were excised immediately following euthanasia, snap-frozen in liquid nitrogen and stored at –80°C until use. The remaining 10 experimental mice from each strain were infected with 1 x 10^6 freshly isolated *P. berghei* ANKA parasitized erythrocytes by intraperitoneal (IP) injection. Parasitemia was monitored daily with thin blood smears. Five mice from each strain were sacrificed at each time interval (day 3 and day 6 following infection). Blood and organs were harvested as above.

Experimental factor value for each experimental factor:

Time: Day 0 (prior to infection)
Day 3 (post infection)
Day 6 (post infection)

P. berghei ANKA infection: infected with 1 x 10^6 parasitized erytrocytes IP
not infected

Genetic variation: BALB/c (cerebral malaria resistant)
C57BL/6 (cerebral malaria susceptible)

Technical protocols for preparing the hybridization extract and labeling:

Total RNA was extracted by homogenizing organs in Trizol reagent. Total RNA samples were denatured and loaded onto a 0.25ml Oligo-dT cellulose column in 40mM Tris pH7.5, 1M NaCl, 2mM EDTA and 0.2% SLS. The columns were washed and RNA was eluted in TE. This process was repeated. Eluted mRNA was ethanol precipitated, resuspended in dH_20 and stored at –80°C until use. Integrity of RNA was assessed by formaldehyde agarose gel electrophoresis.

cDNA was reverse-transcribed from 1-2 μg mRNA using Superscript II reverse transcriptase (Invitrogen) with 1 μg random nonamer and 0.25 μg T_{18}VN primers per reaction. The reaction mix contained a final concentration of 1X RT Buffer, 10mM DTT, 0.5 mM each dNTP and 0.5mM 5-(3-aminoallyl)-2'deoxyuridine-5'-triphosphate (AA-dUTP). Following the RT reaction, RNA was hydrolyzed using NaOH/EDTA and cDNA was purified using QIAquick PCR Purification columns, washed with 80% ethanol and eluted in water. Purified cDNA was coupled with N-hydroxy succinimide esters of Cy3 or Cy5 in bicarbonate buffer. Dye reactions were quenched by adding 4M hydroxylamine. Labeled cDNAs were separated from free dye molecules using QIAquick columns, washed with PE buffer and eluted in EB.