

Data Extraction and Normalization

Data Extraction:

Intensity measurements were extracted from the TIFF images using GenePix 3.0 (Axon Instruments).

Normalization:

The median intensity measurements extracted from the GenePix files were spatially detrended. To compensate for potential cross-hybridization of mouse mRNA to malaria probes, we subtracted from the intensity value of every malaria probe the day 0 intensity value of the same probe in the same tissue. After the cross-hybridization removal step, all negative values were set to zero. We then applied variance stabilizing normalization (VSN) version 1.6.3 in Bioconductor (R version 2.1.1) and transformed to log₂ scale. The intensity scale data shown in Figures is median-subtracted across tissues. The log ratios were obtained after loess smoothing of VSN-normalized arrays.

Data Analysis:

To analyze the data, we applied linear models for microarray data to the normalized log ratios using the Limma software package version 1.9.6 in Bioconductor. B6 samples were taken as reference in the design matrix and two contrast matrices were used. The first contrast matrix was used to estimate the difference between susceptible and resistant mice at day 0; and the second contrast matrix was used to estimate the difference in the response to the infection between susceptible and resistant mice (i.e., to compare the difference between the expression level at day 0 and the average expression level of day 3 and 6 between the two mouse strains). Design and contrast matrices are available upon request. Pvalues for the four tissues were combined in a pvalue per probe using the F-distribution. F-pvalues of probes mapped to the same gene were combined using Fischer method. Genes with a combined F-pvalue < 0.05 were considered differentially expressed. Pvalues were not corrected for multiple testing.