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Gene expression profiling in a mouse model for African trypanosomiasis

S Kierstein^{1,2}, H Noyes³, J Naessens¹, Y Nakamura^{1,4}, C Pritchard⁵, J Gibson^{1,6}, S Kemp^{1,3}, and A Brass⁷

¹International Livestock Research Institute, Nairobi, Kenya

²School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

³School of Biological Sciences, University of Liverpool, Liverpool, UK

⁴Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan

⁵MGU Harwell, Didcot, UK

⁶Institute for Genetics and Bioinformatics, University of New England, Armidale, Australia

⁷School of Biological Sciences, University of Manchester, Manchester, UK

Abstract

This study aimed to provide the foundation for an integrative approach to the identification of the mechanisms underlying the response to infection with *Trypanosoma congolense*, and to identify pathways that have previously been overlooked. We undertook a large-scale gene expression analysis study comparing susceptible A/J and more tolerant C57BL/6 mice. In an initial time course experiment, we monitored the development of parasitaemia and anaemia in every individual. Based on the kinetics of disease progression, we extracted total RNA from liver at days 0, 4, 7, 10 and 17 post infection and performed a microarray analysis. We identified 64 genes that were differentially expressed in the two strains in non-infected animals, of which nine genes remained largely unaffected by the disease. Gene expression profiling at stages of low, peak, clearance and recurrence of parasitaemia suggest that susceptibility is associated with high expression of genes coding for chemokines (e.g. Ccl24, Ccl27 and Cxcl13), complement components (C1q and C3) and interferon receptor alpha (Ifnar1). Additionally, susceptible A/J mice expressed higher levels of some potassium channel genes. In contrast, messenger RNA levels of a few immune response, metabolism and protease genes (e.g. Prss7 and Mmp13) were higher in the tolerant C57BL/6 strain as compared to A/J.

Keywords

microarray; gene expression; African trypanosomiasis; parasite infection; host response; susceptibility

Introduction

Tsetse fly-transmitted infection with *Trypanosoma congolense* is a serious constraint on livestock husbandry and economic development in sub-Saharan Africa. Although a number

of control measurements have been implemented for many years, no significant progress has been achieved in the eradication of the disease.¹ African trypanosomes are known for their ability to switch their surface antigens (variant surface glycoprotein) and to manipulate the host's immune system by a variety of immunosuppressive and -evasive mechanisms.^{2,3} The development of a vaccine has been particularly challenging and so far unsuccessful.⁴ A better understanding of trypanotolerance, the ability of some indigenous breeds of cattle and other ruminants to resist sickness despite latent infection, seems to be the most promising approach to disease control.⁵⁻⁷ A mouse model of genetic control of trypanotolerance exists based on A/J as a susceptible strain and C57BL/6 as a tolerant strain. This model is widely accepted and has led to the identification of five major quantitative trait loci (QTL) on mouse chromosomes 1, 5 and 17, associated with survival time.^{8,9} Until recently, most investigators have focused their research on the innate and adaptive immune response to *T. congolense* infection, investigating components such as trypanosome-specific and nonspecific antibody production, subsets of T cells, complement pathway, cytokine and nitric oxide production, and specific proteins such as heat-shock protein 70.1 and arginase.¹⁰⁻¹⁸ Although these studies have led to important findings, the measurement of a small number of components in any one study has limited the ability to integrate individual results. Microarray-based gene expression assays provide the ability to study the expression of large numbers of genes simultaneously. We undertook a microarray study of gene expression in A/J and C57BL/6 mice to explore the ability of a more integrated analysis of genetics of trypanotolerance and identify pathways involved in trypanotolerance that had been previously overlooked.

Results

Kinetics of *T. congolense* infection in A/J and C57BL/6 mice

A small number of blood parasites was observed in a few animals at day 4, but almost all animals had significant numbers of trypanosomes in their blood at day 6. The difference between strains in parasite numbers was significant ($P = 0.0005$) with an average of 5.24×10^6 and 3.02×10^6 trypanosomes/ml blood in A/J and C57BL/6, respectively. As shown in Figure 1a, mice of both strains reached their peak parasitaemia at around day 8 post infection. At this stage, A/J mice had an average of at least 1.07×10^8 trypanosomes/ml compared to 3.83×10^7 trypanosomes/ml in C57BL/6 mice. Thus, mice of the susceptible A/J strain had approximately three times higher parasite load at this time point (t -test, $P < 0.0001$). Animals of both strains were able to significantly reduce their parasitaemia by day 10, at which stage there was no significant difference between the two strains. A/J mice showed a rapid and extensive recurrence of parasitaemia that was almost 100 times higher than C57BL/6 at 18 days post infection. C57BL/6 also showed a recurrence of parasites, but the parasitaemia was significantly lower than in A/J at all stages (two-way analysis of variance (ANOVA), $P < 0.0001$). Taken together, A/J and C57BL/6 mice significantly differed in the development and degree of parasitaemia (two-way ANOVA, $P < 0.0001$).

Haemoglobin levels in A/J mice were significantly higher than in C57BL/6 pre-infection and this difference remained over the entire study period (Figure 1b, two-way ANOVA, $P < 0.0001$). Both strains developed first signs of anaemia at day 4, at the time point when the first parasites were seen in the blood stream with significant reductions over time (two-way ANOVA, $P < 0.0001$). The reduction of haemoglobin post infection did not differ significantly between the two strains.

A/J and C57BL/6 baseline differential gene expression

The gene expression profiling was designed to characterize gene expression differences at four defined stages of trypanosomiasis, namely 'no infection', 'peak parasitaemia',

'clearance' and 'recurring parasitaemia' (Figure 1a). Expression differences that were initially identified on microarrays using RNA from one set of mice were confirmed by quantitative polymerase chain reaction (PCR) from a second set of mice from an independent experiment. We identified 64 out of 7000 genes that were at least two-fold differentially expressed in uninfected A/J vs C57BL/6 mice. The expression of nine of these strain-specific genes was not further changed during trypanosome infection (Table 1). Eighteen genes were more than two-fold higher expressed in A/J mice as compared to C57BL/6, of which four are involved in immunologic processes. Among the in A/J overexpressed genes was phosphatidylethanolamine binding protein (Pbp). Real-time PCR confirmed that this gene was on average 2.14-fold (range 2.02-2.27) more highly expressed in A/J than C57BL/6 at all time points ($n = 10$ /strain). We observed 46 genes that had significantly higher expression levels in uninfected C57BL/6 mice. Several of these genes are involved in metabolism and biosynthesis (13 out of 46), and another five genes play a role in immunological pathways. Table 1 gives a summary of the differentially expressed genes in non-infected mice, including their chromosomal localization. The nine genes for which expression was largely unaffected by the disease are marked with an asterisk (*).

Time course and expression patterns during the disease progress

Day 4—At this time point, where individuals had very few parasites in their blood, A/J mice appeared to have higher expression levels of genes of metabolic, biosynthesis and molecular transport pathways. Among these genes (marked with $\diamond$ in Table 2) were glucose-6-phosphatase (G6pc) and carbonic anhydrase 3 (Car3). Out of the 34 genes with at least two-fold higher expression in A/J vs C57BL/6 mice, a remarkably high number of genes was involved in cell growth and differentiation (anti-apoptosis) as well as increased expression of cell surface receptors (marked with # in Table 2). The relative gene expression changes between days 0 (not infected) and 4 are depicted as a scatter plot in Figure 2a. A list of the 50 differentially expressed genes is given in Table 2.

Day 7—The 48 genes recorded in Table 3 represent the smallest number of differentially expressed genes between the two strains at any of the time points investigated. Whereas mice of the susceptible strain continued to overexpress genes of metabolism-related pathways ($\diamond$), they also overexpressed the interferon alpha/beta receptor (Ifnar1) and the C1q complement receptor gene (both marked with ∞) when compared to C57BL/6 mice. Among the newly upregulated genes was apolipoprotein A4 (Apo A4) gene. Real-time PCR confirmed the upregulation of this gene on days 7, 10 and 17 with an average of 11.85-fold higher expression in A/J than C57BL/6 (range 4.85-21.00, $n = 6$ /strain).

On day 7, in comparison to A/J, C57BL/6 showed increased expression of genes associated with signal transduction and transcriptional regulation (marked with §) such as nuclear factor of activated T cells (NFAT5) and presenilin 2 (Psen2). Chemotaxis and inflammatory response genes (marked with ∞) were also higher expressed in C57BL/6 mice. The scatter plot in Figure 2b illustrates the changes in differential gene expression occurring between day 4 and 7.

Day 10—Sixty-eight genes were more than two-fold differentially expressed between the susceptible A/J and the resistant C57BL/6 strain (Table 4). Compared to previous time points, at this stage, when parasitaemia was not significantly different between the two groups, both strains appear to have activated a number of genes of the acute phase and inflammatory response. Genes that were more highly expressed in A/J included serum amyloid A2 (Saa2), which was confirmed by real-time PCR (average difference 4.133-fold, range 2.38-5.88, $n = 4$ /strain), and Cxcl13, Ccl6 and Ccl27 (∞). In contrast to A/J mice which mainly overexpressed chemokine genes, only two out of six C57BL/6-specific host-

defence genes (∞) belonged to the chemokine family. One of these genes was Cxcl9. Real-time PCR of Cxcl9 showed a 3.09-fold (range 2.18-4.35, $n = 10$ /strain) difference between the two strains at all time points. This was an interesting finding, as the microarray data suggested a significant upregulation only at day 10 post infection. Further, the trypanotolerant strain overexpressed several genes coding for proteases ($\div$), including matrix metalloproteinase 13 as compared to the susceptible strain. C57BL/6 also continued to maintain a high metabolic activity ($\diamond$). Changes in differential gene expression between days 7 and 10 are shown as a scatter plot in Figure 2c.

Day 17—At this stage, when we observed a massive recurrence of trypanosomes in the blood of A/J mice, we found the highest number (91) of differentially expressed genes (Table 5). Most genes are more highly expressed in the susceptible A/J strain. Of the 70 such differentially expressed genes, a large number of them are chemokine genes, with a total of 17 immune response genes (∞), 13 metabolism-related genes ($\diamond$), and another three genes involved in proteolytic processes ($\div$). A/J mice also showed higher expression levels of potassium channel genes ($\S$) compared to C57BL/6. Twentyone genes were more highly expressed in C57BL/6 compared to A/J, and most of these genes had been differentially expressed in the uninfected mice. The scatter plot displayed in Figure 2d illustrates a shift to higher gene expression levels in the A/J strain. In the real-time PCR, macrophage receptor/collagenous structure (Marco) and complement component 3 (C3) were 3.48- to 5.44-fold and 4.25- to 6.20-fold more highly expressed in A/J compared to C57BL/6 at this time point.

The hierarchical cluster in Figure 3 illustrates very distinct time-specific differential expression patterns, with clear clusters of higher expression in A/J (red) at days 7, 10 and 17. Time-specific clusters were less pronounced for genes that were more highly expressed in C57BL/6 mice (green). Overall, we observed 35 genes with a strong strain association (Figure 4). Among the 20 genes more highly expressed in C57BL/6 mice were major urinary proteins 1 and 4 (Mup1, Mup4), haemolytic component (Hc) and Bcl2-interacting killer-like (Biklk). The expression of another 15 genes appeared generally higher in the susceptible phenotype of A/J mice. These include genes coding for Pbp, Apo A4 and serum amyloid P component (Apcs) among others.

Pathways associated with trypanotolerance

We carried out pathway analyses to put the differentially expressed genes into a functional context that would provide information on which mechanisms might be important in controlling resistance to the infection with *T. congolense*. Statistically significant ($P < 0.0001$) association with the trypanotolerant C57BL/6 strain was seen in the pathways for bile acid and cholesterol synthesis with an upregulation of genes coding for alcohol dehydrogenase 1a (Adh1a), acyl-CoA oxidase 1 (Acox1), aldoketo reductase 1c1 (Akr1c1), 3beta-hydroxysteroid dehydrogenase (Hsd3b1), cytochrome P450 enzyme 7b1 (Cyp7b1), and acyl-CoA:cholesterol acyltransferase 2 (Soat2). Additionally, C57BL/6 mice also showed much more of the classical complement response genes C5, C5a and C9 as compared to A/J. Susceptibility in the A/J strain was associated with an upregulation relative to C57BL/6 of genes involved in acute-phase response and chemotaxis. Generally, the transcriptional response in A/J mice is much more extensive with an activation of a striking number of chemokine genes, including Ccl24, Ccl27, Ccl8, Ccl9, Cxcl1 and Cxcl13. Our pathway analysis did not reveal any significant linkage between susceptibility and a specific pathway.

Integrating QTL mapping and gene expression profiling

Based on the previously published fine mapping of QTL associated with survival after *T. congolense* infection (trypanosome infection response, Tir), we were able to identify three differentially expressed genes that are within 1 cM of one of the three QTLs. Complement component C3 with a chromosomal localization on MMU17 (34.3 cM) is located within the QTL region designated Tir1. C3 is one of the genes specifically upregulated in A/J mice during the recurrence of the infection (day 17). Its differential expression was also confirmed by real-time PCR that indicated an average fold difference of 5.22 (range 4.25-6.19, $n = 2/\text{strain}$). Serum amyloid P component (Apcs) is located on MMU1 at 94.2 cM, within the Tir 3c locus. The third gene, serine/threonine kinase 25 (Stk25) has been mapped to MMU1 (58 cM), which is in close proximity to the Tir 3a locus with its peak at 59 cM.

Discussion

The present study provides the foundation for a more integrative approach towards the understanding of trypanotolerance. In order to identify the most informative time points, we undertook a time course study where the main characteristics of *T. congolense* infection, parasitaemia and anaemia, were monitored in susceptible A/J and tolerant C57BL/6 mice for up to 18 days. Based on these data, we performed a microarray-based gene expression analysis comparing the two mouse strains at days 0 (not infected), 4 (pre-peak parasitaemia), 7 (peak parasitaemia), 10 (clearance) and 17 (recurrence) post infection. Our findings indicated that susceptibility is associated with higher expression of genes coding for chemokines, alternative complement components and interferon receptors during the recurrence of parasitaemia. Additionally, the expression of some potassium channel genes was higher in A/J mice at this stage. In contrast, the tolerant C57BL/6 strain overexpressed fewer genes, with a focus on the classical complement cascade and genes of the bile acid and cholesterol pathways.

A/J mice are known to have a functional null allele (Hc^0) of the complement C5 component. The gene is expressed but the protein is not secreted.^{19,20} We observed a two-fold overexpression of this gene in C57BL/6 which have an intact copy of the gene, suggesting that the mutation in A/J caused by a two base pair deletion near the 5'-end of the gene may also lead to some non-sense-mediated decay. It is possible that the absence of a functional copy of this gene leads to knock-on effects elsewhere in the complement cascade such as the overexpression of C3 in A/J mice, which may be a consequence of a feedback control loop stimulating higher expression of C3 in the absence of C5.

Recent findings suggest that regulation of cholesterol metabolism in macrophages impacts antimicrobial response, cytokine secretion and phagocytosis.²¹⁻²³ Many functions in immune cells are coordinated from lipid rafts in the plasma membrane. Although the contribution of cholesterol-dependent lipid assemblies to this complex is still controversial, it might be the link between the innate immune response and metabolic adjustments.²⁴ Macrophages and Kupffer cells have previously been shown to be mainly responsible for the clearance of trypanosomes. These cells have a high capacity for cholesterol recycling and turnover and the upregulation of genes involved in this pathway might be the critical transcriptional adaptation required for trypanotolerance. Interestingly, two of the Tir QTLs, namely Tir2 and Tir3, are located in chromosomal regions where QTLs for cholesterol absorption and high-density lipoprotein levels are mapped.²⁵⁻²⁹ Although trypanotolerance was significantly associated with the activation of the classical complement cascade and cholesterol and bile acid pathways, no specific pathway seemed to be linked to susceptibility. Failure to control parasitaemia might be owing to the inadequate metabolic adaptation and an overactivation of the immune system rather than specific initiation. In this

context, the immune response might be detrimental for the host but insufficient to clear the parasites.

Our initial kinetics study shows that the infection with *T. congolense* is following a similar course in both susceptible and tolerant strains. Although some studies indicate that the ability to control anaemia is an important factor in progression of the disease,³⁰ we were unable to detect significant differences between the strains caused by trypanosome infection. One explanation might be that anaemia is usually becoming a critical factor in the late stage of the disease and our experiment did not cover these late time points. The choice of time points was somewhat difficult, as the physiological parameters measured at a given stage, can be regarded as the result of the gene expression, which might have occurred minutes, hours or days before. However, the number of differentially expressed genes at a given time point correlated well with the differences in disease status observed in the two strains. The number of differentially expressed genes was lowest at day 7, anticipating the clearance of parasites with no significant difference in parasitaemia between A/J and C57BL/6 at day 10. The most obvious strain differences in both transcriptional level and disease status were observed during the recurrence phase.

It is noteworthy that the gene expression differences seen in the microarray study were consistently confirmed by real-time PCR of selected genes. The RNA samples used in the real-time PCR were from different animals to those used in the microarray experiments. Thus, we were able to confirm not just that microarray assays were accurate but also that the differential expression was reproducible in two independent experiments.

Whether or not the differential expression of genes in uninfected animals is relevant to the outcome of the infection with *T. congolense* remains unknown at this stage. Some of these genes do not show differential expression levels at some stages post infection. Only nine of the differentially expressed genes in uninfected mice remained differentially expressed at all times post infection (Table 1).

This study was a first attempt to integrate transcriptional regulation and QTL mapping in a mouse model of trypanotolerance. Although, we identified three differentially expressed genes that map within 1 cM of one of the Tir loci, the possibility of this occurring by chance is relatively high. Additionally, the number of genes investigated by this microarray study was approximately 7000, which in turn restricted the number of genes that are located within QTL interval that are represented on the microarray. Nevertheless, our results indicate at least one pathway, namely complement activation, is related to differences in resistance.^{15,31} In the case of complement activation, complement component B is situated in close proximity to C3 (Tir1) but is not on the microarray. Our results also substantiate the findings of others, indicating that interferons^{13,32} and arginase¹² are factors associated with susceptibility or resistance. However, the study by Duleu *et al.* suggested that macrophages of the susceptible strain express higher levels of arginase I and II, whereas we found that arginase I was more highly expressed in the tolerant C57BL/6 strain. This difference might be a time point-dependent effect or due to differences between *in vivo* and *ex vivo* analysis. Our data support the hypothesis that it is metabolic pathways and protease activity that should be explored as new anti-parasitic mechanisms.³³ Most importantly, our study strongly suggests for the first time a possible role of chemokines and specific ion channels in the host response to trypanosome infection.

Materials and methods

Animals

All animals were housed in the animal facility of the International Livestock Research Institute (ILRI), Nairobi, Kenya. C57BL/6 and A/J mice were used at 5-8 weeks of age. Animals received food and water *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at ILRI.

Murine model of African trypanosomiasis

T. congolense clone IL 118034 was grown in sub-lethally irradiated Sprague-Dawley rats, and trypanosomes were isolated from infected rat blood by anion exchange column.³⁵ C57BL/6 and A/J mice were infected by intraperitoneal injection of 1×10^4 parasites in 200 μ l of phosphate-buffered saline (pH 8.0) containing 1.5% glucose. Naive animals from each strain were used as controls.

Monitoring the disease progress, parasitaemia and anaemia

The first experiment was designed to define the kinetics of the disease and to compare the main characteristics of parasitaemia and anaemia between A/J and C57BL/6 mice. Each mouse was monitored for parasitaemia and anaemia every second day by collecting 5 μ l of blood from the tail. Additionally, one group of animals per strain ($n = 6$) was killed every second day until day 18 post infection and the liver was snap frozen in liquid nitrogen for subsequent RNA isolation. In the second experiment (microarray study), individuals were randomly checked for parasitaemia. Groups of mice ($n = 6$) were killed at 0, 4, 7, 10 and 17 days post infection, enabling us to group the animals according to five distinct phases of the disease as defined by the first experiment. The stages of disease were: stage 1=non-infected; stage 2=infected, low but increasing parasite counts; stage 3=peak parasitaemia (usually parasite counts 10^7); stage 4=clearance with low parasite counts after the peak; stage 5=recurrence, increasing parasite counts after first peak and first clearance. Again, the liver was snap frozen in liquid nitrogen for subsequent RNA extraction.

Assessment of parasitaemia and anaemia

To determine the animal parasite load, tail blood samples (3 μ l) were collected and diluted 1:200 in Alsevers solution (3 mM citric acid, 114 mM dextrose, 72 mM sodium chloride and 27 mM sodium citrate). Parasitaemia, expressed as number of trypanosomes/ml, was evaluated manually by counting the number of parasites in the blood using a haemocytometer. Anaemia was defined indirectly by measuring the relative haemoglobin concentrations. Samples of 2 μ l blood per animal were collected from the tail and diluted in 150 μ l of distilled water in a 96-well round bottom plate (Costar 3799, Corning Inc., Corning, NY, USA) followed by a 30 min incubation at room temperature. Cell debris was pelleted by centrifugation at 600 *g* for 10 min. A total of 100 μ l of the supernatant was transferred to a new plate and the optical density was measured at 540 nm in an enzyme-linked immunosorbent assay plate reader (Multiscan MCC/340, Titertrek Instruments, Huntsville, AL, USA). Sampling and measurements were carried out in triplicate from each mouse.

RNA isolation

Total RNA from liver was isolated using Trizol Reagent as described previously³⁶

Microarray hybridization

Gene expression differences were assessed in two independent experiments. For the microarray experiment, a pool of liver RNA from groups of five animals per strain and time point was produced to reduce individual variability. Approximately, 60 μ g of total RNA was

reverse transcribed using oligo dT (T₂₃) primer and MMLV II reverse transcriptase (Invitrogen, Paisley, UK). First-strand cDNA was purified using PCR purification columns (Qiagen, Crawley, UK) and eluted in 50 μ l water. From each sample, 20 μ l cDNA was labelled with either Cy3 or Cy5 dye (1 mM) and purified with ProbQuant G-50 microcolumns (Amersham, Little Chalfont, UK). For each hybridization, differently labelled cDNAs from both strains at a given time point were combined and hybridized to Compugen 7000 'known gene' oligo arrays at 45°C overnight. Each hybridization (time point) was conducted in triplicate including one colour swap. Slides were washed in a series of 2 $\times$ SSC, 0.1 $\times$ SSC/0.1% SDS and 0.1 $\times$ SSC. Normalization and data analysis was performed using MaxD software (<http://umber.sbs.man.ac.uk/microarray/maxd/>).

Real-time PCR

Individual RNA samples obtained from an experiment other than the microarray study were used for real-time PCR of a small number of genes showing interesting differences in the microarray assays. Approximately 1 μ g of total RNA was treated with DNaseI (Ambion, Huntingdon, UK) according to the manufacturer's protocol. The reverse transcription reaction was performed with Stratascript Reverse Transcription kit using 200 ng random hexamer primers. Approximately, 100 ng cDNA was used in each PCR reaction with 6 pmol of the gene-specific forward and reverse primers in a total volume of 20 μ l. Reactions were carried out in a Corbett RotorGene thermocycler (Corbett Life Sciences, Cambridge, UK) using Quantitect SYBRgreen PCR mix (Qiagen). PCR reactions were performed in triplicate per individual. Two individuals/strain/group were used and standardized against the beta-actin gene. We used the comparative quantitation ($\Delta\Delta C_T$) to express messenger RNA level differences at each time point between the two strains.

Data analysis

MaxD software was used for the analysis of the microarray data.³⁷ Normalization was performed using standard methodologies.³⁸ For other statistical analysis, we used Prism4 software (GraphPad Inc., San Diego, CA, USA), ANOVA with post tests using the Bonferroni method and test for linear trend. Student's *t*-test was used for two-group comparisons. Data are expressed as mean $\pm$ s.e.m. and *P*<0.05 was considered statistically significant.

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References

1. Torr SJ, Hargrove JW, Vale GA. Towards a rational policy for dealing with tsetse. *Trends Parasitol.* 2005; 21:537–541. [PubMed: 16140579]
2. Mansfield JM, Paulnock DM. Regulation of innate and acquired immunity in African trypanosomiasis. *Parasite Immunol.* 2005; 27:361–371. [PubMed: 16179030]
3. Omamo SW, d'Ieteren GD. Managing animal trypanosomosis in Africa: issues and options. *Rev Sci Tech.* 2003; 22:989–1002. [PubMed: 15005555]
4. Nyame AK, Kwarar ZS, Cummings RD. Antigenic glycans in parasitic infections: implications for vaccines and diagnostics. *Arch Biochem Biophys.* 2004; 426:182–200. [PubMed: 15158669]
5. Hill EW, O'Gorman GM, Agaba M, Gibson JP, Hanotte O, Kemp SJ, et al. Understanding bovine trypanosomiasis and trypanotolerance: the promise of functional genomics. *Vet Immunol Immunopathol.* 2005; 105:247–258. [PubMed: 15808304]

6. Maichomo MW, Ndungu JM, Ngare PM, Ole-Mapenay IM. The performance of Orma Boran and Maasai Zebu cross-breeds in a trypanosomiasis endemic area of Nguruman, south western Kenya. Onderstepoort J Vet Res. 2005; 72:87–93. [PubMed: 15991707]
7. Williams DJ, Taylor K, Newson J, Gichuki B, Naessens J. The role of anti-variable surface glycoprotein antibody responses in bovine trypanotolerance. Parasite Immunol. 1996; 18:209–218. [PubMed: 9223176]
8. Clapcott SJ, Teale AJ, Kemp SJ. Evidence for genomic imprinting of the major QTL controlling susceptibility to trypanosomiasis in mice. Parasite Immunol. 2000; 22:259–263. [PubMed: 10792765]
9. Iraqi F, Clapcott SJ, Kumari P, Haley CS, Kemp SJ, Teale AJ. Fine mapping of trypanosomiasis resistance loci in murine advanced intercross lines. Mamm Genome. 2000; 11:645–648. [PubMed: 10920233]
10. Baetselier PD, Namangala B, Noel W, Brys L, Pays E, Beschin A. Alternative versus classical macrophage activation during experimental African trypanosomiasis. Int J Parasitol. 2001; 31:575–587. [PubMed: 11334945]
11. Buza J, Sileghem M, Gwakisa P, Naessens J. CD5+ B lymphocytes are the main source of antibodies reactive with non-parasite antigens in *Trypanosoma congolense*-infected cattle. Immunology. 1997; 92:226–233. [PubMed: 9415031]
12. Duleu S, Vincendeau P, Courtois P, Semballa S, Lagroye I, Daulouede S, et al. Mouse strain susceptibility to trypanosome infection: an arginase-dependent effect. J Immunol. 2004; 172:6298–6303. [PubMed: 15128819]
13. Hertz CJ, Mansfield JM. IFN-gamma-dependent nitric oxide production is not linked to resistance in experimental African trypanosomiasis. Cell Immunol. 1999; 192:24–32. [PubMed: 10066343]
14. Nakamura Y, Naessens J, Takata M, Taniguchi T, Sekikawa K, Gibson J, et al. Susceptibility of heat shock protein 70.1-deficient C57BL/6 J, wild-type C57BL/6 J and A/J mice to *Trypanosoma congolense* infection. Parasitol Res. 2003; 90:171–174. [PubMed: 12756556]
15. Otesile EB, Lee M, Tabel H. Plasma levels of proteins of the alternative complement pathway in inbred mice that differ in resistance to *Trypanosoma congolense* infections. J Parasitol. 1991; 77:958–964. [PubMed: 1838113]
16. Tabel H, Kaushik RS, Uzonna J. Experimental African trypanosomiasis: differences in cytokine and nitric oxide production by macrophages from resistant and susceptible mice. Pathobiology. 1999; 67:273–276. [PubMed: 10725801]
17. Uzonna JE, Kaushik RS, Gordon JR, Tabel H. Immunoregulation in experimental murine *Trypanosoma congolense* infection: anti-IL-10 antibodies reverse trypanosome-mediated suppression of lymphocyte proliferation *in vitro* and moderately prolong the lifespan of genetically susceptible BALB/c mice. Parasite Immunol. 1998; 20:293–302. [PubMed: 9651931]
18. Uzonna JE, Kaushik RS, Gordon JR, Tabel H. Cytokines and antibody responses during *Trypanosoma congolense* infections in two inbred mouse strains that differ in resistance. Parasite Immunol. 1999; 21:57–71. [PubMed: 10101716]
19. Miller CG, Justus DE, Jayaraman S, Kotwal GJ. Severe and prolonged inflammatory response to localized cowpox virus infection in footpads of C5-deficient mice: investigation of the role of host complement in poxvirus pathogenesis. Cell Immunol. 1995; 162:326–332. [PubMed: 7743560]
20. Ooi YM, Colten HR. Genetic defect in secretion of complement C5 in mice. Nature. 1979; 282:207–208. [PubMed: 492335]
21. Joseph SB, Bradley MN, Castrillo A, Bruhn KW, Mak PA, Pei L, et al. LXR-dependent gene expression is important for macrophage survival and the innate immune response. Cell. 2004; 119:299–309. [PubMed: 15479645]
22. Kay JG, Murray RZ, Pagan JK, Stow JL. Cytokine secretion via cholesterol-rich lipid raft-associated SNAREs at the phagocytic cup. J Biol Chem. 2006; 281:11949–11954. [PubMed: 16513632]
23. Manley PN, Ancsin JB, Kisilevsky R. Rapid recycling of cholesterol: the joint biologic role of C-reactive protein and serum amyloid A. Med Hypotheses. 2006; 66:784–792. [PubMed: 16337748]
24. Hancock JF. Lipid rafts: contentious only from simplistic standpoints. Nat Rev Mol Cell Biol. 2006; 7:456–462. [PubMed: 16625153]

25. Machleder D, Ivandic B, Welch C, Castellani L, Reue K, Lusis AJ. Complex genetic control of HDL levels in mice in response to an atherogenic diet. Coordinate regulation of HDL levels and bile acid metabolism. *J Clin Invest.* 1997; 99:1406–1419. [PubMed: 9077551]
26. Schwarz M, Davis DL, Vick BR, Russell DW. Genetic analysis of intestinal cholesterol absorption in inbred mice. *J Lipid Res.* 2001; 42:1801–1811. [PubMed: 11714849]
27. Suto J, Takahashi Y, Sekikawa K. Quantitative trait locus analysis of plasma cholesterol and triglyceride levels in C57BL/6J × RR F2 mice. *Biochem Genet.* 2004; 42:347–363. [PubMed: 15524312]
28. Wang X, Le Roy I, Nicodeme E, Li R, Wagner R, Petros C, et al. Using advanced intercross lines for high-resolution mapping of HDL cholesterol quantitative trait loci. *Genome Res.* 2003; 13:1654–1664. [PubMed: 12805272]
29. Wittenburg H, Lyons MA, Li R, Kurtz U, Wang X, Mossner J, et al. QTL mapping for genetic determinants of lipoprotein cholesterol levels in independent and combined crosses of inbred mouse strains. *J Lipid Res.* 2006; 47:1780–1790. [PubMed: 16685081]
30. Naessens J, Kitani H, Nakamura Y, Yagi Y, Sekikawa K, Iraqi F. TNF-alpha mediates the development of anaemia in a murine *Trypanosoma brucei* rhodesiense infection, but not the anaemia associated with a murine *Trypanosoma congolense* infection. *Clin Exp Immunol.* 2005; 139:405–410. [PubMed: 15730385]
31. Authie E, Pobel T. Serum haemolytic complement activity and C3 levels in bovine trypanosomiasis under natural conditions of challenge - early indications of individual susceptibility to disease. *Vet Parasitol.* 1990; 35:43–59. [PubMed: 2343531]
32. Shi M, Pan W, Tabel H. Experimental African trypanosomiasis: IFN-gamma mediates early mortality. *Eur J Immunol.* 2003; 33:108–118. [PubMed: 12594839]
33. Lakhdar-Ghazal F, Blonski C, Willson M, Michels P, Perie J. Glycolysis and proteases as targets for the design of new anti-trypanosome drugs. *Curr Top Med Chem.* 2002; 2:439–456. [PubMed: 11966466]
34. Nantulya VM, Musoke AJ, Rurangirwa FR, Barbet AF, Ngaira JM, Katende JM. Immune depression in African trypanosomiasis: the role of antigenic competition. *Clin Exp Immunol.* 1982; 47:234–242. [PubMed: 7075022]
35. Lanham SM. Separation of trypanosomes from the blood of infected rats and mice by anion-exchangers. *Nature.* 1968; 218:1273–1274. [PubMed: 5656665]
36. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987; 162:156–159. [PubMed: 2440339]
37. Hancock D, Wilson M, Velarde G, Morrison N, Hayes A, Hulme H, et al. maxdLoad2 and maxdBrowse: standards-compliant tools for microarray experimental annotation, data management and dissemination. *BMC Bioinformatics.* 2005; 6:264. [PubMed: 16269077]
38. Fang Y, Brass A, Hoyle DC, Hayes A, Bashein A, Oliver SG, et al. A model-based analysis of microarray experimental error and normalisation. *Nucleic Acids Res.* 2003; 31:e96. [PubMed: 12907748]

Figure 1.

Kinetics of *T. congolense* infection in A/J and C57BL/6 mice. Mice of the susceptible A/J and the resistant C57BL/6 strain were infected with *T. congolense* by i.p. injection of 1×10^4 parasites. Tail blood was collected every other day from each individual. (a) Parasites were counted from 3 μ l of blood, diluted 1:200 in Alsevers solution, under the microscope using a haemocytometer. Average parasite counts are expressed as number of parasites per ml. A/J mice had significantly higher parasite counts at day 8 (*t*-test, $P < 0.0001$), but both strains were able to reduce their trypanosome load by day 10 at which stage there was no significant difference between the two strains. However, the parasite numbers were again significantly higher in A/J mice at all following time points (two-way ANOVA, $P < 0.0001$). (b) To determine blood haemoglobin content, an additional 2 μ l of blood was collected and diluted in 150 μ l distilled water. After lysis of erythrocytes and removal of the cell debris, supernatants were transferred into 96-well plates, and optical density at 540 nm was determined using an enzyme-linked immunosorbent assay plate reader. Sampling and measurements were carried out in triplicate for each mouse. A/J and C57BL/6 mice had

significantly different levels of haemoglobin (two-way ANOVA, $P<0.0001$) and maintained this difference throughout the study period. *Indicates statistically significant difference.

Figure 2.

Scatter plots illustrating gene expression differences between A/J and C57BL/6 mice. From each strain, groups of mice ($n = 5$) were killed at days 0, 4, 7, 10 and 17 and liver tissue was snap frozen in liquid nitrogen. Total RNA from liver was extracted and pools of RNA consisting of five individuals/time point/strain were reverse transcribed and labelled with either Cy3 or Cy5 dyes. Differentially labelled cDNAs from each strain were hybridized onto 7000 gene oligo arrays in three replicates/time point. Hybridization signal were analysed using MaxD microarray analysis software. (a) Scatter plot of A/J vs C57BL/6 ratios on days 0 and 4 with every dot representing an individual gene. Among others, genes labelled Apcs, Pbp and abhydrolase domain containing 1 (Abhd1) were higher expressed in A/J mice (ratio >0) as compared to C57BL/6 mice. *Vice versa*, as examples of genes that were higher expressed in C57BL/6 mice (ratio <0) nicotinamide *N*-methyltransferase (Nnmt), major urinary protein 4 (Mup4), haemolytic complement (Hc) and Bcl2-interacting killer-like (Biklk) genes are labelled. (b) Scatter plot of A/J vs B57BL/6 ratios on days 4 and 7. Genes such as cytochrome *P*450, family member 4 (Cyp4a14), carbonic anhydrase (Car3) and catalytic glucose-6-phosphatase (G6pc) are upregulated in A/J, genes labelled adipose differentiation related protein (Adfp), presenilin 2 (Psen2), structural *p*-lysozyme (Lzp-s) and steaoryl-coenzyme A desaturase 1 (Scd1) are upregulated in C57BL/6 mice. (c) Scatter plot of A/J vs C57BL/6 ratios on days 7 and 10. More higher expressed genes were (among others) interferon alpha/beta receptor 1 (Ifnar1), chemokine (C-X-C motif) ligand 13 (Cxcl13), chemokine (C-C motif) ligands 8 and 6 in A/J and chemokine (C-X-C motif) ligand 9, interferon-activated gene 203 (Ifi203) and bone marrow stromal cell antigen 1 (Bst1) in C57BL/6. (d) Scatter plot of A/J vs C57BL/6 ratios on days 10 and 17. Serum amyloid A 2 (Saa2), Apo A4, K inward rectifying channel family J 10 (Kcnj10), chemokine (C-C motif) ligand 24 and orosomucoid 1 (Orm1) appeared higher expressed in the A/J, whereas selen

binding protein 2(Selenbp2), serin/threonin kinase 25 (Stk25) and glutathione *S*-transferase alpha 2 (Gsta2) were higher expressed in the C57Bl/6 strain.

Figure 3.
Hierarchical cluster of mean gene expression levels. Single-linkage hierarchical cluster analysis of the genes upregulated in A/J (red) or C57BL/6 mice (green) over each of the time points was performed using the MaxD software.

Figure 4.

Cluster analysis revealing strain-specific expression patterns. Single-linkage hierarchical cluster analysis of the genes upregulated in A/J (red) or C57BL/6 mice (green) over each of the time points was performed using the MaxD software.

Table 1
Genes that were two-fold up-regulated in A/J (top part) and C57BL/6 (bottom part, next page) at day 0

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
NM_011318	*	Apes	1	94.2	
NM_021304	*	Abhd1	5	83	Biological_process unknown
NM_018858	*	Pbp	5	63	
NM_007822	*	Cyp4a14	4	49.5	Electron transport
NM_016981		Slc9a1	4	64.6	Transport; regulation of pH; sodium ion transport
NM_007846		Defers			
NM_015762		Thioredoxin reductase 1	10	72	Electron transport; thioredoxin pathway
NM_008766		Slc22a6	19	0	Transport; ion transport; anion transport
NM_019545		Hao3	3	110	Electron transport; protein targeting
X81439					
NM_007598		Cap1	4	82.5	Cellular morphogenesis
NM_009286		Sth2			Steroid metabolism
M19226					
NM_010585					
NM_010091		Dvl1	4	82	Development; intracellular signalling cascade
NM_011696		Vdac3	8	8	Behavioural fear response; learning; anion transport
NM_008093		Gata5	2	106	Regulation of transcription, DNA-dependent
NM_011338		Ccl9	11	47.4	Signal transduction; chemotaxis; immune response
NM_009688		Birc4	X	55	Apoptosis; antiapoptosis
NM_008648	*	Mup4	4	27.8	Transport
NM_011183	*	Presenilin 2	1		Intracellular signalling cascade
NM_010406	*	Hc	2	23.5	Cytolysis; inflamm response; complement activation
NM_019414	*	Selenbp2	3	50.8	
NM_016978	*	Oat	7	63	Amino-acid metabolism
M27608		Mup1			Transport; immediate hypersensitivity response
NM_010924		Nnmt	9	29	
NM_013541		Gstp2	19	0	Glutathione conjugation reaction
NM_008293		Hsd3b1	3	49.1	C21-steroid hormone biosynthesis
NM_016974		Dbp	7	23	Circadian rhythm; regulation of transcription

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
AF221095	Ndst3	N-deacetylase/N-sulphotransferase	3	110	
NM_007546	Biklk	Bcl2-interacting killer-like	15	64	
NM_021489	F12	Coagulation factor XII (Hageman factor)	13	60	Proteolysis and peptidolysis
NM_007819	Cyp3a13	Cytochrome P450, family 3, subfamily a	5	83	Electron transport
X71479	Cyp4a12	Cytochrome P450, family 4, subfamily x	4	49.5	Electron transport
NM_007482	Arg1	Arginase 1, liver	10	72	Urea cycle; arginine metabolism; arginine catabolism
NM_008182	Gsta2	Glutathione S-transferase, alpha 2 (Yc2)	9	44	
NM_007703	Elovl3	Elongation of very long fatty acids-like 4	19	47	Fatty acid biosynthesis
NM_019455	Ptgsd2	Prostaglandin D2 synthase 2, haematopoietic	6	88	Prostaglandin metabolism; prostaglandin biosynthesis
X05475	C9	Complement component 9	15	64	Cytolysis; complement activation
AB041540	Tm4sf10	Transmembrane 4 superfamily member 13	X	55	
M92417	Stfa1	Stefin A1			
NM_011765	Zfp97	Zinc finger protein 97			
NM_009127	Scd1	Stearoyl-coenzyme A desaturase 1	19	43	Fatty acid biosynthesis
NM_011979	Vnn3	Vanin 3	10	72	Nitrogen metabolism
AF187099	Cml5	Camello-like 5	6	88	Negative regulation of cell adhesion
U27014	Sdh1	Sorbitol dehydrogenase 1	2	66	
NM_015729	Acox1	Acyl-coenzyme A oxidase 1, palmitoyl	11	77	Electron transport; fatty acid metabolism
NM_018853	Rplp1	Ribosomal protein, large, P1	8	72.4	Protein biosynthesis; translational elongation
NM_011433	Soat2	Sterol O-acyltransferase 2	15	61.7	Metabolism
NM_010401	Hal	Histidine ammonia lyase	10	51	Histidine metabolism; his catabolism
NM_010288	Gja1	Gap junction membrane protein epsilon 1	10	29	Cell-cell signalling; regulation of heart rate
AB028071	Keg1	Kidney expressed gene 1	19	56	
NM_009581	Zp3r	Zona pellucida 3 receptor	1	67	Fertilization; binding of sperm to zona pellucida
NM_009732	Avp	Arginine vasopressin	2	73.2	Regulation of blood pressure
NM_011125	Pltp	Phospholipid transfer protein	2	93	Lipid transport
NM_013593	Mb	Myoglobin	15	43.3	Transport; oxygen transport
NM_019639					
AF290877	Wasf1	WASP family 1	10	25	Cell motility; cellular morphogenesis;
NM_008843	Pip	Prolactin-induced protein	6	88	
NM_021537	Stk25	Serine/threonine kinase 25	1	58	Protein amino-acid phosphorylation

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
NM_013806	Abcc2	ATP-binding cassette, sub-family C	19	43	Transport
NM_013797	Slc21a1	Solute carrier family 21	6	88	Transport; ion transport; organic anion transport
M59912	Kitl	Kit ligand	10	57	Cell adhesion; germ-cell development
NM_013917	Ptg1	Pituitary tumour-transforming 1	11	77	Cell growth and/or maintenance; mitosis; DNA repair

Table 2
Genes that were 2-fold up-regulated in A/J (top part) and C57BL/6 (bottom part) at day 4

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
NM_008061	◇ G6pc	Glucose-6-phosphatase, catalytic	11	77	Glycogen biosynthesis
NM_007834	◇ Dscr3	Down syndrome critical region gene 3	16	63	Intracellular protein transport
NM_007606	◇ Car3	Carbonic anhydrase 3	3	11.7	One-carbon compound metabolism
NM_019545	◇ Hao3	Hydroxyacid oxidase (glycolate oxidase) 3	3	110	Electron transport; protein targeting
NM_007618	◇ Serpina6	Serine (or cysteine) proteinase inhibitor	12	51	Transport
NM_011318	Apes	Serum amyloid P-component	1	94.2	
NM_021304	Abhd1	Abhydrolase domain containing 1	5	83	Biological process unknown
NM_018858	Pbp	Phosphatidylethanolamine binding protein	5	63	
NM_008766	Slc22a6	Solute carrier family 22	19	0	Transport; ion transport; anion transport
NM_007846	Defers	Defensin-related cryptidin, related sequence			
NM_007822	Cyp4a14	Cytochrome P450, family 4	4	49.5	Electron transport
NM_009376	TgN737Rpw	Transgene 737, polycystic kidney disease	14	21	Ant/post pattern formation; digit morphogenesis
AB049623	0610025L15Rik	RIKEN cDNA 0610025L15 gene	7	74.5	
NM_007559	Bmp8b	Bone morphogenetic protein 8b	4	57.4	Spermatogenesis; TGFbeta receptor signalling
NM_009155	Sepp1	Selenoprotein P, plasma, 1			Biological process unknown
AF248643	Actn2	Actinin alpha 2	13	7	Muscle contraction
NM_007408	# Adip	Adipose differentiation-related protein	4	38.9	Biological process unknown
AF304118	# Ptdsr	Phosphatidylserine receptor	11	75	Cell surface receptor signal transduc; apoptosis
NM_019578	# Extl1	Exostosins (multiple)-like 1	4	60	Cell growth and/or maintenance
NM_007419	# Adrb1	Adrenergic receptor, beta 1	19	51	G-protein-coupled receptor protein signalling
NM_009688	# Birc4	Baculoviral IAP repeat-containing 4	X	55	Apoptosis; antiapoptosis
NM_013590	# Lzp-s	P lysozyme structural			Carbohydrate metabolism; cell wall catabolism
NM_010496	# Idb2	Inhibitor of DNA binding 2	12	7	Development; lymph gland development
M16355	Mup1	Major urinary protein 1			Transport; immediate hypersensitivity response
NM_010924	Nmnt	Nicotinamide N-methyltransferase	9	29	Transport; immediate hypersensitivity response
M27608	Mup1	Major urinary protein 1			
AF221095	Ndst3	N-deacetylase/N-sulphotransferase	3	110	Intracellular signalling cascade
NM_011183	Psen2	Presenilin 2			
NM_008648	Mup4	Major urinary protein 4	4	27.8	Transport

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
NM_010406	Hc	Haemolytic complement	2	23.5	Cytolysis; inflammm respon; complement activation
NM_007825	Cyp7b1	Cytochrome <i>P450</i> , family 7	3	1	Electron transport; cholesterol metabolism
NM_010401	Hal	Histidine ammonia lyase	10	51	Biosynthesis; his metabolism; his catabolism
X05475	C9	Complement component 9	15	64	Cytolysis; complement activation
NM_013541	Gsp2	Glutathione <i>S</i> -transferase, pi 2	19	0	Glutathione conjugation reaction
NM_009127	Scd1	Stearoyl-coenzyme A desaturase 1	19	43	Fatty acid biosynthesis
NM_007819	Cyp3a13	Cytochrome <i>P450</i> , family 3	5	83	Electron transport
NM_008293	Hsd3b1	Hydroxysteroid dehydrogenase	3	49.1	Steroid biosynthesis
NM_007546	Biklk	Bcl2-interacting killer-like	15	64	
NM_019639					
NM_016978	Oat	Ornithine aminotransferase	7	63	Amino acid metabolism
NM_021537	Stk25	Serine/threonine kinase 25 (yeast)	1	58	Protein amino-acid phosphorylation
NM_019414	Selenbp2	Selenium binding protein 2	3	50.8	
X71479	Cyp4a12	Cytochrome <i>P450</i> , family 4	4	49.5	Electron transport
NM_018853	Rplp1	Ribosomal protein, large, P1	8	72.4	Protein biosynthesis; translational elongation
NM_008843	Pip	Prolactin-induced protein	6	88	
NM_009578	Zafn1a1	Zinc finger protein (Ikaros)	11	6	Regulation of transcription, DNA-dependent
NM_020564	pending	Sulphotransferase-related gene XI	8		
NM_008496	Lgals7	Lectin, galactose binding, soluble 7	7	74.5	
AB041997	Ptges	Prostaglandin E synthase	2	24	Prostaglandin metabolism
NM_013797	Slc21a1	Solute carrier family 21	6	88	Transport; ion transport; transport

Table 3
Genes that were two-fold upregulated in A/J (top part) and C57BL/6 (bottom part) at day 7

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
NM_010508	Ifnar1	Interferon (alpha/beta) receptor 2	16	63.2	
NM_007574	C1qg	Complement component 1 q receptor 1	4	66.1	Complement activation; classical pathway
NM_007468	Apoa4	Apolipoprotein A-IV	9	27	Lipid transport; reg of cholesterol absorption
X15684	Slc2a2	Solute carrier family 2	3	14.4	Transport; carbohydrate transport
AF312938	Atf5	Activating transcription factor 5	7	74.5	Regulation of cell proliferation; antiapoptosis
NM_010699	Ldh1	Lactate dehydrogenase 1, A chain	7	23.5	Glycolysis
AB018421	Cyp4a10	Cytochrome P450, family 4	4	49.5	
NM_016772	Ech1	Enoyl coenzyme A hydratase 1	7	74.5	Metabolism; fatty acid metabolism
NM_008218	Hba-a1	Haemoglobin alpha, adult chain 1	11		Transport; oxygen transport
U63146	Rbp4	Retinol binding protein 4, plasma	19	38	Transport
U85511	Nme1	Expressed in non-metastatic cells 1	11	77	GTP; CTP; UTP biosynthesis
NM_008086	Gas1	Growth arrest specific 1	13	37	Cell cycle arrest
NM_008490	Lcat	Lecithin cholest acyltransferase	8	53	Lipid metabolism
NM_007822	Cyp4a14	Cytochrome P450, family 4	4	49.5	Electron transport
NM_018858	Pbp	Phosphatidylethanolamine bindg prot	5	63	
NM_011318	Apcs	Serum amyloid P-component	1	94.2	
NM_010991	Olfir49	Olfactory receptor 49	14	75	G-protein signalling pathway; olfaction
NM_009286	Stn2	Sulphotransferase, hydroxysteroid 2			Steroid metabolism
NM_007606	Car3	Carbonic anhydrase 3	3	11.7	One-carbon compound metabolism
NM_021304	Abhd1	Abhydrolase domain containing 1	5	83	Biological process unknown
NM_007846	Defcrs12	Defensin-related sequence 7			
AJ401619	2610037M15Rik	RIKEN cDNA 2610037M15 gene	6	88	
NM_021564	Fetub	Fetuin beta	16	63	
NM_011546			1		
NM_008210	H3f3a	H3 histone, family 3A	13	60	
NM_008328	Ifi203	Interferon-activated gene 203	1	95.2	Immune response
NM_009916	§ Cer4	Chemokine (C-C motif) receptor 4	9	61	Chemotaxis; inflammatory response
AF228706	§ Nfat5	Nuclear factor-activated T-cells 5	8	53	Regulation of transcription, DNA-dependent
U79525	§ Cmkrl1	Chemokine-like receptor 1	5	83	Chemotaxis; G-protein signalling pathway

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
NM_008955	§ Pxl1	Placenta specific homeobox 1	X	55	Regulation of transcription, DNA-dependent
NM_018831	§ Dclrela	DNA cross-link repair 1A, homologue	19	56	Nucleotide-excision repair
NM_011183	§ Psen2	Presenilin 2	1		Intracellular signalling cascade
M27608	Mup1	Major urinary protein 1			Transport; immediate hypersensitivity response
NM_010924	Nnmt	Nicotinamide N-methyltransferase	9	29	
NM_008648	Mup4	Major urinary protein 4	4	27.8	Transport
NM_010406	Hc	Haemolytic complement	2	23.5	Cytolysis; inflammm respon; complement activation
AF221095	Ndst3	N-deacetylase/N-sulphotransferase	3	110	
NM_013590	Lzp-s	P lysozyme structural			Carbohydrate metabolism; cell wall catabolism
NM_011979	Vnn3	Vanin 3	10	72	Nitrogen metabolism
AF011413	V2r3	Vomer nasal 2, receptor, 3			
NM_009688	Birc4	Baculoviral IAP repeat-containing 4	X	55	Apoptosis; antiapoptosis
NM_021537	Stk25	Serine/threonine kinase 25 (yeast)	1	58	Protein amino-acid phosphorylation
NM_019518	Grasp	GRP1-associated scaffold protein	15	64	Intracell signal cascade; intracell prot transport
NM_009127	Scd1	Stearyl-coenzyme A desaturase 1	19	43	Fatty acid biosynthesis
L11333	Es31	Esterase 31	8		
NM_019414	Selenbp2	Selenium binding protein 2	3	50.8	
NM_007819	Cyp3a13	Cytochrome P450, family 3	5	83	Electron transport
NM_016978	Oat	Ornithine aminotransferase	7	63	Amino-acid metabolism

Table 4
Genes that were two-fold upregulated in A/J (top part) and C57BL/6 (bottom part) at day 10

Gene Id	Symbol	Gene name	MMU	cM	Biological process	
NM_018866	∞	Cxcl13	Chemokine (C-X-C motif) ligand 13	5	83	Chemotaxis; inflammatory response; immune response
NM_021443	∞	Ccl8	Chemokine (C-C motif) ligand 8	11	47	Signal transduction; chemotaxis; inflammm; immune response
NM_011314	∞	Saa2	Serum amyloid A 2	7	23.5	Acute-phase response
NM_011336	∞	Ccl27	Chemokine (C-C motif) ligand 27			Immune response
NM_009139	∞	Ccl6	Chemokine (C-C motif) ligand 6	11	47.51	Signal transduction; chemotaxis; immune response
NM_010508		Ifnar1	Interferon (alpha/beta) receptor 2	16	63.2	
NM_007468		Apoa4	Apolipoprotein A-IV	9	27	Lipid transport; regulation of cholesterol absorption
NM_011318		Apcs	Serum amyloid P-component	1	94.2	
NM_007598		Cap1	Adenylyl cyclase-assoc CAP prot homologue	4	82.5	Cellular morphogenesis
NM_018858		Pbp	Phosphatidylethanolamine binding prot	5	63	
NM_009221		Snea	Synuclein, alpha	6	29	
X62705				1		
NM_011414		Slpi	Secretory leucocyte protease inhibitor	2	106.6	
NM_008509		Lpl	Lipoprotein lipase	8	33	Lipid metabolism; lipid transport; lipid catabolism
NM_019659		Kcnj1	K inwardly-rectifying channel, subf J	9	78.8	Ion transport; potassium ion transport
AJ007909				1		
NM_021304		Abhd1	Abhydrolase domain containing 1	5	83	Biological_process unknown
NM_009997	◇	Cyp2a4	Cytochrome P450, family 2, subfamily a			Electron transport
NM_013806	◇	Abcc2	ATP-binding cassette, sub-family C	19	43	Transport
NM_007482	◇	Arg1	Arginase 1, liver	10	72	Urea cycle; arginine metabolism; arginine catabolism
NM_011433	◇	Soat2	Sterol O-acyltransferase 2	15	61.7	Protein amino-acid prenylation; cholesterol metabolism
NM_008030	◇	Fmo3	Flavin containing monooxygenase 3			Electron transport
NM_007494	◇	Ass1	Argininosuccinate synthetase 1	2		Urea cycle; arginine biosynthesis
NM_008777	◇	Pah	Phenylalanine hydroxylase	10	47	Metabolism; phenylalanine catabolism
NM_007409	◇	Adh1	Alcohol dehydrogenase 1	3	71.2	Retinoid metabolism
NM_008761	◇	Fxyd5	FXYD domain-cont ion transport regul 7	7	74.5	Ion transport
NM_019552	◇	Abcb10	ATP-binding cassette, sub-family B	8	72.4	Transport
M62766	◇	Hmgcr	3-hydroxy-3-methylglutaryl-Co A synth 2	13	49	Biosynthesis; cholesterol biosynthesis
NM_015760	◇	Nox4	NADPH oxidase 4	7	74.5	Electron transport; bone resorption; superoxide release

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
NM_007448	◇	Angiogenin-like			Angiogenesis
NM_013593	◇	Myoglobin	15	43.3	Transport; oxygen transport
AF187099		Camello-like 5	6	88	Negative regulation of cell adhesion
NM_009763		Bst1	5	25	Defense response
NM_008328		Interferon activated gene 203	1	95.2	Immune response
NM_013653		Chemokine (C-C motif) ligand 5	11	47.4	Signal transduction; chemotaxis; inflammm; immune response
NM_009735		Beta-2 microglobulin	2	69	Defense response; endogenous antigen via MHC class I
NM_008599		Chemokine (C-X-C motif) ligand 9	5	53	Inflammatory response; immune response
NM_021489	∴	Coagulation factor XII (Hageman factor)	13	60	Proteolysis and peptidolysis
NM_009258	∴	Serine protease inhibitor, Kazal type 4	18	56	
NM_008941	∴	Protease, serine, 7 (enterokinase)	16	63	Proteolysis and peptidolysis
NM_008607	∴	Matrix metalloproteinase 13	9	78.8	Proteolysis and peptidolysis; collagen catabolism
M27608		Major urinary protein 1			Transport; immediate hypersensitivity response
NM_009688		Baculoviral IAP repeat-containing 4	X	55	Apoptosis; antiapoptosis
NM_008182		Glutathione S-transferase, alpha 2	9	44	
NM_010406		Haemolytic complement	2	23.5	Cytolysis; inflammatory response; complement activation
NM_007819		Cytochrome P450, family 3, subfamily a	5	83	Electron transport
NM_010924		Nicotinamide N-methyltransferase	9	29	
NM_016978		Omitine aminotransferase	7	63	Amino-acid metabolism
NM_015729		Acyl-coenzyme A oxidase 1, palmitoyl	11	77	Electron transport; fatty acid metabolism; spermatogenesis
NM_018853		Ribosomal protein, large, P1	8	72.4	Protein biosynthesis; translational elongation;
L11333		Esterase 31	8		
NM_011183		Presenilin 2	1		Intracellular signalling cascade
NM_012058		Signal recognition particle 9			
NM_019414		Selenium binding protein 2	3	50.8	
AF221095		N-deacetylase/N-sulphotransferase	3	110	
NM_008648		Major urinary protein 4	4	27.8	Transport
NM_009581		Zona pellucida 3 receptor	1	67	Fertilization; binding of sperm to zona pellucida
NM_021537		Serine/threonine kinase 25 (yeast)	1	58	Protein amino-acid phosphorylation
U27014		Sorbitol dehydrogenase 1	2	66	
NM_007546		Bcl2-interacting killer-like	15	64	

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
NM_009349	Temt	Thioether S-methyltransferase	6	88	
NM_010401	Hal	Histidine ammonia lyase	10	51	Biosynthesis; histidine metabolism; histidine catabolism
NM_009203	Slc22a2	Solute carrier family 22-like 2	19	56	Transport
NM_009578	Znfn1a1	Zinc finger protein (Ikaros)	11	6	Regulation of transcription, DNA-dependent
NM_011393	Slc1a2	Solute carrier family 1, member 2	2	54	Transport; dicarboxylic acid transport
NM_021273	Ckb	Creatine kinase, brain	16	55	
NM_019639					
U24680					

Table 5
Genes that were two-fold upregulated in A/J (top part) and C57BL/6 (bottom part, next page) at day 17

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
X14607	◇	Len2	2	27	Transport
Z71189	◇	Acadvl	11	38	Electron transport; fatty acid metabolism
X03766	◇	Actl			Muscle contraction; cytoskeleton org and biogenesis
NM_009171	◇	Shmt1	11	77	One-carbon compound; amino-acid metabolism
L02331	◇	Sult1a1	7	4	Steroid metabolism
NM_011868	◇	Peci	13	60	Metabolism; peroxisome organization and biogenesis
NM_010763	◇	Man1b	3	110	Carbohydrate metabolism; N-linked glycosylation
AF218416	◇	Ttpa	4	82.5	Transport; embryo implantation; vitamin E metabolism
NM_019573	◇	Wwox	8	72.4	Metabolism; induction of apoptosis
NM_011044	◇	Pck1	2	103	Gluconeogenesis
NM_018829	◇	Ap3m1	14	2.5	Intracellular protein transport; protein-lysosome targeting
NM_009286	◇	Sth2			Steroid metabolism
NM_008489	◇	Lbp	2	83	Xenobiotic metabolism; lipid transport
NM_008768	◇∞	Orn1	4	31.4	Transport; acute-phase response
U9889	◇∞	Hpxn	7	74.5	Transport; acute-phase response
NM_019577	∞	Ccl24	5	83	Chemotaxis; inflammatory response; immune response
NM_018866	∞	Cxcl13	5	83	Chemotaxis; inflammatory response; immune response
NM_010392	∞	H2-Q2	-1		Defense response
NM_011338	∞	Ccl9	11	47.4	Signal transduction; chemotaxis; immune response
NM_007827	∞	Daf2	1	67.6	Complement activation, classical pathway
NM_011316	∞	Saa4	7	23.5	Acute-phase response
NM_021443	∞	Ccl8	11	47	Signal transduc; chemotaxis; inflammi; immune response
NM_010740	∞	C1qr1	2	84	Cell adhesion; defense response
NM_007643	∞	Cd36	5	2	Cell adhesion; transport
NM_011579	∞	Tgtp	11	77	
NM_010766	∞	Marco			
NM_009921	∞	Camp	9	61	Xenobiotic metabolism; defense response
M83218	∞	S100a8	3	43.6	Chemotaxis
NM_019948	∞	Clecst9	6	59.6	

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
K02782	∞	Complement component 3	17	34.3	Inflammatory response; complement activation
NM_017370	∞∞	Haptoglobin	8	55	Acute-phase response; proteolysis and peptidolysis
NM_010517	∞∞	Igfbp4	11	77	Regulation of cell growth
NM_008176	∞∞	Chemokine (C-X-C motif) ligand 1	5	51	Cell growth/maintenance; inflammm; immune response
NM_009984	∞	Cathepsin L	13	30	Proteolysis and peptidolysis
NM_021445	∞	Cathepsin 6	13	35.5	Proteolysis and peptidolysis
NM_019447	∞	Hepatocyte growth factor activator	5	83	Proteolysis and peptidolysis
NM_020269	‡	K inwardly-rectifying channel, subfam J	1	93.5	Ion transport; potassium ion transport
AF020711	‡	K large conductance Ca-activ channel	11	77	Potassium ion transport
NM_019659	‡	K inwardly-rectifying channel, subfam J	9	78.8	Ion transport; potassium ion transport
NM_007502	‡	ATPase, Na+/K+ trans, beta 3 polypeptide	9	51	Potassium ion transport; sodium ion transport
NM_010656	&	Sarcospan	6	71.55	Cell growth/maintenance; regulation of cell cycle
NM_011318		Serum amyloid P-component	1	94.2	
NM_010508		Interferon (alpha and beta) receptor 2	16	63.2	
NM_007598		Adenyl cyclase-assoc CAP prot homologue	4	82.5	Cellular morphogenesis
NM_021304		Abhydrolase domain containing 1	5	83	Biological_process unknown
NM_011314		Serum amyloid A 2	7	23.5	Acute-phase response
NM_011336		Chemokine (C-C motif) ligand 27			Immune response
NM_007846		Defensin-related sequence 7			
NM_018858	Pbp	Phosphatidylethanolamine binding protein	5	63	
NM_007752	Cp	Ceruloplasmin	3	55	
NM_009221	Snca	Synuclein, alpha	6	29	
AB018421	Cyp4a10	Cytochrome P450, family 4	4	49.5	
NM_010442	Hmxo1	Heme oxygenase (decycling) 1	8	35	Heme oxidation
NM_007822	Cyp4a14	Cytochrome P450, family 4	4	49.5	Electron transport
NM_018777	Cldn6	Claudin 6	17	71.3	
AF290877	Wasf1	WASP family 1	10	25	Cell motility; protein complex assembly; cell morphogen
NM_021398	pending	Embryonic epithelial gene 1	2	106.6	
NM_016974	Dbp	D site albumin promoter binding protein	7	23	Circadian rhythm; regulation of transcription
NM_010091	Dvl1	Dishevelled, dsh homolog 1 (Drosophila)	4	82	Development; Wnt receptor signalling pathway
NM_011620	Tnnt3	Troponin T3, skeletal, fast	7	69	Regulation of muscle contraction; muscle development

<i>Gene Id</i>	<i>Symbol</i>	<i>Gene name</i>	<i>MMU</i>	<i>cM</i>	<i>Biological process</i>
NM_007468	Apoa4	Apolipoprotein A-IV	9	27	Lipid transport; regulation of cholesterol absorption
NM_015779					
NM_009376	TgN737Rpw	Transgene inser737, polycystic kidney dis	14	21	Ant/post pattern formation; digit morphogenesis
NM_009747	Bdkrb2	Bradykinin receptor, beta 2	12	53	G-protein coupled receptor protein signalling pathway
NM_013933	Vapa	Vesicle-associated membrane protein	17	71.3	Biological_process unknown; cell motility
NM_019744	Ncoa4	Nuclear receptor coactivator 4	14	75	Biological_process unknown
NM_009114	S100a9	S100 calcium binding protein A9	3	43.6	
NM_009175	Siat1	Sialyltransferase 1	16	15.5	Protein amino-acid glycosylation
NM_007411	Adk	Adenosine kinase	14	75	Purine salvage
NM_013532	Gp49b	Glycoprotein 49 B	10	32	
M27608	Mup1	Major urinary protein 1			Transport; immediate hypersensitivity response
NM_009688	Birc4	Baculoviral IAP repeat-containing 4	X	55	Apoptosis; antiapoptosis
NM_008648	Mup4	Major urinary protein 4	4	27.8	Transport
NM_010406	Hc	Haemolytic complement	2	23.5	Cytolysis; inflammatory response; complement activation
NM_011183	Psen2	Presenilin 2			Intracellular signalling cascade
D00232					
L11333	Es31	Esterase 31	8		
NM_011341	Sdf4	Stromal cell-derived factor 4	4	82.5	
NM_007546	Bcllk	Bcl2-interacting killer-like	15	64	
NM_021537	Stk25	Serine/threonine kinase 25 (yeast)	1	58	Protein phosphorylation
NM_013653	Ccl5	Chemokine (C-C motif) ligand 5	11	47.4	Signal transduc; chemotaxis; inflammm; immune response
AF221095	Ndst3	N-deacetylase/N-sulphotransferase	3	110	
AF246218	Shkbp1	Sh3kbp1 binding protein 1	7	74.5	Biological_process unknown
NM_010924	Nnmt	Nicotinamide N-methyltransferase	9	29	
NM_007606	Car3	Carbonic anhydrase 3	3	11.7	One-carbon compound metabolism
NM_008182	Gsta2	Glutathione S-transferase, alpha 2 (Yc2)	9	44	
NM_019414	Selenbp2	Selenium binding protein 2	3	50.8	
AJ290944	Usmg2	Upreg during skeletal muscle growth			
M83538					
AF022856	Nrp2	Neuropilin 2			Cell adhesion
NM_007925	Eln	Elastin	5	75	