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The white-footed deermouse, an infection-tolerant reservoir for several zoonotic agents, tempers interferon responses to endotoxin in comparison to the mouse and rat

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Abstract

The white-footed deermouse *Peromyscus leucopus*, a long-lived rodent, is a key reservoir for agents of several zoonoses, including Lyme disease. While persistently infected, this deermouse is without apparent disability or diminished fitness. For a model for inflammation elicited by various pathogens, the endotoxin lipopolysaccharide (LPS) was used to compare genome-wide transcription in blood by *P. leucopus*, *Mus musculus* and *Rattus norvegicus* and adjusted for white cell concentrations. Deermice were distinguished from the mice and rats by LPS response profiles consistent with non-classical monocytes and alternatively-activated macrophages. LPS-treated *P. leucopus*, in contrast to mice and rats, also displayed little transcription of interferon-gamma and lower magnitude fold-changes in type 1 interferon-stimulated genes. This was associated with comparatively reduced transcription of endogenous retrovirus sequences and cytoplasmic pattern recognition receptors in the deermice. The results reveal a mechanism for infection tolerance in this species and perhaps other animal reservoirs for agents of human disease.

eLife assessment

This study provides a comprehensive whole genome transcriptomic analysis of three small mammals, including Peromyscus leucopus, after exposure to endotoxin lipopolysaccharide. The authors find that the inflammatory response of the three species is complex and that P. leucopus responds differently compared to mice and rats. The data are **convincing** and constitute an **important** advance in our understanding of inflammatory responses in animals that serve as reservoirs for relevant pathogens.

Introduction

How does the white-footed deermouse *Peromyscus leucopus* continue to thrive while sustaining infections with disease agents it serves as reservoir for [4]? The diverse tickborne pathogens (and diseases) for humans include the extracellular bacterium *Borreliella burgdorferi* (Lyme disease), the intracellular bacterium *Anaplasma phagocytophilum* (anaplasmosis), the protozoan *Babesia microti* (babesiosis), and the Powassan flavivirus (viral encephalitis). Most deermice remain persistently infected but display scant inflammation in affected tissues[23, 52, 64], and without apparent consequence for fitness [83, 92].

A related question—conceivably with the same answer--is what accounts for the two-to-three fold longer life span for *P. leucopus* than for the house mouse, *Mus musculus* [48, 79]? The abundance of *P. leucopus* across much of North America [34, 66] and its adaptation to a variety of environments, including urban areas and toxic waste sites [11, 50, 67], indicates successful adjustment to changing landscapes and climate. *Peromyscus* species, including the hantavirus reservoir *P. maniculatus* [65], are more closely related to hamsters and voles in family Cricetidae than to mice and rats of family Muridae [15].

As a species native to North America, *P. leucopus* is an advantageous alternative to the Eurasian-origin house mouse for study of natural variation in populations that are readily accessible [9, 53]. A disadvantage for the study of any *Peromyscus* species is the limited reagents and genetic tools of the sorts that are applied for mouse studies. As an alternative, we study *P. leucopus* with a non-reductionist approach that is comparative in design and agnostic in assumptions [3]. The genome-wide expression comparison for *P. leucopus* is with *M. musculus* and, added here, the brown rat *Rattus norvegicus*. Given the wide range of pathogenic microbes that deermice tolerate, we use the bacterial endotoxin lipopolysaccharide (LPS) as the primary experimental treatment because the inflammation it elicits within a few hours has features common to different kinds of serious infections, not to mention severe burns and critical injuries [96].

We previously reported that a few hours after injection of LPS, *P. leucopus* and *M. musculus* had distinguishing profiles of differentially expressed genes (DEG) in the blood, spleen, and liver [3]. In brief, the inflammation phenotype of deermice was consistent with an “alternatively activated” or M2-type macrophage polarization phenotype instead of the expected “classically activated” or M1-type polarization phenotype that was observed for *M. musculus* [69]. The deermice also differed from mice in displaying evidence of greater neutrophil activation and degranulation after LPS exposure. The potentially damaging action from neutrophil proteases and reactive oxygen species appeared to be mitigated in part in *P. leucopus* by proteins like secretory leukocyte protease inhibitor (Slpi) and superoxide dismutase 2 (Sod2).

Here, we first address whether the heightened transcription of neutrophil-associated genes in *P. leucopus* is attributable to differences in numbers of white cells in the blood. To better match for genetic diversity, we substituted outbred *M. musculus* for the inbred BALB/c mouse of the previous study. We retained the experimental protocol of short-term responses to LPS. This main experiment was supplemented by a study of rats under the similar conditions, by an investigation of a different dose of LPS and duration of exposure in another group of deermice, and by analysis of deermice infected with a bacterium lacking LPS. The focus was on the blood of these animals, not only because the distinctions between species in their transcriptional profiles were nearly as numerous for this specimen as for spleen and liver [3], but also because for ecological and immunological studies of natural populations of *Peromyscus* species blood is obtainable from captured-released animals without their sacrifice.

The results inform future studies of *Peromyscus* species, not only with respect to microbial infections and innate immunity, but conceivably also determinants of longevity and resilience in the face of other stressors, such as toxic substances in the environment. The findings pertain as

well to the phenomenon of infection tolerance broadly documented in other reservoirs for human disease agents, such as betacoronaviruses and bats [57]. Less directly, the results provide for insights about maladaptive responses among humans to microbes, from systemic inflammatory response syndrome (SIRS) to post-infection fatigue syndromes.

Results

LPS experiment and hematology studies

Twenty adult animals each for *P. leucopus* and *M. musculus* and equally divided between sexes received by intraperitoneal injection either purified *E. coli* LPS at a dose of 10 μg per g body mass or saline alone (**Table 1**). Within 2 h LPS-treated animals of both species displayed piloerection and sickness behavior, i.e. reduced activity, hunched posture, and huddling. By the experiment's termination at 4 h, 8 of 10 *M. musculus* treated with LPS had tachypnea, while only one of ten LPS-treated *P. leucopus* displayed this sign of the sepsis state ($p = 0.005$).

Within a given species there was little difference between LPS-treated and control animals in values for erythrocytes. But overall the deermice had lower mean (95% confidence interval) hematocrit at 42 $^{(36\text{-}48)}$%, hemoglobin concentration at 13.8 g/dL (12.1-15.5), and mean corpuscular volume for erythrocytes at 49 fL $^{(47\text{-}51)}$ than *M. musculus* with respective values of 56 $^{(51\text{-}62)}$%, 16.1 g/dL (14.6-17.7), and 60 fL $^{(58\text{-}62)}$ ($p < 0.01$). These hematology values for adult CD-1 *M. musculus* and LL stock *P. leucopus* in this study were close to what had been reported for these colony populations [18, 94].

In contrast to red blood cells, the mean numbers of white blood cells in the LPS groups in both species were lower than those of control groups (**Figure 1**). Controls had a mean 4.9 (3.5-6.4) x 10^3 white cells per μl among *M. musculus* and 5.8 (4.2-7.4) x 10^3 white cells per μl among *P. leucopus* (p = 0.41). For the LPS-treated animals the values were 2.1 (1.5-2.7) x 10^3 for mice and 3.1 (0.9-5.4) x 10^3 for deermice ($p = 0.39$). However, there was difference between species among LPS-treated animals in the proportions of neutrophils and lymphocytes in the white cell population. The ratios of neutrophils to lymphocytes were 0.25 (0.14-0.45) and 0.20 (0.13-0.31) for control *M. musculus* and *P. leucopus*, respectively ($p = 0.53$). But under the LPS condition. the neutrophil-to-lymphocyte ratio was 0.18 (0.11-0.28) for mice and 0.64 (0.42-0.97) for deermice ($p = 0.0006$). The regression curves for plots of neutrophils and lymphocytes for LPS-treated and control *P. leucopus* and LPS-treated *M. musculus* had similar slopes, but the *y*-intercept was shifted upwards towards a higher ratio of neutrophils to lymphocytes for blood from the LPS group of deermice. Control group mice and deermice and LPS-treated mice had similar percentages (~5%) of monocytes in their blood; the mean monocyte percentage rose to 10% in LPS treated deermice ($p = 0.12$). Eosinophil percentages tended to be higher in deermice at a mean 3.4 (2.1-4.7)% than mice at 1.2 (0.5-1.9)% under either condition ($p = 0.004$).

In the *P. leucopus* experiment with a 10-fold lower dose of LPS and a 12 hour duration, the mean (95% confidence interval) white blood cell count (x 10^3) at termination 3.5 (2.5-4.5) in controls and 7.9 (6.0-9.7) in the LPS-treated ($p = 0.01$). Even with the higher overall white blood cell count the increase white cells was proportionately higher for neutrophils than for lymphocytes, as was seen in the deermice in the higher dose LPS experiment. The ratio of neutrophils-to-lymphocytes was 0.20 (0.07-0.32) in the controls and 0.38 (0.26-0.50) in the LPS-treated ($p = 0.10$).

The higher neutrophil to lymphocyte ratio in the deermice exposed to LPS was consistent with the greater neutrophil activation noted by transcriptional analysis [3]. But many individual genes that constitute this and related gene ontology (GO) terms had transcription levels in the deermice

Animal	Genus	Sex	Age (d)	Mass (g)	Treatment	Tachypnea	Hct * (%)	MCV *	WBC *	Neutrophils	Lymphocytea	Monocytes	Eosinophils	Neutrophil/ Lymphocyte
MM19	*Mus*	female	149	60.4	control	no	71	62	3800	570	2926	190	114	0.19
MM21	*Mus*	female	149	51.4	control	no	51	59	8600	826	4897	177	0	0.17
MM23	*Mus*	female	149	30.6	control	no	51	.	1500	135	1305	60	0	0.10
MM25	*Mus*	female	149	42.1	control	no	65	61	7900	1106	6557	237	0	0.17
MM27	*Mus*	female	149	39.7	control	no	58	62	3700	962	2257	333	148	0.43
MM1	*Mus*	male	149	50.9	control	no	67	59	3300	726	2376	198	0	0.31
MM17	*Mus*	male	149	40.8	control	no	51	.	7000	4970	2030	0	0	2.45
MM3	*Mus*	male	149	45.8	control	no	58	59	4300	344	3655	301	0	0.09
MM5	*Mus*	male	149	41.2	control	no	60	57	5400	810	4266	324	0	0.19
MM7	*Mus*	male	149	44.1	control	no	47	58	3800	798	2736	266	0	0.29
MM31	*Mus*	female	149	65.5	LPS	yes	53	58	1900	209	1539	114	38	0.14
MM33	*Mus*	female	149	48.4	LPS	yes	62	62	3200	256	2784	96	64	0.09
MM35	*Mus*	female	149	40.5	LPS	yes	49	64	2200	528	1518	88	66	0.35
MM37	*Mus*	female	149	38.5	LPS	no	54	65	1900	152	1634	57	57	0.09
MM39	*Mus*	female	149	40.2	LPS	yes	61	63	600	60	510	12	18	0.12
MM11	*Mus*	male	149	57.4	LPS	yes	59	58	2600	572	1898	104	0	0.30
MM13	*Mus*	male	149	58.5	LPS	yes	66	59	3700	1258	2183	259	0	0.58
MM15	*Mus*	male	149	51.0	LPS	yes	33	56	1800	90	1602	90	0	0.06
MM29	*Mus*	male	149	39.1	LPS	no	53	59	1800	306	1368	72	54	0.22
MM9	*Mus*	male	149	44.1	LPS	yes	58	.	1300	260	858	182	0	0.30
24841	*Peromyscus*	female	162	19.9	control	no	58	48	5100	204	4590	102	204	0.04
24842	*Peromyscus*	female	164	18.3	control	no	47	48	3900	663	3003	117	117	0.22
24843	*Peromyscus*	female	162	20.5	control	no	45	46	4200	942	3150	42	84	0.30
24845	*Peromyscus*	female	161	18.7	control	no	49	48	9100	2002	6916	182	0	0.29
24853	*Peromyscus*	female	160	22.8	control	no	42	.	4800	1008	2880	864	1	0.35
24852	*Peromyscus*	male	162	19.4	control	no	44	46	7100	994	4970	284	852	0.20
24861	*Peromyscus*	male	157	20.8	control	no	28	50	1300	104	1053	91	52	0.10
24863	*Peromyscus*	male	157	17.1	control	no	26	58	7200	720	5904	288	288	0.12
24869	*Peromyscus*	male	143	29.0	control	no	48	52	6000	1260	4260	180	240	0.30
24876	*Peromyscus*	male	142	16.1	control	no	54	48	9700	2716	6208	194	485	0.44
24846	*Peromyscus*	female	162	22.7	LPS	no	23	47	1100	231	718	44	44	0.32
24847	*Peromyscus*	female	162	16.7	LPS	no	39	.	1500	570	675	180	75	0.84
24848	*Peromyscus*	female	166	16.2	LPS	no	43	49	2700	918	1701	27	54	0.54
24850	*Peromyscus*	female	157	19.4	LPS	no	46	.	3300	1551	1683	66	0	0.92
24851	*Peromyscus*	female	161	25.4	LPS	no	24	47	2300	552	1242	437	69	0.44
24855	*Peromyscus*	male	165	27.1	LPS	no	51	51	2100	1281	714	42	63	1.79
24860	*Peromyscus*	male	160	17.8	LPS	yes	42	54	13200	6996	3696	1320	1056	1.89
24865	*Peromyscus*	male	160	16.7	LPS	no	49	46	1800	396	1080	288	0	0.37
24873	*Peromyscus*	male	145	23.2	LPS	no	43	48	2200	550	1430	110	110	0.38
24879	*Peromyscus*	male	145	22.0	LPS	no	40	.	1100	220	538	352	0	0.41

* Abbreviations: Hct, hematocrit; MCV, mean cellular volume of erythrocytes; WBC, white blood cell count

Table 1.

Characteristics (including hematology values) and treatments (with or without LPS) of *Mus musculus* CD-1 and *Peromyscus leucopus* LL stock

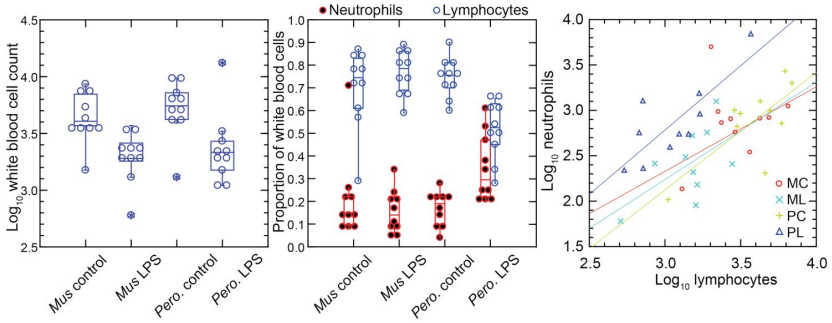

Figure 1.

Total white blood cells, neutrophils, and lymphocytes of *Mus musculus* (M) and *Peromyscus leucopus* (P) with or without (control; C) treatment with 10 µg lipopolysaccharide (LPS; L) per g body mass 4 h previous. The box plots of left and center panels show values of individual animals and compiled median, quartiles, and extreme values. The linear regressions of the right panel are color-coded according to the species and treatment designations. The outlier value for a *M. musculus* control (MM17) was excluded from the linear regression for that group.

that far exceeded a three-fold difference in neutrophil counts. For some genes the differences were a hundred or more fold, which suggested that the distinctive LPS transcriptional response profile between species was not attributable solely to neutrophil counts.

Genome-wide expression in blood of deermice and mice

We used the respective transcript sets from the reference genomes for *P. leucopus* and *M. musculus* for deep coverage RNA-seq with paired-end ~150 nt reads (Dryad Tables D1 and D2). Principle component analyses (PCA) of the *P. leucopus* data and *M. musculus* data revealed that untreated controls had coherent profiles within each species (**Figure 2**). With the exception of one mouse, the LPS-treated *M. musculus* were also in a tight PCA cluster. In contrast, the LPS-treated deermice displayed a diversity of genome-wide transcription profiles and limited clustering.

For both species the number of genes with higher expression with LPS exposure exceeded those with lower or unchanged expression (Dryad Tables D3 and D4). For *P. leucopus* and *M. musculus* the mean fold-changes were 1.32 (1.29-1.35) and 1.30 (1.24-1.36), respectively (p = 0.31). For GO term analysis the absolute fold-change criterion was ≥ 2. Because of the ~3-fold greater number of transcripts for the *M. musculus* reference set than the *P. leucopus* reference set, application of the same false-discovery rate (FDR) threshold for both datasets would favor the labeling of transcripts as DEGs in *P. leucopus*. Accordingly, the FDR p values were arbitrarily set at $<5 \times 10^{-5}$ for *P. leucopus* and $<3 \times 10^{-3}$ for *M. musculus* to provide approximately the same number of DEGs for *P. leucopus* (1154 DEGs) and *M. musculus* (1266 DEGs) for the GO term comparison.

Figure 3 shows the GO terms for the top 20 clusters by ascending p-value for up-regulated and down-regulated in *P. leucopus* and the corresponding categories for *M. musculus*. The up-regulated gene profile for *P. leucopus* featured terms associated with “neutrophil degranulation”, “myeloid leukocyte activation”, “leukocyte migration”, and “response to molecule of bacterial origin”. Other sets of up-regulated genes for the deermice were “negative regulation of cytokine production” and “regulation of reactive oxygen species metabolic process”. None of these were among the top 20 up-regulated clusters for *M. musculus*. Indeed, “leukocyte activation” and “leukocyte migration” were GO terms for down-regulated DEGs in *M. musculus*. Distinctive GO terms for up-regulated genes distinguishing mice from deermice were “response to virus”, “response to interferon-beta”, “response to interferon-gamma”, “response to protozoan”, and “type II interferon signaling”.

By arbitrary criterion of 100 for the top DEGs by ascending p value for each species, 24 genes were shared between species (Supplementary Materials Table S1). These included up-regulated Bcl3, Ccl3, Cxcl1, Cxcl2, Cxcl3, Cxcl10, Il1rn, and Sod2. Among the 100 mouse DEGs, 20 were constituents of GO terms “response to virus” or “response to interferon-beta” and only 3 were members of GO term sets “response to molecule of bacterial origin” or “response to lipopolysaccharide”. In contrast, among the top 100 deermouse DEGs, there were only 2 associated with the virus or type 1 interferon GO terms, but 12 were associated with either or both of the bacterial molecule GO terms.

We confirmed the sex identification for each sample with sex-specific transcripts of Xist for females and Ddx3y for males [3]. For female and male *P. leucopus* there were 5012 transcripts out of 54,466 in the reference set for which there were TPM values of ≥10 in at least one animal in each of the sexes under either condition (Dryad Table D5). The comparison of females to males by fold-changes between LPS-treated and control animals revealed transcripts that were differentially expressed between sexes under LPS treatment (Table S2 and **Figure 4**). Some were down-regulated in one sex while unchanged in expression in the opposite sex. Of note in this category were different isoforms or variants of Lilra6 (leukocyte immunoglobulin-like receptor, subfamily A, member 6), one of a family of orphan receptors of myeloid cells [8]. The opposite case was exemplified by the Dnajc15 and Hspa8 genes for two chaperones: DnaJ heat shock protein family (Hsp40) member C15 and heat shock protein 8, respectively. These were substantially lower in transcription in the LPS-treated females than in untreated animals, but little

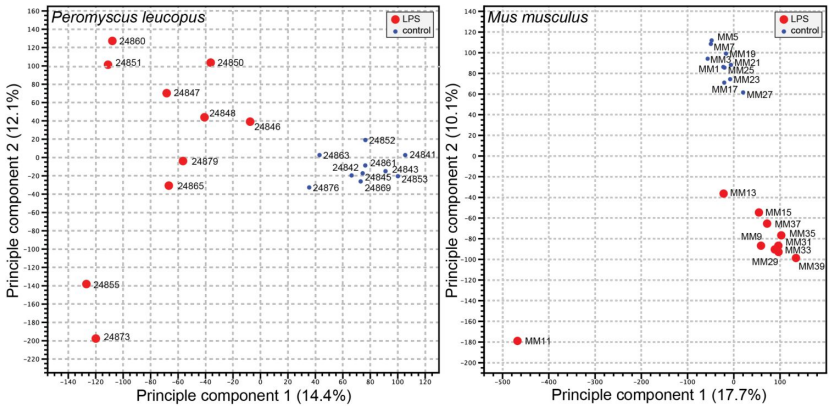

Figure 2.

Principle component analysis of genome-wide RNA-seq data of *P. leucopus* or *M. musculus* with or without (blue dot) treatment with LPS 4 h previous. The individual animals listed in **Table 1** are indicated on the graphs. The insets indicate the size and color of the symbol for the experimental condition (LPS-treated or control).

changed in LPS-treated males. Coordinates for some other genes, e.g. Saa5 and Cxcl2, fell outside the prediction limits at the extreme end of up-regulation, but their vectors were within 20-25° of each other. While these and other sex-associated differences merit attention for future studies, overall they were not of sufficient number or magnitude in our view to warrant division by sex for the subsequent analyses, which had the aim of identifying differences applicable for both females and males.

Targeted RNA seq analysis

The emerging picture was of *P. leucopus* generally responding to LPS exposure as if infected with an extracellular bacterial pathogen, including with activated neutrophils. While *M. musculus* animals of both sexes shared with *P. leucopus* some features of an antibacterial response, they also displayed type 1 and type 2 interferon type response profiles associated with infections with viruses and intracellular bacteria and parasites.

Going forward, the challenge for a cross-species RNA-seq was commensurability between annotated transcripts of reference sets. Orthologous genes can be identified, but mRNA isoforms and their 5' and 3' untranslated regions may not fully correspond. Accordingly, we limited targeted RNA-seq to protein coding sequences of mRNAs for the corresponding sets of *P. leucopus* and *M. musculus* sequences.

The 113 mRNA coding sequences, which are listed in Methods, were drawn from the identified DEGs for *P. leucopus* and *M. musculus* from the genome-wide RNA-seq. For cross-species normalization we first evaluated three methods: (1) normalization using the ratio of mean total reads for all samples to total reads for a given sample, (2) the ratio of reads mapping to a given target gene (i.e. the numerator) to the reads to the mitochondrial 12S rRNA gene (i.e. the denominator), or (3) when the denominator instead was the myeloid cell marker CD45 (protein tyrosine phosphatase, receptor type C) encoded by the Ptprc gene. In humans, mice, and hamsters, Ptprc is expressed by nucleated hematopoietic cells, and CD45 is commonly used as a white cell marker for flow cytometry ([82]). The coefficients of determination (R^2) between comparison pairs (e.g. normalized total reads vs. Ptprc ratio) within a species were ≥ 0.95 (Supplementary Materials Figure S1 and Table S3). There was also little difference between the choice of Ptprc or 12S rRNA transcripts as denominator with respect to cross-species comparisons of LPS-treated to control fold changes. Given the widespread adoption of CD45 for flow cytometry, we chose Ptprc as denominator and as an adjustment for white cell numbers in the samples. Pearson correlation between log-transformed total white blood cell counts and normalized reads for Ptprc across 40 animals representing both species, sexes, and treatments was 0.40 (p = 0.01).

Figure 5 comprises plots of the log-transformed mean ratios for the 10 *P. leucopus* controls and 10 *M. musculus* controls and for the 10 *P. leucopus* and 10 *M. musculus* treated with LPS (Table S4). For untreated animals (left panel) there was high correlation and a regression coefficient of ~1 between the paired data for deermice and mice. The mitochondrial cytochrome oxidase 1 gene (MT-Co1) and S100a9, a subunit of calprotectin, were comparably transcribed. But, there were other coding sequences that stood out for either their greater or lesser transcription in untreated deermice than mice. Two examples of greater expression were Arg1 and MX dynaminin-like GTPase 2 (Mx2), an ISG, while two examples of lesser expression were matrix metalloprotease 8 (Mmp8) and Slpi. There was low to undetectable transcription of Nos2 and interferon-gamma (Ifng) in the blood of controls of both species.

For the LPS-treated animals (right panel **Figure 5**) there was, as expected for this selected set, higher expression of the majority genes and greater heterogeneity among *P. leucopus* and *M. musculus* animals in their responses for represented genes. In contrast to the findings with controls, Ifng and Nos2 had higher transcription in treated mice. In deermice the magnitude of difference in the transcription between controls and LPS-treated was less. A comparatively

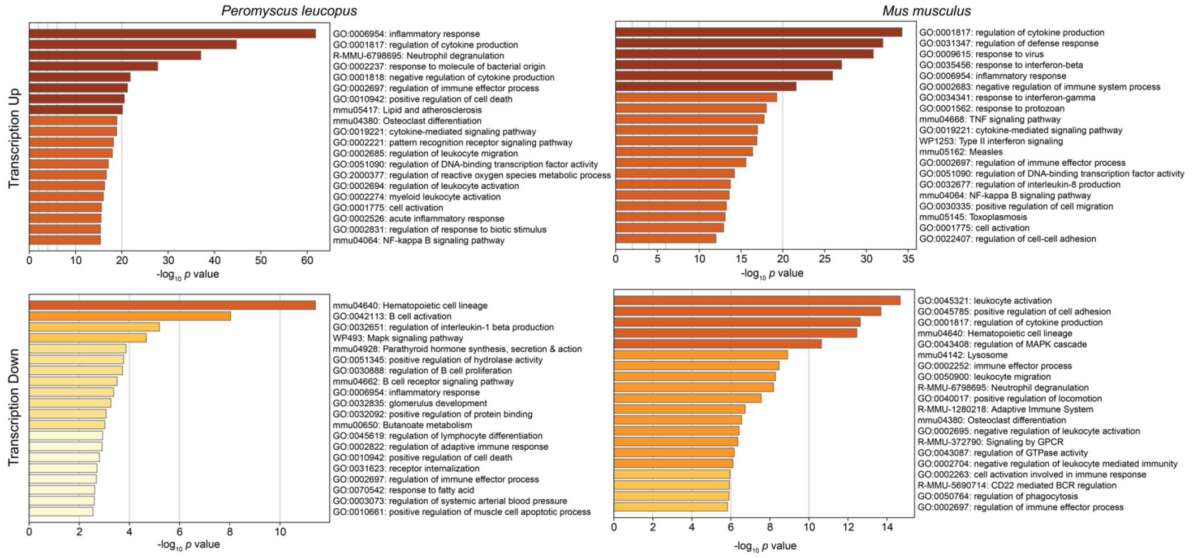

Figure 3.

Gene Ontology (GO) term clusters associated with up-regulated genes (upper panels) and down-regulated genes (lower panels) of *P. leucopus* (left panels) and *M. musculus* (right panels) treated with LPS in comparison with untreated controls of each species. The scale for the *x*-axes for the panels was determined by the highest -log10 *p* values in each of the 4 sets. The horizontal bar color, which ranges from white to dark brown through shades of yellow through orange in between, is a schematic representation of the -log10 *p* values.

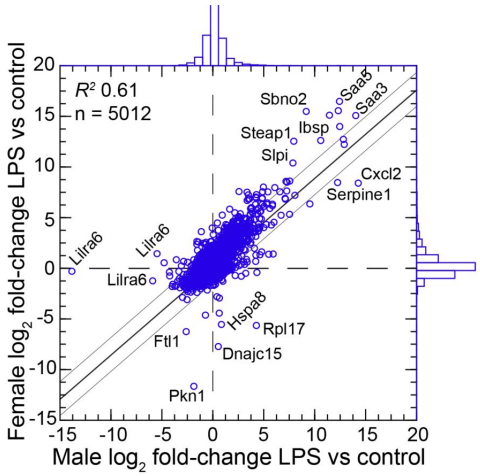

Figure 4.

Scatter plot with linear regression of pairs of log2-transformed mean fold-changes between LPS-treated and control *P. leucopus* by male and female sex. The 5012 reference transcripts from the genome reference set are defined in the text and listed in Table S2 and Dryad Table D5. The coefficient of determination (R^2), the 95% upper and lower prediction limits for the regression line, and distributions of the values on the *x*-and *y*-axes are shown. Selected genes for which their *x*-*y* coordinates fall outside the limits of prediction are labeled. Cxcl2, Ibsp, Saa3, Saa5, Sbno2, Serpine1, Slpi, and Steap1 were noted as up-regulated DEGs for the groups with both sexes (Table S1).

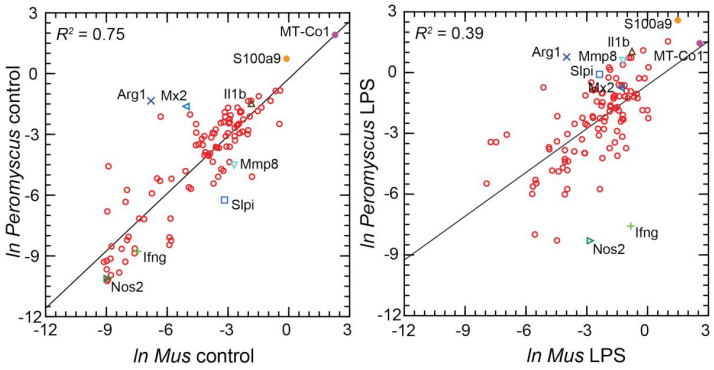

Figure 5.

Scatter plots with linear regression of pairs of log-transformed (*ln*) normalized RNA-seq reads for selected coding sequences for control *P. leucopus* and *M. musculus* (left panel) and LPS-treated *P. leucopus* and *M. musculus* (right panel). The R^2 values and selected genes (each with a different symbol) are indicated in each graph.

restrained transcriptional response in deermice was also noted for Mx2. On the other hand, there were greater fold-change from baseline in *P. leucopus* than in *M. musculus* for interleukin-1 beta (Il1b), Mmp8, Slpi, and S100a9.

Table 2 lists all the selected targets with the means and confidence intervals for the normalized values for controls and LPS-treated *M. musculus* and controls and LPS-treated *P. leucopus* (Table S4). Box plots for a selected 54 of these targets organized by functional characteristics are provided in Figure S2. The fold-changes within each species and between treatments across species are given. The final column is the ratio of the fold-change between LPS to control in *P. leucopus* to the corresponding value for *M. musculus*. This along with the derived heat-map of these ratios, presented in the second column, indicates the genes for which there was little difference between species in their responses to LPS--either up or down--as well as those that were comparatively greater or lesser in one species or the other. Several of these genes are considered in other specific contexts below. Of note here are the places of Nos2 and Ifng at the bottom of the table, and Il1b near the top at position 20.

"Alternatively-activated" macrophages and "nonclassical" monocytes in *P. leucopus*

While we could not type single cells using protein markers, we could assess relative transcription of established indicators of different white cell subpopulations in whole blood. The present study, which incorporated outbred *M. musculus* instead of an inbred strain, confirmed the previous finding of differences in Nos2, the gene for inducible nitric oxide synthase and Arg1, the gene for arginase 1, expression between *M. musculus* and *P. leucopus* (**Figure 5**; **Table 2**). Results similar to the RNA-seq findings were obtained with specific RT-qPCR assays for Nos2 and Arg1 transcripts for *P. musculus* and *M. musculus* (**Table 3**).

Low transcription of Nos2 in both in controls and LPS-treated *P. leucopus* and an increase in Arg1 with LPS was also observed in another experiment for present study where the dose of LPS was 1 μg/g body mass instead of 10 μg/g and the interval between injection and assessment was 12 h instead of 4 h (**Table 4**).

In addition to the differences in Nos2 and Arg1 expression for typing macrophage and monocyte subpopulations, there are also the relative expressions of three other pairs of genes: (1) IL12 and IL10, where a lower IL12/IL10 ratio is more characteristic of alternatively activated or M2 type (68, 90); (2) the proto-oncogene kinases Akt1 and Akt2, where the associations are Akt1 with M2-type and Akt2 with M1-type macrophages (1, 91); and (3) CD14 and CD16 (low affinity immunoglobulin gamma Fc region receptor III; Fcgr3), where low expression of CD14 and high expression of CD16/Fcgr3 is associated with "non-classical" monocytes (70). There is evidence that nonclassical monocytes can change to M2-type macrophages (42).

These four relationships, which are presented as log-transformed ratios of Nos2/Arg1, IL12/IL10, Akt1/Akt2, and CD14/CD16, are shown in **Figure 6**. We confirmed the difference between *P. leucopus* and *M. musculus* in the ratios of Nos2/Arg1 and IL12/IL10 (3) with outbred mice and normalization for white cells. In both species the Akt1/Akt2 ratio declined in LPS-treated animals, but for *P. leucopus* the ratio remained > 1.0 even among LPS-treated animals, while in the blood of *M. musculus* the ratio was < 1.0 at baseline and declined further in the LPS-treated animals.

An ortholog of Ly6C (13), a protein used for typing mouse monocytes and other white cells, has not been identified in *Peromyscus* or other Cricetidae family members. Therefore, for this study the comparison with Cd14 is with Cd16 or Fcgr3, which deermice and other cricetines do have. In mice the Cd14/Cd16 ratio increased from baseline in the LPS group. In the deermice the ratio in

Gene *	Heatmap †	Mus control mean (95% CI) ‡	Mus LPS mean (95% CI)	Mus FC LPS vs control**	Mus FDR p value**	Peromyscus control mean (95% CI)	Peromyscus LPS mean (95% CI)	Peromyscus FC LPS v control	Peromyscus FDR p value	Peromyscus v Mus control FC	Peromyscus v Mus LPS FC	Peromyscus v Mus LPS/control †
Ibsp		0.51 (0.28-0.93)	5.99 (2.04-17.6)	11.7	2E-03	0.17 (0.06-0.47)	471 (305-728)	2720	3E-10	0.34	78.6	233
Slpi		42.0 (32.6-54.0)	93.2 (44.5-195)	2.22	7E-02	1.94 (0.96-3.94)	921 (636-1333)	474	1E-10	0.05	9.88	213
Steap1		0.13 (0.10-0.16)	0.58 (0.21-1.60)	4.52	1E-02	0.09 (0.05-0.17)	31.7 (16.3-61.4)	335	1E-09	0.73	54.3	74.2
Saa3		2.82 (0.89-8.95)	458 (165-1267)	162	7E-06	0.21 (0.12-0.37)	2052 (1273-3306)	9934	2E-13	0.07	4.48	61.2
Lpo		0.13 (0.09-0.20)	0.37 (0.19-0.72)	2.78	2E-02	0.03 (0.03-0.04)	4.10 (1.44-11.7)	118	1E-07	0.26	11.2	42.4
Mmp8		67.4 (34.3-133)	298 (187-477)	4.43	3E-03	10.9 (4.26-27.8)	1823 (1311-2534)	168	3E-08	0.16	6.11	37.9
Lrg		40.8 (22.1-75.3)	107 (52.2-219)	2.62	7E-02	8.01 (2.98-21.5)	353 (268-464)	44.1	2E-06	0.20	3.30	16.8
Mmp9		222 (147-335)	153 (90.1-258)	0.69	3E-01	154 (96.8-245)	1258 (903-1753)	8.17	2E-06	0.69	8.25	11.9
Csf3		0.14 (0.10-0.19)	7.85 (3.07-20.1)	57.1	6E-07	0.04 (0.03-0.05)	24.5 (8.95-67.1)	648	2E-09	0.27	3.12	11.4
Serpine1		0.51 (0.27-0.93)	11.7 (3.04-45.2)	23.2	9E-04	0.14 (0.06-0.32)	32.8 (4.82-223)	239	9E-05	0.27	2.80	10.3
Fpr2		36.9 (25.8-52.7)	246 (168-362)	6.68	2E-06	8.74 (6.48-11.8)	535 (399-717)	61.2	5E-12	0.24	2.17	9.16
Cxcl3		0.32 (0.17-0.59)	19.6 (12.3-31.3)	60.8	2E-08	0.09 (0.04-0.21)	47.2 (15.9-140)	523	2E-07	0.28	2.41	8.59
Retn		0.35 (0.18-0.68)	0.45 (0.26-0.77)	1.28	6E-01	3.13 (1.80-5.46)	31.8 (22.0-45.9)	10.1	4E-06	8.96	71.1	7.93
Csf3r/Cd114		669 (455-982)	302 (221-411)	0.45	7E-03	423 (318-563)	1445 (1024-2040)	3.42	6E-05	0.63	4.79	7.58
Fcgr3/Cd16		45.7 (31.7-65.8)	70.1 (35.1-140)	1.53	3E-01	44.7 (34.1-58.6)	409 (338-497)	9.17	1E-09	0.98	5.84	5.97
Ptx		0.24 (0.17-0.33)	4.06 (0.94-17.6)	17.0	2E-03	0.05 (0.03-0.09)	4.88 (1.10-21.6)	92.2	4E-05	0.22	1.20	5.42
Fcgr2a		78.8 (59.1-105)	149 (93.8-237)	1.89	4E-02	75.0 (62.0-90.8)	766 (597-983)	10.2	2E-10	0.95	5.14	5.40
Cd177		25.0 (11.1-56.1)	158 (80.2-310)	6.31	4E-03	4.29 (2.12-8.69)	144 (107-194)	33.5	1E-07	0.17	0.91	5.31
Ncf4		134 (112-160)	204 (172-242)	1.52	5E-03	49.1 (38.4-62.9)	317 (258-389)	6.46	5E-09	0.37	1.55	4.24
Il1b		158 (102-244)	464 (341-631)	2.94	1E-03	230 (146-362)	2845 (1998-4052)	12.4	2E-07	1.45	6.14	4.22
Fcgr2b		32.7 (27.4-39.0)	111 (78.2-156)	3.38	1E-05	24.9 (22.4-27.8)	352 (302-409)	14.1	2E-14	0.76	3.18	4.17
Hk3		35.8 (25.7-50.0)	63.6 (46.6-86.8)	1.78	3E-02	85.5 (66.8-109)	602 (468-773)	7.04	1E-08	2.39	9.46	3.96
Fgr		138 (110-174)	161 (125-207)	1.17	4E-01	251 (205-308)	1131 (987-1295)	4.50	3E-09	1.82	7.02	3.85
Tlr4		11.9 (8.62-16.4)	11.9 (6.23-22.8)	1.00	1E+00	29.7 (22.9-38.6)	113 (90.7-141)	3.81	1E-06	2.50	9.49	3.80
Glrx		54.8 (43.1-69.7)	122 (65.4-227)	2.22	4E-02	12.4 (11.1-13.9)	88.4 (71.6-109)	7.11	5E-11	0.23	0.73	3.20
Slc11a1/Nramp		54.9 (41.6-72.5)	75.3 (60.0-94.6)	1.37	1E-01	104 (88.0-123)	424 (308-582)	4.07	1E-06	1.89	5.62	2.97
Ccl3		3.07 (2.20-4.30)	227 (161-320)	73.9	2E-11	0.26 (0.14-0.48)	54.6 (33.2-89.8)	213	1E-09	0.08	0.24	2.88
Tlr1		71.8 (63.3-81.5)	52.2 (37.4-72.8)	0.73	1E-01	36.0 (29.8-43.4)	73.3 (61.6-87.1)	2.04	5E-05	0.50	1.40	2.81
Hmox1		16.0 (12.5-20.6)	27.8 (13.7-56.7)	1.74	2E-01	17.4 (14.9-20.4)	78.2 (51.5-119)	4.49	6E-06	1.09	2.81	2.58
Tnfrsf1a		70.8 (57.2-87.7)	69.3 (45.5-106)	0.98	9E-01	44.6 (34.1-58.4)	105 (89.0-123)	2.34	6E-05	0.63	1.51	2.40
Olfm4		7.41 (3.43-16.0)	162 (98.8-266)	21.9	6E-06	3.56 (1.96-6.44)	181 (101-326)	51.0	1E-07	0.48	1.12	2.33
Sod2		58.1 (48.3-69.9)	412 (262-650)	7.10	8E-07	124 (80.5-192)	2030 (1666-2475)	16.3	5E-09	2.14	4.92	2.30
Il2ra/Cd25		12.3 (3.95-38.3)	8.23 (3.06-22.2)	0.67	6E-01	4.33 (3.80-4.94)	5.77 (4.38-7.61)	1.33	9E-02	0.35	0.70	1.99
Lcn2		29.6 (14.9-58.9)	2797 (1832-4271)	94.5	1E-08	25.2 (11.4-55.9)	4571 (3268-6393)	181	3E-09	0.85	1.63	1.92
Sting		51.2 (42.7-61.3)	47.8 (38.5-59.3)	0.93	7E-01	181 (160-204)	317 (277-363)	1.76	2E-05	3.53	6.64	1.88
Jak2		166 (150-185)	355 (267-472)	2.13	2E-04	6.02 (5.43-6.66)	23.4 (16.5-33.2)	3.89	2E-06	0.04	0.07	1.82
Lmk2		93.9 (82.2-107)	83.5 (66.1-105)	0.89	4E-01	153 (125-188)	236 (163-340)	1.54	6E-02	1.63	2.82	1.73
Il1rn		18.6 (12.1-28.6)	325 (185-572)	17.5	7E-07	17.4 (13.0-23.3)	523 (408-670)	30.0	2E-11	0.94	1.61	1.72
Csf1		8.18 (5.53-12.1)	17.5 (12.0-25.5)	2.14	2E-02	3.32 (2.43-4.55)	12.1 (8.04-18.1)	3.63	1E-04	0.41	0.69	1.70
Cfb		6.28 (4.26-9.25)	111 (46.0-269)	17.7	2E-05	7.93 (3.52-17.9)	225 (140-362)	28.4	3E-06	1.26	2.03	1.60
Itgam/Cd11b		265 (207-339)	361 (283-461)	1.37	1E-01	183 (135-250)	396 (278-564)	2.16	5E-03	0.69	1.10	1.58
Timp1		108 (94.0-124)	192 (117-316)	1.78	5E-02	102 (91.0-114)	272 (226-326)	2.67	1E-07	0.94	1.41	1.50
Socs3		19.9 (12.8-30.8)	254 (213-304)	12.8	2E-08	16.8 (12.1-23.4)	304 (224-413)	18.1	1E-09	0.84	1.20	1.42
Il6		0.27 (0.17-0.42)	9.27 (5.27-16.3)	34.5	5E-08	0.17 (0.09-0.33)	7.77 (1.74-34.6)	45.5	3E-04	0.64	0.84	1.32
Ltf		10.5 (5.82-18.8)	824 (445-1526)	78.8	5E-08	28.5 (12.0-68.0)	2930 (2069-4151)	103	5E-08	2.73	3.56	1.30
S100a9		912 (539-1543)	4540 (2426-8497)	4.98	2E-03	2036 (1240-3344)	12972 (10079-16695)	6.37	6E-06	2.23	2.86	1.28
Cxcl1		0.13 (0.09-0.19)	20.3 (9.03-45.7)	158	9E-09	0.06 (0.03-0.13)	12.3 (3.08-49.0)	198	6E-06	0.48	0.60	1.25
Il10		0.11 (0.10-0.13)	3.57 (1.86-6.87)	31.7	3E-08	0.09 (0.05-0.15)	3.53 (1.13-11.0)	39.6	3E-05	0.79	0.99	1.25
Gapdh		646 (591-707)	1014 (558-1845)	1.57	2E-01	667 (590-754)	1295 (923-1818)	1.94	2E-03	1.03	1.28	1.24
Csf1r/Cd115		233 (201-270)	47.3 (31.7-70.7)	0.20	2E-06	320 (262-390)	78.3 (44.7-137)	0.24	2E-04	1.37	1.66	1.21
Nfkb1		149 (133-168)	336 (283-399)	2.25	1E-06	118 (114-122)	300 (209-431)	2.54	1E-04	0.79	0.89	1.13
Nr3c1		124 (105-148)	119 (101-139)	0.95	7E-01	47.0 (41.5-53.1)	49.9 (38.3-65.2)	1.06	7E-01	0.38	0.42	1.11
Akt1		98.1 (82.8-116)	68.1 (54.1-85.7)	0.69	3E-02	148 (133-164)	113 (88.8-144)	0.76	6E-02	1.51	1.66	1.10
Pkm		538 (469-618)	884 (506-1544)	1.64	1E-01	417 (364-479)	741 (558-983)	1.77	2E-03	0.78	0.84	1.08
Il4ra/Cd124		91.2 (80.2-104)	247 (198-309)	2.71	1E-06	51.7 (47.2-56.7)	150 (122-184)	2.90	8E-08	0.57	0.61	1.07
Foxr2		104 (70.7-153)	15.7 (10.0-24.5)	0.15	1E-05	55.7 (47.6-65.1)	8.93 (4.96-16.1)	0.16	2E-05	0.54	0.57	1.06
Camp		3.12 (1.76-5.54)	285 (145-558)	91.2	3E-08	5.42 (2.26-13.0)	524 (354-776)	96.7	8E-08	1.74	1.84	1.06
Hif1a		53.4 (46.5-61.5)	191 (144-254)	3.58	8E-07	83.6 (77.0-90.8)	299 (203-440)	3.58	1E-05	1.56	1.57	1.00
Padi4		15.9 (8.70-29.0)	41.2 (22.3-76.0)	2.59	5E-02	44.6 (34.1-58.4)	105 (89.0-123)	2.34	6E-05	2.81	2.54	0.90
Bcl3		21.1 (14.8-30.0)	209 (168-260)	9.92	1E-08	18.9 (15.1-23.8)	167 (127-219)	8.80	3E-09	0.90	0.80	0.89
Mt2		0.38 (0.19-0.77)	89.1 (33.8-235)	235	1E-07	0.31 (0.13-0.75)	61.2 (20.5-182)	199	2E-06	0.81	0.69	0.84
Myd88		50.8 (39.5-65.4)	127 (96.0-168)	2.50	2E-04	51.5 (46.6-56.8)	108 (99.7-116)	2.09	5E-09	1.01	0.85	0.84
Thy1		35.0 (22.6-54.2)	25.2 (13.3-48.0)	0.72	5E-01	503 (457-553)	297 (240-368)	0.59	4E-04	14.4	11.8	0.82
Jun		31.4 (23.9-41.2)	157 (105-234)	5.01	6E-06	6.02 (5.43-6.66)	23.4 (16.5-33.2)	3.89	2E-06	0.19	0.15	0.78
Icam		24.1 (20.3-28.6)	301 (257-352)	12.5	3E-12	12.6 (10.5-15.0)	121 (67.3-217)	9.61	2E-06	0.52	0.40	0.77
Cx3cr1		27.9 (21.8-35.7)	3.46 (1.99-6.00)	0.12	4E-06	26.1 (19.0-35.9)	2.45 (1.44-4.15)	0.09	1E-06	0.94	0.71	0.76
Cxcl2		0.16 (0.09-0.26)	119 (76.0-186)	762	6E-12	0.10 (0.04-0.26)	60.2 (19.6-185)	574	2E-07	0.67	0.51	0.75
Akt2		138 (117-163)	275 (197-415)	1.99	9E-03	59.4 (57.1-61.7)	87.6 (71.5-107)	1.47	2E-03	0.43	0.32	0.74
Jak1		415 (386-447)	623 (467-830)	1.50	2E-02	256 (232-281)	277 (214-359)	1.09	6E-01	0.61	0.45	0.72
ERV Gag-pol		3210 (2406-4283)	13633 (10083-18433)	4.3	4E-06	233 (195-278)	692 (548-873)	2.97	2E-06	0.07	0.05	0.70
Pbib		93.7 (82.3-107)	97.2 (58.5-161)	1.04	9E-01	143 (134-153)	102 (71.2-147)	0.71	9E-02	1.53	1.05	0.69
Nox1		0.14 (0.11-0.18)	0.98 (0.65-1.48)	7.01	8E-07	10.1 (8.12-12.5)	45.6 (34.4-60.5)	4.53	3E-07	71.8	46.4	0.65
Tnf		2.99 (2.26-3.96)	19.5 (14.0-27.1)	6.52	3E-07	0.76 (0.42-1.40)	3.10 (1.62-5.95)	4.06	7E-03	0.26	0.16	0.62
Tlr2		26.1 (18.1-37.5)	165 (126-215)	6.31	6E-07	50.8 (37.2-69.5)	194 (128-294)	3.83	1E-04	1.95	1.18	0.61
Cd8		23.8 (19.6-29.0)	17.3 (13.6-22.1)	0.73	7E-02	52.6 (41.6-66.5)	21.3 (10.2-44.7)	0.41	4E-02	2.21	1.23	0.56
Stat1		160 (132-193)	449 (247-814)	2.81	6E-03	256 (221-297)	387 (323-465)	1.51	3E-03	1.60	0.86	0.54
Mapk1		71.1 (65.5-77.0)	92.6 (65.0-132)	1.30	2E-01	67.5 (63.9-71.3)	44.2 (37.4-52.3)	0.65	2E-04	0.95	0.48	0.50
Arg1		1.12 (0.36-3.42)	18.4 (5.99-56.7)	16.5	4E-03	261 (154-441)	2161 (1709-2732)	8.28	2E-06	234	117	0.50
Ccl2		0.16 (0.10-0.27)	12.7 (4.87-33.0)	77.7	8E-07	0.19 (0.07-0.50)	7.42 (3.23-17.0)	38.5	3E-05	1.18	0.58	0.50
Rigi/Ddx58		47.3 (41.9-53.3)	293 (177-491)	6.20	0.00	28.0 (20.5-34.4)	98.9 (76.6-127)	3.72	0.00	0.60	0.29	0.49
MT-Co1		10015 (7976-12576)	13316 (8322-28047)	1.33	5E-01	6608 (4976-8775)	4164 (2563-6765)	0.63	1E-01	0.66	0.31	0.47
Cd4		55.6 (45.7-67.6)	27.5 (19.6-38.8)	0.50	4E-03	88.5 (75.5-104)	19.9 (10.4-38.3)	0.23	5E-04	1.59	0.72	0.45
Socs1		0.82 (0.59-1.13)	45.4 (25.6-80.4)	55.6	3E-09	0.74 (0.53-1.04)	18.4 (9.19-36.9)	24.8	5E-07	0.91	0.41	0.45
Cd3d		81.9 (71.8-93.4)	46.7 (32.8-66.5)	0.57	1E-02	77.1 (64.1-92.7)	18.3 (8.91-37.6)	0.24	2E-03	0.94	0.39	0.42
Mtor		27.9 (24.3-31.9)	40.2 (34.9-46.3)	1.44	2E-03	62.1 (55.6-69.4)	36.3 (27.4-48.1)	0.58	3E-03	2.23	0.90	0.41
Il18		1.78 (1.18-2.70)	4.24 (2.84-6.32)	2.38	1E-02	4.89 (4.22-5.67)	4.15 (2.40-7.18)	0.85	6E-01	2.74	0.98	0.36
Stat2		41.0 (36.6-45.9)	229 (144-364)	5.59	2E-06	89.1 (75.3-105)	177 (153-206)	1.99	2E-05	2.17	0.77	0.36
Il7r/Cd127		36.8 (29.2-46.3)	39.6 (28.4-55.1)	1.08	7E-01	59.2 (50.5-69.3)	22.3 (15.5-32.0)	0.38	2E-04	1.61	0.56	0.35
Tgfb		0.13 (0.09-0.19)	8.02 (3.82-16.8)	60.6	4E-08	1.08 (0.78-1.50)	22.1 (11.5-42.5)	20.4	5E-07	8.18	2.76	0.34
Acod1		1.20 (0.60-2.37)	388 (289-521)	324	2E-10	2.64 (1.72-4.05)	276 (202-378)	105	2E-11	2.21	0.71	0.32
Csf2		0.16 (0.11-0.23)	3.94 (2.09-7.43)	24.9	3E-07	0.05 (0.03-0.07)	0.33 (0.11-0.98)	7.10	4E-03	0.29	0.08	0.28
Stat4		55.4 (50.4-60.8)	110 (95.3-128)	1.99	8E-07	26.4 (21.8-32.0)	14.4 (10.4-19.9)	0.55	6E-03	0.48	0.13	0.27
Dhx58/Lgp2		11.3 (9.76-13.2)	156 (77.1-317)	13.8	2E-06	20.2 (14.3-28.4)	73.1 (55.9-95.5)	3.63	3E-05	1.78	0.47	0.26
Isg15		81.5 (65.6-101)	600 (219-1646)	7.37	2E-03	183 (104-322)	342 (175-668)	1.87	2E-01	2.24	0.57	0.25
Oas1		10.6 (8.90-12.6)	106 (49.4-225)	9.98	3E-05	81.1 (51.9-127)	191 (127-285)	2.35	1E-02	7.67	1.81	0.24
Cgas		1.55 (1.19-2.02)	17.3 (14.3-21.0)	11.2	4E-10	5.53 (4.52-6.77)	13.7 (10.8-17.4)	2.47	3E-05	3.57	0.79	0.22
Gbp4		86.1 (73.9-100)	795 (523-1208)	9.23	4E-08	81.5 (70.5-94.1)	153 (112-209)	1.88	2E-03	0.95	0.19	0.20
Il12		2.85 (2.19-3.71)	11.7 (7.75-17.8)	4.12	4E-05	0.30 (0.17-0.53)	0.25 (0.14-0.43)	0.82	6E-01	0.11	0.02	0.20
ERV Env		913 (661-1261)	3398 (2240-5155)	3.7	2E-04	48.1 (42.9-53.9)	33.1 (25.7-47.5)	0.69	2E-02	0.05	0.01	0.18
Irf7		43.5 (34.1-55.5)	528 (276-1010)	12.1	2E-06	145 (83.0-253)	296 (186-469)	2.04	7E-02	3.33	0.56	0.17
Fos		16.6 (11.9-23.2)	17.3 (9.38-31.8)	1.04	9E-01	20.2 (14.5-28.0)	2.39 (1.61-3.55)	0.12	5E-07	1.21	0.14	0.11
Ifih1/Mda5		8.39 (6.56-10.7)	181 (82.4-395)	21.5	2E-06	43.3 (35.5-52.8)	92.1 (75.8-112)	2.13	6E-05	5.16	0.51	0.10
Cxcl10		0.35 (0.21-0.59)	231 (81.4-655)	659	1E-08	0.26 (0.12-0.59)	16.2 (5.83-44.9)	61.7	1E-05	0.75	0.07	0.094
Mpo		0.33 (0.19-0.57)	19.5 (8.56-44.6)	59.9	6E-07	1.73 (0.71-4.26)	7.91 (2.61-24.0)	4.56	5E-02	5.32	0.41	0.076
Myc		22.4 (18.0-27.7)	294 (209-413)	13.1	3E-09	41.6 (35.9-48.3)	41.4 (25.2-68.1)	1.00	1E+00	1.86	0.14	0.076
Ngp		7.82 (4.03-15.2)	1071 (580-1977)	137	1E-08	130 (77.8-216)	1275 (1022-1589)	9.83	5E-07	16.6	1.19	0.072
Ifit1		10.7 (8.18-13.9)	309 (96.6-991)	29.0	5E-05	53.1 (32.8-85.8)	96.7 (59.0-158)	1.82	1E-01	4.98	0.31	0.063
Mx2		6.29 (5.53-7.16)	270 (158-460)	42.9	1E-09	198 (121-323)	476 (300-757)	2.41	2E-02	31.5	1.76	0.056
Cd69		14.1 (11.5-17.3)	203 (155-266)	14.4	1E-10	39.3 (34.6-44.6)	31.1 (17.3-55.9)	0.79	5E-01	2.78	0.15	0.055
Ccl4		1.84 (1.10-3.09)	217 (126-376)	118	3E-09	117 (75.4-181)	740 (487-1126)	6.33	2E-05	63.5	3.40	0.054
Gzmb		14.7 (9.75-22.1)	1063 (603-1873)	72.4	3E-09	28.8 (15.8-52.6)	103 (56.2-188)	3.57	1E-02	1.96	0.10	0.049
Tnfrsf9/Cd137		0.64 (0.46-0.89)	97.5 (47.3-201)	152	3E-09	0.76 (0.42-1.40)	3.10 (1.62-5.95)	4.06	7E-03	1.19	0.03	0.027
Cd14		44.5 (25.5-77.7)	667 (408-1091)	15.0	2E-06	88.5 (75.5-104)	19.9 (10.4-38.3)	0.23	5E-04	1.99	0.03	0.015
Nos2		0.13 (0.10-0.17)	58.9 (22.1-157)	459	4E-09	0.04 (0.03-0.05)	0.25 (0.09-0.69)	6.16	3E-03	0.31	0.00	0.013
Ifng		0.58 (0.33-1.02)	438 (178-1080)	751	3E-09	0.15 (0.08-0.29)	0.51 (0.14-1.89)	3.30	1E-01	0.26	0.00	0.004

* Gene: Mus musculus reference genome GenBank name/other name in literature. The list includes the 113 coding sequences listed in Methods and two endogenous retrovius (ERV) proteins, Env and Gag-pol.
† Heatmap (red-white-blue scale) of log-transformed "Peromyscus v Mus LPS/control" values (last column). This is ratio of "Peromyscus v Mus LPS FC" (2nd column from right) to "Peromyscus v Mus control FC" (3rd column from right)
‡ Mean unique reads for given gene normalized for reads for Ptprc (Cd45) gene for a sample. The 95% confidence intervals (CI) are asymmetric. Actual [gene]/Ptprc ratios are X 10^-3.
** Other abbreviations: Peromyscus, Peromyscus leucopus; Mus, Mus musculus FC, fold-change; FDR, false discovery rate

Table 2.

Targeted RNA-seq of blood of *Peromyscus leucopus* and *Mus musculus* 4 hours after intraperitoneal injection of lipopolysaccharide (LPS) or saline control

Blood mRNA source	Control mean copies (95% CI)	LPS mean copies (95% CI)	Fold difference LPS/control	*t* test *p* value/Mann-Whitney *p* value
P. leucopus				
Gapdh	1.22 (0.40-3.74) x 10^5	2.38 (1.10-5.16) x 10^5	1.95	0.35/0.49
Nos2	191 (141-260)	138 (61-315)	0.72	0.47/0.53
Arg1	4.62 (3.09-6.92) x 10^3	12.3 (3.22-47.1) x 10^3	2.66	0.18/0.55
M. musculus				
Gapdh	6.10 (2.32-16.0) X 10^6	1.77 (0.80-3.89) X 10^6	0.29	0.06/0.02
Nos2	101 (68-151)	1891 (866-4130)	18.6	<0.00001/0.002
Arg1	27 (15-20)	16 (8-34)	0.59	0.29/0.45

Table 3.

RT-qPCR of blood of LPS-treated *P.leucopus* and *M.musculus*

Gene	Control (n=3) mean (95% CI)	LPS (n=3) mean (95% CI)	Fold change	FDR *p* value
Akt1	220 (12-4202)	514 (321-825)	2.3	0.05
Akt2	145 (97-217)	336 (236-478)	2.3	0.04
Arg1	146 (58-367)	2812 (273-28925)	19	0.018
Cd14	82 (12-569)	914 (161-5197)	11	0.05
Cd69	165 (87-310)	68 (14-329)	0.42	0.15
ERV Env	25 (3-242)	40 (7-224)	1.6	0.86
ERV Gag-pol	3085 (132-14695)	2768 (533-1278)	0.9	0.66
Fcgr3/Cd16	40 (25-66)	841 (533-1278)	21	0.008
Gapdh	7176 (3504-15142)	23811 (5827-97306)	3.3	0.07
Gbp4	97 (6-1551)	439 (33-5819)	4.5	0.05
Ifit1	367 (51-2663)	1373 (374-5047)	3.7	0.05
Ifng	0 (0-0)	0 (0-0)	.	.
Il1b	258 (18-3652)	1432 (183-11220)	5.6	0.1
Irf7	121 (93-157)	11405 (530-245616)	94	0.003
Isg15	429 (184-1001)	19505 (11140-34152)	45	0.005
Mx2	157 (73-341)	1310 (323-5315)	8.3	0.04
Nos2	0 (0-0)	0 (0-0)	.	.
Oas1	65 (31-138)	1458 (367-5795)	22	0.03
Rigi/Ddx58	38 (3-504)	173 (35-845)	4.5	0.07
Saa3	2 (0-250)	6683 (1494-29896)	3372	0.03
Slpi	6 (1-46)	779 (326-1864)	123	0.03
Sod2	180 (54-607)	3406 (698-16633)	19	0.03

Table 4.

Taegeted RNA-seq of *P. leucopus* blood in 12 h and *1 ug/g* LPS experiment

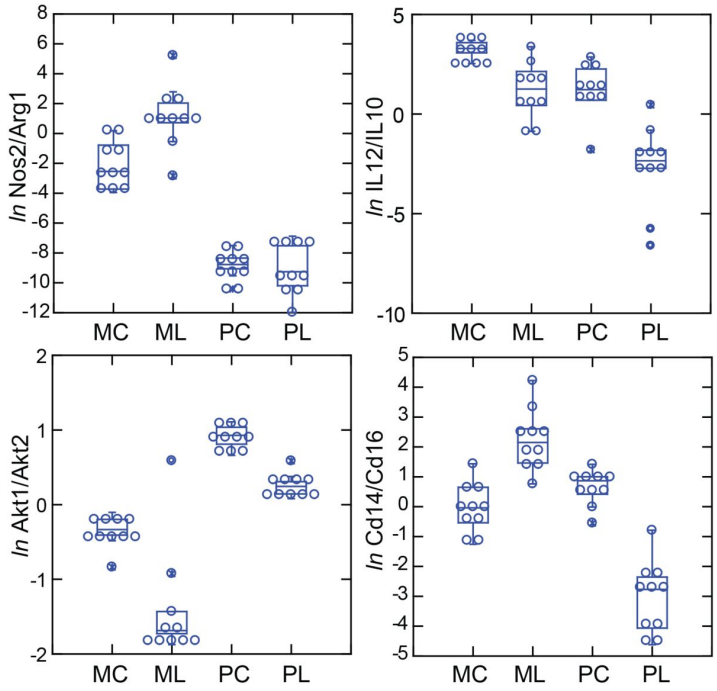

Figure 6.

Box plots of log-transformed ratios of four pairs of gene transcripts from targeted RNA-seq analysis of blood of *P. leucopus* (P) or *M. musculus* (M) with (L) or without (C) treatment with LPS. The values are from Table S4. Upper left, Nos2/Arg1. Upper right, IL12/IL10. Lower left, Akt1/Akt2. Lower right, Cd14/Cd16.

control animals was midway between the two groups of mice but there was a marked decrease in the LPS-treated deermice (**Figure 6**). This was not associated with a fall in the absolute numbers or percentages of monocytes in the blood of these animals.

Taken together, the Nos2-Arg1, Il12-Il10, Akt1-Akt2, and CD14-CD16 relationships document a disposition toward alternatively-activated macrophages and nonclassical monocytes in *P. leucopus* both before and after exposure to LPS. This contrasts with profiles consistent with a predominance of classically-activated macrophages and classical monocytes in mice.

Interferon-gamma and interleukin-1 beta dichotomy between deermice and murids

For mice the Ifng transcript was one of the top ranked DEGs by both fold-change and adjusted *p* value by genome-wide RNA-seq (**Table 2**). In contrast, for *P. leucopus* Ifng was far down the list, and the comparably ranked DEG instead was Il1b. This inversion of relationships between two pro-inflammatory cytokines was confirmed by analysis of the individual animals of both species (**Figure 7**). There was little or no detectable transcription of Ifng in the blood of deermice in which Il1b expression was high. There was also scant to no transcription of Ifng in the blood of *P. leucopus* 12 h after injection of LPS (**Table 4**).

The up-regulation of Ifng within 4 hours of exposure to LPS was not limited to the species *M. musculus*. In an experiment with the rat *R. norvegicus*, we used two different LPS doses (5 μg/g and 20 μg/g), but the same 4 h endpoint and whole blood as the sample. Both groups of LPS-treated rats had lowered total white blood cells and, like the mice, lower neutrophil-to-lymphocyte ratios compared to controls (**Table 5**). There were also elevations of interferon-gamma, interleukin-6 and interleukin-10 proteins from undetectable levels in the blood of the treated rats (Dryad Table D5). The values for rats receiving 5 μg/g or 20 μg/g doses were similar (Figure S3), so these groups were combined. By targeted RNA-seq there were 24x fold-changes between the LPS-treated rats and control rats for Ifng and Nos2 but only ~3x fold-change for Il1b (**Table 5**).

We asked why the Infg response observed in CD-1 mice and rats here was not as pronounced in BALB/c mice (3). Accordingly, we used the RNA-seq reads from the prior study in combination with the reads of the present study and carried out targeted RNA-seq (Figure S4). The BALB/c inbred mice had, like the CD-1 mice, modest elevations of Il1b transcription. Ifng expression was also elevated in the BALB/c animals but not to the degree noted in CD-1 mice or rats. One explanation is an inherent difference of BALB/c mice from other strains in their lower interferon-gamma response $_{\text{to LPS}}$ (47, 84).

Interferon-gamma and inducible nitric oxide synthase

Interferon-gamma is a determinant of Nos2 expression (55, 81). So, the scant transcription of Ifng in *P. leucopus* conceivably accounted for the low expression of Nos2 in that species. The analysis shown in upper left panel of **Figure 8** shows a tight correlation between the levels of transcription of Ifng and Nos2 for both species and both experimental conditions (Figure S5). A significant correlation was also observed for the combined set of animals between the ratios of Nos2 to Arg1 and Ifng to Il1b (upper right panel), an indication of co-variation between Ifng expression and macrophage polarization.

The plausible sources of Ifng mRNA in whole blood are T-cells, Natural Killer cells, and Type 1 Innate Lymphoid Cells (75). A DEG for *M. musculus* by both genome-wide and targeted RNA-seq (**Table 2**; Table S1) was Cd69, a C-type lectin protein and an early activation antigen for these cells(38). In *P. leucopus* transcription Cd69 occurred in the blood of control *P. leucopus*, but it was the same or only marginally different for the LPS-treated animals (lower left panel). In contrast, in *M. musculus* the baseline transcription of Cd69 was below that of *P. leucopus*, but in the LPS-

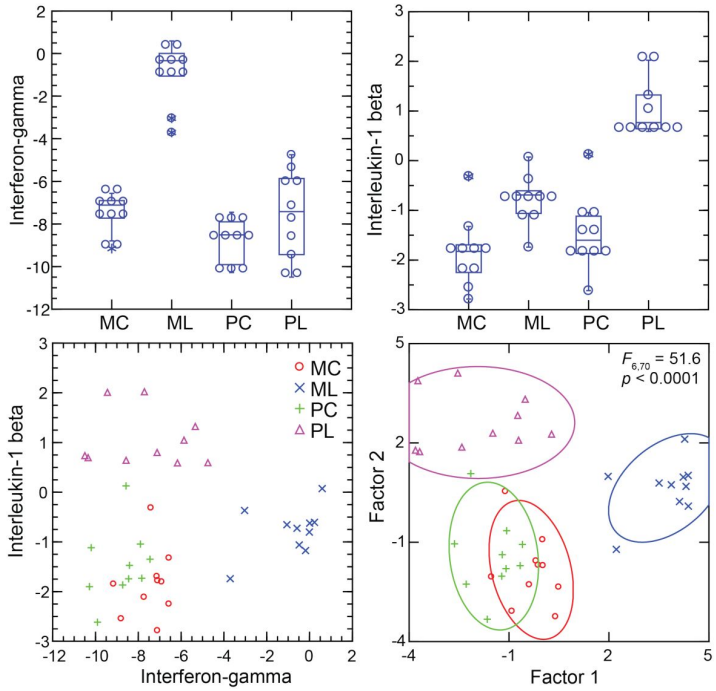

Figure 7.

Transcripts of interferon-gamma and interleukin-1 beta by targeted RNA-seq of the blood of *P. leucopus* (P) or *M. musculus* (M) with (L) or without (C) treatment with LPS. The top panels are box plots of the individual values. The lower left panel is a scatter plot of interleukin-1 ý on interferon-ψ values. The lower right panel is a Discriminant Analysis of these pairs of values where Factor 1 corresponds to interferon-gamma, and Factor 2 corresponds to interleukin-1 beta. Values for analysis are in Table S4.

Variable	Control (n=5) mean (95% CI)*	LPS (n=11) mean (95% CI)*,†	Fold change	FDR *p* value
Hematology				
Hematocrit (%)	48 (46-50)	48 (46-49)	1.0	1E+00
White blood cells	7660 (7200-8310)	4980 (2020-7940)	0.75	2E-01
Neutrophils	3680 (3260-4090)	1410 (520-2290)	0.38	4E-03
Lymphocytes	3170 (3010-3330)	2830 (1230-4430)	0.89	8E-01
Neutrophil/lymphocyte	1.17 (1.01-1.33)	0.49 (0.44-0.55)	0.42	7E-09
Plasma cytokines (pg/ml)				
Interleukin-6	0 (0-0)	36933 (21676-52190)	.	5E-15
Interleukin-10	9 (1-17)	640 (477-802)	71	2E-09
Interferon-gamma	0 (0-0)	9091 (7126-11056)	.	9E-20
Targeted RNA-seq				
Akt1	35.8 (29.8-42.9)	24.3 (21.5-27.5)	0.68	4E-03
Akt2	50.8 (44.9-57.4)	109 (95.2-125)	2.2	9E-06
Arg1	0.04 (0.02-0.08)	0.21 (0.10-0.44)	4.8	2E-02
Cd14	7.7 (5.3-11.2)	43.4 (29.6-63.6)	5.6	9E-05
Cd177	0.89 (0.43-1.8)	190 (143-251)	213	2E-10
Cd3	32.3 (27.7-37.6)	21.9 (18.5-25.8)	0.68	1E-02
Cd69	17.9 (16.7-19.2)	58.9 (49.1-70.7)	3.3	9E-07
Cgas	1.5 (1.3-1.6)	12.8 (10.8-15.1)	8.7	3E-10
Cxcl10	0.43 (0.34-0.56)	130 (93.7-181)	302	1E-11
Dhx58	7.1 (6.7-7.6)	113 (97.3-130)	15.8	3E-12
ERV Env	8.4 (7.5-9.3)	713 (624-815)	85.3	1E-14
ERV gag-pol	6.0 (5.4-6.7)	506 (449-570)	84.3	7E-15
Fcgr2a	114 (87.0-150)	764 (631-925)	6.7	4E-08
Fcgr2b	32.5 (24.6-43.1)	161 (127-204)	4.9	2E-06
Fcgr3/Cd16	15.2 (13.6-17.0)	13.9 (11.8-16.3)	0.91	5E-01
Gapdh	327 (237-451)	1643 (1385-1949)	5.0	2E-07
Gbp4	35.7 (33.6-38.0)	269 (237-306)	7.5	3E-11
Ibsp	0.41 (0.31-0.55)	0.61 (0.47-0.80)	1.5	1E-01
Ifih1	18.7 (17.1-20.4)	165 (149-184)	8.8	2E-12
Ifit1	102 (70.0-147)	756 (677-844)	7.4	3E-09
Ifng	0.47 (0.32-0.67)	10.3 (6.4-16.5)	22.1	1E-06
Il10	0.12 (0.07-0.21)	4.5 (3.4-5.8)	38.2	5E-09
Il12	0.07 (0.04-0.11)	3.2 (2.0-4.9)	45.9	7E-08
Il1b	58.6 (39.7-86.4)	618 (503-760)	10.6	2E-08
Il6	0.06 (0.05-0.08)	4.4 (2.9-6.6)	70.9	6E-09
Irf7	44.8 (36.9-54.3)	443 (372-528)	9.9	6E-10
Isg15	15.6 (13.1-18.7)	624 (534-729)	39.9	6E-13
Itgam?Cd11b	66.3 (52.5-83.7)	208 (161-269)	3.1	9E-05
Mmp8	75.2 (50.4-112)	519 (438-615)	6.9	7E-08
Mx2	40.7 (35.9-46.2)	900 (780-1039)	22.1	1E-12
Nos2	32.4 (17-60.6)	2990 (2491-3589)	92.4	1E-10
Oas1	23.1 (18.8-28.4)	151 (140-164)	6.6	3E-11
Rigi/Ddx58	8.6 (7.8-9.4)	151 (135-168)	17.6	1E-13
S100a9	298 (190-466)	2884 (2269-3666)	9.7	2E-07
Saa1	0.60 (0.49-0.73)	699 (552-884)	1167	4E-14
Slpi	20.2 (13.1-31.3)	262 (197-347)	12.9	1E-07
Sod2	63.8 (51.1-79.6)	901 (759-1070)	14.1	2E-10
Tlr4	5.3 (4.6-6.0)	20.1 (17.2-23.6)	3.8	8E-08
Tnf	1.1 (0.63-1.9)	78.8 (62.6-99.1)	72.8	2E-10
Ratios				
Akt1/Akt2	0.70 (0.65-0.76)	0.22 (0.19-0.25)	0.31	2E-08
Cd14/Fcgr3	0.51 (0.34-0.76)	3.14 (2.26-4.36)	6.16	1E-05
IL12/IL10	0.54 (0.22-1.37)	0.70 (0.51-0.95)	1.30	5E-01
Nos2/Arg1	741 (403-1362)	14244 (7615-26646)	19.2	5E-05

* For targeted RNA-seq it is mean unique reads for given gene normalized for reads for Ptprc (Cd45) gene for a sample. The 95% confidence intervals (CI) are asymmetric. Actual [gene]/Ptprc ratios are X
† The results for rats receiving 5 μg/g (n=6) and 20 μg/g (n=5) were combined

Table 5.

Hematology, cytokines, and targeted RNA-seq of LPS-treated and control rats

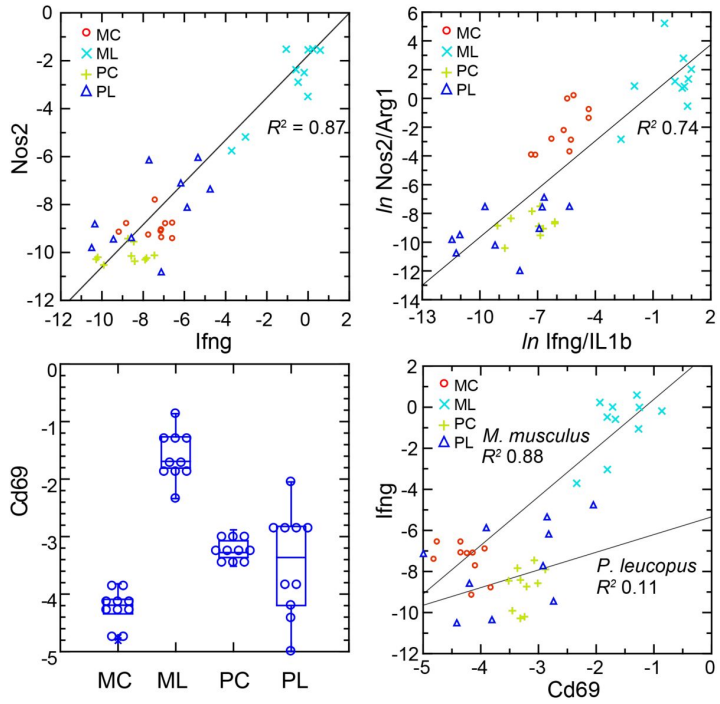

Figure 8.

Normalized transcripts of Nos2, Ifng, and Cd69 in targeted RNA-seq analysis of blood of *P. leucopus* (P) or *M. musculus* (M) with (L) or without (C) treatment with LPS. Upper left: scatter plot of individual values for Nos2 on Ifng with linear regression curve and coefficient of determination (R^2). Upper right: natural logarithm (*ln*) of ratios of Nos2/Argi1 on Ifng/IL1b with regression curve and R^2. Lower left: Box plots of individual values of normalized transcripts of Cd69. Lower right: Scatter plot of Ifng on Cd69 with separate regression curves and R^2 values for *M. musculus* and *P. leucopus*. Values for analysis are in Table S4. Box plots for Nos2 and Arg1 are provided in Figure S5, and box plots for Ifng and Il1b are provided in **Figure 7**.

treated mice it was many fold higher. In mice transcripts for Cd69 correlated tightly with Ifng transcription, while in the deermice there was little correlation between Cd69 and Ifng expression at those low levels (lower right panel).

The findings are consistent with CD69-positive cells being a source of Ifng in mice. Cd69 transcription was comparatively higher in control deermice than in control mice, so we presume that deermice have CD69-positive cells at baseline. One explanation then for the comparatively few Ifng transcripts in the deermice after LPS is a diminished responsiveness of these cells. Tlr4 expression increased ~3 -fold more in *P. leucopus* than in *M. musculus* after LPS (**Table 2**), but the magnitude of the decline in expression of Cd14 in deermice than mice was even greater. CD14 is required for LPS-stimulated signaling through surface TLR4 [58], and, as such, its decreased availability for this signaling pathway is a possible explanation for the moderated response to LPS in *P. leucopus*.

Interferon-stimulated genes and RIG-I-like receptors

As noted, GO terms differentiating mice from deermice included "response to interferon-beta" and "response to virus" (**Figure 3**). There was also the example of Mx2, an ISG with anti-viral activity on its own, that showed a greater fold-change from baseline in mice than in deermice (**Figure 5**). Five other ISGs--guanylate binding protein 4 (Gbp4), interferon-induced protein with tetratricopeptide repeat (Ifit1), interferon regulatory factor 7 (Irf7), ubiquitin-type modifier ISG15 (Isg15), and 2'-5' oligoadenylate synthase 1A (Oas1a)—had higher transcription in all the LPS-treated animals. But the magnitude of fold change was less in the deermice, ranging from 6-25% of what it was in the LPS group of mice (**Table 2**; Figure S6).

The up-regulation of these ISGs was evidence of an interferon effect, but transcripts for interferon-1 beta (Ifnb) or -alpha (Ifna) themselves were scarcely detectable in deermice or mice in the blood under either condition. We then considered pattern recognition receptors (PRR) that might be part of a signaling pathway leading to ISG expression. Among the DEGs from the genome-wide analyses were four cytoplasmic PRRs: (1) Rigi (formerly Ddx58), which encodes the RNA helicase retinoic acid-inducible I (RIG-I); (2) Ifih1, which encodes interferon induced with helicase C domain 1, also known as MDA5 and a RIG-I-like receptor; (3) Dhx58, also known LGP2 and another RIG-I-like receptor; and (4) cGAS (cyclic GMP-AMP synthase), which is part of the cGAS-STING sensing pathway (Table S4).

All four cytoplasmic PRRs were upregulated in the blood of LPS-treated mice and deermice (**Table 2**). But, again, for each of them the magnitude of fold change was less by 50-90% in treated *P. leucopus* than in *M. musculus*. The coefficients of determination for the 6 ISGs and the 4 PRRs are provided in **Figure 9**. For most of the pairs there was evidence of covariation across all 40 animals. When the correlation was low across all the data, e.g. between the ISG Mx2 and the PRR Rigi or the ISGs Mx2 and Gbp4, it was high within a species.

These findings were evidence that pathways in *P. leucopus* for PRR signaling and ISG expression functioned similarly to those in *M. musculus* but differed under these experimental conditions in magnitude of the changes, being more moderate in the deermice.

Endogenous retroviruses in deermice, mice, and rats after LPS exposure

The six ISGs are nonexclusive consequences of activity of type 1 interferons. What we could document was the association of transcription of the gene for the cytoplasmic PPRs, including RIG-I, and the ISGs in both species, as well as the distinction between deermice in the magnitude of the responses of both PRRs and ISGs. These findings led us to ask could be a pathogen-associated molecular pattern (PAMP) for signaling pathways leading to expression of type 1 interferons.

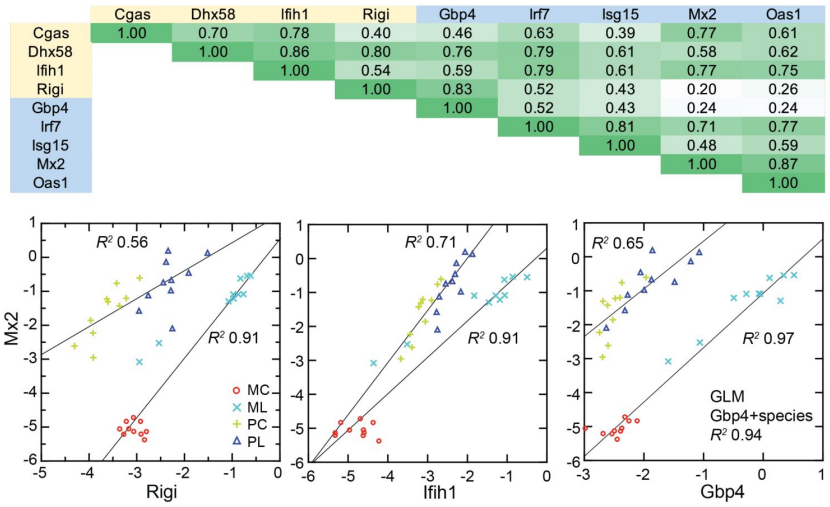

Figure 9.

Co-variation between transcripts for selected PRRs and ISGs in the blood of *P. leucopus* (P) or *M. musculus* (M) with (L) or without (C) LPS treatment. Top panel: matrix of coefficients of determination (R^2) for combined *P. leucopus* and *M. musculus* data. PRRs are indicated by yellow fill and ISGs by blue fill on horizontal and vertical axes. Shades of green of the matrix cells correspond to R^2 values, where cells with values less than 0.30 have white fill and those of 0.90-1.00 have deepest green fill. Bottom panels: scatter plots of log-transformed normalized Mx2 transcripts on Rigi (left), Ifih1 (center), and Gbp4 (right). The linear regression curves are for each species. For the right-lower graph the result from the General Linear Model (GLM) estimate is also given. Values for analysis are in Table S4; box plots for Gbp4, Irf7, Isg15, Mx2, and Oas1 are provided in Figure S6.

One of these is endogenous retroviruses (ERV). The activity of these diverse, abundant, and pervasive elements have been recognized as one of the drivers of innate immune responses to a microbe [41, 51, 76]. Our attention was drawn to ERVs by finding in the genome-wide RNA-seq of LPS-treated and control rats. Two of the three highest scoring DEGs by FDR *p* value and fold-change were a *gag-pol* polyprotein of a leukemia virus with 131x fold-change from controls and a mouse leukmia virus (MLV) envelope (Env) protein with 62x fold-change (Dryad Table D5).

We returned to the mouse and deermouse data. There were four MLV or other ERV Env proteins among the 1266 genome-wide RNA-seq DEGs for *M. musculus*. But, there was no ERV Env protein identified as such among the 1154 DEGs identified for *P. leucopus* (Table S1). One possible explanation for the difference was an incomplete annotation of the *P. leucopus* genome. We took three approaches to rectify this. The first was to examine the DEGs for *P. leucopus* that encoded a polypeptide ≥ 200 amino acids and was annotated for the genome as "uncharacterized". A search with these candidates of both the virus and rodent proteins databases identified two that were homologous with *gag-pol* polyproteins of ERVs, mainly leukemia viruses, of mammals.

For a second approach we carried out a de novo transcript assembly of mRNA reads from blood of LPS-treated and control *P. leucopus* and used the resultant contigs as the reference set for RNA-seq analysis. This identified two contigs that were measurably transcribed in the blood, differentially expressed between conditions, and were homologous to ERV sequences. One was revealed to be an Env protein that was identical to a *P. leucopus* protein annotated as "MLV-related proviral Env protein" (XP_037065362). The second was a *gag-pol* protein. The latter was near-identical to the *gag-pol* protein identified by the first approach.

The third approach was to scan the *P. leucopus* genome for nonredundant sequences, defined as <95% identity, that were homologous with ERV *gag-pol* sequences, which are not typically annotated because of masking for repetitive sequences. This analysis yielded 615 unique sequences. These were used in turn as a reference set for RNA-seq. There were 4 sequences that met the criterion of FDR *p* value <0.01. Three were transcribed at 5-to 40-fold higher levels in LPS-treated deermice than in controls. But all three, as well as the fourth, a down-regulated DEG, were ERV relics with truncations, frame shifts, and in-frame stop codons. These were assessed as non-coding RNAs and not further pursued in this study.

To represent *P. leucopus* in a targeted RNA-seq comparison with mice and rats we settled on the Env protein and *gag-pol* coding sequences identified present in the blood mRNA and as DEGs. Representing *M. musculus* were highest ranked MLV Env and *gag-pol* protein sequences among the DEGs. For rats we chose Env and *gag-pol* proteins that were second and third ranked DEGs identified in the genome-wide RNA-seq. Because of length differences for the coding sequences, the unit used for cross-species analysis was reads per kilobase before normalization for Ptprc transcription.

The left panel of **Figure 10** shows the striking transcriptional fold-change in LPS-treated rats of both Env and *gag-pol* sequences over controls. Of lesser magnitude but no less significant was the fold-change observed *M. musculus* for both Env and *gag-pol* sequences. In both mice and rats Env and *gag-pol* read values were highly correlated across conditions. In contrast, in *P. leucopus* the magnitudes of fold-change upwards for *gag-pol* was less than in mice or rats, and transcription of the Env protein sequence was actually lower in LPS-treated animals than in controls. While there was a tight association between Env protein and the PRR Rigi transcription in the *M. musculus*, this was not observed in *P. leucopus*. Rigi transcription was moderately higher at the time that Env protein's transcription was lower in the LPS group.

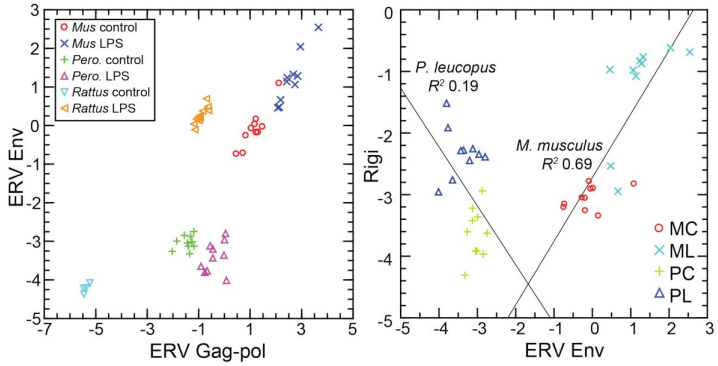

Figure 10.

Scatter plots of endogenous retrovirus (ERV) Env and Gag-pol protein gene transcription (left) and association of ERV Env with Rigi transcription (right) in the blood of *P. leucopus* (*Pero*; P), *M. musculus* (*Mus*; M), or *R. norvegicus* (*Rattus*) with (L) or without (control; C) treatment with LPS. In right panel the linear regression curve and coefficients of determination (R^2) for *P. leucopus* and *M. musculus* are shown. Values for analysis are in Table S4; box plots for ERV Env and ERV Gag-pol are provided in Figure S7.

Borrelia hermsii infection of P. leucopus

The phenomena reported so far were consequences of exposures to a particular PAMP--bacterial lipopolysaccharide with its hallmark lipid A moiety--recognized by a particular PRR, TLR4. While the focus was primarily on events downstream from that initial signaling, we asked in a concluding study whether the profile observed in *P. leucopus* applied in circumstances when the PAMP or PAMPs did not include LPS. This question is germane, given *P. leucopus*' role as a natural host for *B. burgdorferi*. This organism and other members of the spirochete family *Borreliaceae* do not have LPS [5, 88], but they have abundant lipoproteins, which are agonists for TLR2 in a heterodimer with TLR1 [80]. *B. burgdorferi* is transiently blood-borne at low densities in *P. leucopus*, but in its life cycle *B. burgdorferi* is mainly tissue-associated in vertebrate hosts [6]. We previously observed that the blood of *B. burgdorferi*-infected *P. leucopus* manifested few DEGs in comparison to skin [52]. More comparable to the LPS experimental model is infection of *P. leucopus* with a relapsing fever *Borrelia* species, which commonly achieve high densities in the blood. *P. leucopus* is a reservoir for *Borrelia miyamotoi*, which causes hard tick-borne relapsing fever [6], and the related *P. maniculatus* is a natural host for the soft tick-borne relapsing fever agent *B. hermsii* [43].

Accordingly, we used blood RNA-seq reads, which were taken from a prior study of *B. hermsii* infection of *P. leucopus* [3], for targeted analysis with the same reference set employed for the LPS analyses (**Table 6** and Table S5). The blood samples were taken from infected and uninfected animals on day 5, when bacteremia was at its peak, as documented by microscopy of the blood, qPCR of the spleen, and transcripts of a *B. hermsii* plasmid in the RNA extracts of the blood. As expected for *B. hermsii* infection [24], the spleen was enlarged in infected animals.

Similarities in the profiles for the LPS-treated and *B. hermsii*-infected deermice were as follows: (1) low levels of transcription of Nos2 and Ifng that contrasted with the high levels for Arg1 and Il1b in the same animals, (2) maintenance of Akt1/Akt2 > 1.0 under both conditions, (3) reduction of the Cd14/Cd16 ratio, (4) decreased transcription of Cd69, and (5) stable, low transcription of ERV Env and gag-pol loci with only marginal increases in transcription of ISGs and RIG-I-like receptors. Other equivalences under the two experimental conditions included increases in expression of genes for superoxide dismutase 2, low-affinity Fc gamma receptors, and secretory leukocyte protease inhibitor. Thus, the responses that distinguish deermice are not confined to the singular case of LPS as the elicitor.

Discussion

Study limitations

The approach was forward and unbiased, looking for differences between species broadly across their transcriptomes. The findings lead to hypotheses, but reverse genetics in service of that testing was not applied here. In selective cases we could point to supporting evidence in the literature on *M. musculus* and the phenotypes of relevant gene knockouts, but there are no such resources for *Peromyscus* as yet. The resource constraint also applies to the availability of antibodies for use with *Peromyscus* for immunoassays for specific proteins, e.g. interferon-gamma, in serum, or for cell markers, e.g. CD69, for flow cytometry of white blood cells.

While a strength of the study was use of an outbred population of *M. musculus* to approximate the genetic diversity of the *P. leucopus* in the study, this meant that some genes of potential relevance might have gone undetected, i.e. from type II error. The variances for a sample of genetically diverse outbred animals, like the LL stock of *P. leucopus* [52, 53], would be expected to be greater than for the same sized sample of inbred animals. For some traits, especially ones that are complex or under balancing selection, even sample sizes of 10 in each group may not have provided sufficient power for discrimination between deermice and mice. For the same reason

Variable	Uninfected (n=3) mean (95% CI)*	Infected (n=4) mean (95% CI)*	Fold change	FDR *p* value
B. hermsii qPCR spleen	.	13615 (1882-98476)	.	.
B. hermsii reads blood	.	3487 (743-16362)	.	.
% spleen/body mass	0.15 (0.12-0.19)	0.36 (0.26-0.51)	2.4	1E-02
Targeted RNA-seq				
Akt1	184 (135-253)	347 (191-630)	1.88	3E-01
Akt2	92.9 (72.4-119)	141 (89.4-222)	1.52	3E-01
Arg1	247 (96.9-630)	1375 (848-2230)	5.57	4E-02
Cd14	308 (119-799)	598 (357-1002)	1.94	3E-01
Cd177	2.11 (0.71-6.27)	35.9 (11.9-108)	17.0	4E-02
Cd3	86.9 (77.7-97.2)	56.2 (16.4-192)	0.65	6E-01
Cd69	133 (114-155)	65 (33.6-128)	0.49	2E-02
Cgas	8.87 (5.40-14.6)	16.8 (8.99-31.4)	1.89	3E-01
Cxcl10	0.33 (0.14-0.76)	4.85 (2.74-8.59)	14.7	2E-02
Dhx58	22.0 (9.26-52.4)	37.0 (13.3-103)	1.68	6E-01
ERV Env	26.8 (22.4-32.1)	29.2 (14.8-57.8)	1.09	9E-01
ERV gag-pol	1853 (1351-2542)	1578 (820-3037)	0.85	8E-01
Fcgr2a	44.8 (24.0-83.6)	715 (303-1689)	16.0	2E-02
Fcgr2b	47.3 (30.2-74.3)	578 (167-2000)	12.2	5E-02
Fcgr3 (Cd16)	30.6 (18.6-50.4)	392 (171-897)	12.8	2E-02
Gapdh	1985 (1142-3447)	5366 (2383-12081)	2.70	2E-01
Gbp4	126 (82.3-193)	289 (130-644)	2.30	3E-01
Ibsp	0.81 (0.56-1.16)	358 (95-1350)	444	1E-02
Ifih1	48.5 (31.9-73.7)	49.1 (26.8-89.7)	1.01	1E+00
Ifit1	223 (107-465)	604 (303-1203)	2.71	2E-01
Ifng	0.58 (0.09-3.92)	2.28 (0.86-6.06)	3.94	3E-01
Il10	0.25 (0.07-0.87)	1.49 (0.31-7.28)	5.94	3E-01
Il12	0.43 (0.23-0.81)	1.13 (0.45-2.88)	2.63	3E-01
Il1b	477 (174-1308)	2828 (1325-6034)	5.93	7E-02
Il6	0.51 (0.18-1.39)	1.80 (0.61-5.28)	3.55	3E-01
Irf7	93.3 (13.4-65)	626 (196-1998)	6.71	2E-01
Isg15	302 (30.1-3030)	1922 (623-5934)	6.36	3E-01
Itgam (Cd11b)	72.2 (44.5-117)	322 (211-492)	4.45	2E-02
Mmp8	7.1 (2.74-18.6)	537 (148-1952)	75.2	2E-02
Mx2	152 (48.6-476)	167 (48.0-582)	1.10	9E-01
Nos2	0.16 (0.08-0.30)	0.32 (0.13-0.80)	2.01	4E-01
Oas1	51.3 (6.75-390)	159 (39.2-643)	3.10	5E-01
Rigi (Ddx58)	38.7 (18.9-79.2)	55.7 (33.9-91.5)	1.44	5E-01
S100a9	1739 (657-4596)	18430 (6546-51883)	10.6	5E-02
Saa3	0.49 (0.13-1.87)	212 (25.9-1733)	431	2E-02
Slpi	0.41 (0.18-0.95)	166 (49.0-566)	401	1E-02
Sod2	104 (51.4-211)	2011 (804-5028)	19.3	2E-02
Tlr1	21.4 (15.0-30.4)	111 (53.9-230)	5.21	4E-02
Tlr2	83.2 (59.7-116)	371 (234-587)	4.46	2E-02
Tlr4	44.8 (26.1-77.0)	256 (138-474)	5.71	3E-02
Tnf	2.86 (1.64-4.96)	78.8 (38.8-160)	27.6	1E-02
Ratios				
Akt1/Akt2	2.0 (1.7-2.3)	2.5 (2.1-2.9)	1.25	1E-01
Cd14/Fcgr3	11.3 (4.8-17.9)	1.7 (0.72-2.8)	0.15	2E-02
IL12/IL10	2.3 (0.00-5.0)	2.8 (0.0-7.6)	1.21	9E-01
Nos2/Arg1	0.001 (0.0-0.002)	0.0001 (0.0-0.0004)	0.28	2E-01

* For targeted RNA-seq it is mean unique reads for given gene normalized for reads for Ptprc (Cd45) gene for a sample. The 95% confidence intervals (CI) are asymmetric. Actual [gene]/Ptprc ratios are X 10^-3.

Table 6.

Targeted RNA-seq of *P. leucopus* with and without *Borrelia hermsii* infection

differences between sexes of a species in their responses might have been undetected. The interpretations applied to mixed-sex groups of deermice and mice. Expression strongly associated with female or male sex could have yielded an average fold change for the whole group that fell below the screen's threshold.

The parameters for the experiment of LPS dose, the route, and duration of experiment each might have had different values under another design. Those particular choices were based on past studies of deermice and mice [3, 49]. In another experiment we found that with doses twice or half those given the deermice the responses by rats to the different doses were indistinguishable by hematology, cytokine assays, and RNA-seq. Thus, there seems to be some latitude in the dose and still achieving replication. We obtained similar results for *P. leucopus* when we looked at a replicate of the experiment with the same conditions [3], when the dose was lower and duration lengthened to 12 h (this study). The analysis here of the *B. hermsii* infection experiment also indicated that the phenomenon observed in *P. leucopus* was not limited to a TLR4 agonist.

While the rodents in these experiments were housed in the same facility and ate the same diet, we cannot exclude inherent differences in gastrointestinal microbiota between species and individual outbred animals as co-variables for the experimental outcomes. We reported differences between the LL stock *P. leucopus* and BALB/c *M. musculus* of the same age and diet in their microbiomes by metagenomic analysis and microbiologic means [61]. This included a commensal *Tritrichomonas* sp. in *P. leucopus* but not in the *M. musculus* in the study. The presence of these protozoa affects innate and adaptive immune responses in the gastrointestinal tract [19, 29], but it is not clear whether there are systemic consequences of colonization by this flagellate.

LPS, ERVs, and interferons

The results confirm previous reports of heightened transcription of ERV sequences in mice or mouse cells after exposure to LPS [35, 44, 87]. Here we add the example of the rat. The LPS was administered in solution and not by means of membrane vesicles. The sensing PRR presumably was surface-displayed, membrane-anchored TLR4 [58]. It follows that a second, indirect of LPS on the mouse is through its provocation of increased ERV transcription intracellularly. ERV-origin RNA, cDNA and/or protein would then be recognized by a cytoplasmic PRR. RIG-I was one associated with ERV transcription in this study. Kong et al. reported that LPS stimulated expression of Rigi in a mouse macrophages but did not investigate ERVs for an intermediary function in this phenomenon [46]. As was demonstrated for LINE type retrotransposons in human fibroblasts, intracellular PRR signaling can trigger a type 1 interferon response [25]. The combination of these two signaling events, i.e. one through surface TLR4 by LPS itself and another through intracellular PPR(s) by to-be-defined ERV products, manifested in mice and rats as a response profile that had features of both a response to a virus with type 1 interferon and ISGs and a response to a bacterial PAMP like LPS with acute phase reactants such as calprotectin and serum amyloid.

This or a similar phenomenon has been observed under other circumstances. In humans there was heightened transcription of retrotransposons in patients with septic shock [63], as well as in peripheral blood mononuclear cells from human subjects experimentally injected with LPS [74]. Bacteria like *Staphylococcus epidermidis* that express TLR2 agonists, such as lipoteichoic acid, promoted expression of ERVs, which in turn modulated host immune responses [51]. A synthetic analog of a *B. burgdorferi* lipoprotein activated human monocytic cells and promoted replication of the latent HIV virus in cells that were persistently infected [72].

P. leucopus does not fit well with this model. Instead of the prominent interferon-gamma response observed in mice and rats, there were prominent responses of interleukin-1 beta and genes associated with neutrophil activation. Instead of the much heightened expression of ISGs, like Mx2 and Isg15, in mice treated with LPS, the deermice under the same condition had a more subdued

ISG transcription profile. Instead of increased expression of ERV Env protein sequences in blood of mice and rats treated with LPS, there was decreased transcription of the homologous MLV Env gene in like-treated *P. leucopus*.

This suppression in the deermice may be attributable to defensive adaptations of *Peromyscus* to repeated invasions of endogenous retroviruses, as Gozashti et al. has proposed for *P. maniculatus* [32]. This includes expanding the repertoire of silencing mechanisms, such as Kruppel-associated box (KRAB) domain-containing zinc finger proteins [97]. Like *P. maniculatus*, *P. leucopus* has an abundance of Long Terminal Repeat retrotransposons, several named for their endogenous retrovirus heritages [52]. Our initial analysis of the *P. leucopus* genome reported a depletion of KRAB domains compared to Muridae [52]. But a subsequent annotation round identified several genes for KRAB domain zinc finger proteins in *P. leucopus*, including Zfp809 (XP_006982432), which initiates ERV silencing [95], and Zfp997 (XP_037067826), which suppresses ERV expression [89]. Another possible adaptation in *P. leucopus* is the higher baseline expression of some ISGs as noted here (**Figure 9** and Figure S6).

Reducing differences between *P. leucopus* and murids *M. musculus* and *R. norvegicus* to a single attribute, such as the documented inactivation of the Fcgr1 gene in *P. leucopus* [7], may be fruitless. But the feature that may best distinguish the deermouse from the mouse and rat is its predominantly anti-inflammatory quality. This characteristic likely has a complex, polygenic basis, with environmental (including microbiota) and epigenetic influences. An individual's placement is on a spectrum or, more likely, a landscape rather than in one or another binary or Mendelian category.

One argument against a purely anti-inflammatory characterization is the greater neutrophil numbers and activity in *P. leucopus* compared to *M. musculus* in the LPS experiment. The neutrophil activation, migration, and phagocytosis would be appropriate early defenses against a pyogenic pathogen. But if not contained, they bring local and systemic risks for the host. This damage would not likely be from nitric oxide and reactive nitrogen species, given the minimal Nos2 transcription. But deermice showed heightened expression of proteases, such as Mmp8, enzymes for reactive oxygen species, such as NADPH oxidase 1 (Nox1), and facilitators of neutrophil extracellular traps, such as PAD4 (Padi4) (**Table 2**). We had previously identified possible mitigators, such as the protease inhibitor Slpi and superoxide dismutase 2 [3]. These findings were replicated here. The topic of neutrophil activation and these and other possible counters is considered in more detail elsewhere.

An anti-inflammatory disposition but at what cost?

An assignment of infection tolerance to a host and pathogen pairing assumes sufficient immunity against the microbe to keep it in check if elimination fails. *P. leucopus* and *P. maniculatus*, are in this sense "immunocompetent" with respect to the microbes they host and with which they may share a long history [40]. Yet, has this balance of resistance and tolerance for certain host-associated microbes been achieved in a trade-off that entails vulnerabilities to other types of agents?

The selection of LPS as the experimental model was meant to cover this contingency, at least for the common denominator of acute inflammation many types of infections elicit. But LPS studies revealed potential weaknesses that some pathogens might exploit. One of these is the low expression of inducible nitric oxide. Although Nos2 gene knockouts in *M. musculus* had lower LPS-induced mortality than their wild-type counterparts, the mutants were more susceptible to the protozoan *Leishmania major* and the facultative intracellular bacterium *Listeria monocytogenes* [56, 93]. While there are no known studies of either of these pathogens in *P. leucopus*, the related species *P. yucatanicus* is the main reservoir for *Leishmania mexicana* in Mexico [17]. Compared with *M. musculus*, which suffer a high fatality rate from experimental infections with *L. mexicana*, *P. yucatanicus* infections are commonly asymptomatic [54].

Given the restrained interferon and ISG response shown by *P. leucopus*, another plausible vulnerability would be viral infections. But other studies suggest that neither RNA nor DNA viruses pose an inordinately high risk for *Peromyscus*. Both tolerance of and resistance to the tickborne encephalitis flavivirus Powassan virus by *P. leucopus* were demonstrated in an experimental model in which mice, by contrast, gwere severely affected [62]. *P. maniculatus* has been successfully infected with the SARS-CoV-2 virus by the respiratory route, but the infected animals displayed only mild pathology, manifested little if any disability, and recovered within a few days [30, 33]. Among natural populations and in the laboratory *P. maniculatus* is noted for its tolerance of hantavirus, which commonly is fatal for infected humans [14, 20]. *P. maniculatus* was permissive of infection with monkeypox virus, but the infection was mild and transient [26].

A distinguishing *P. leucopus* characteristic, which was not expressly examined here, is its aforementioned 2-3 fold greater life span than that of *M. musculus*. While deermice may not be in the same longevity league as the naked mole-rat (*Heterocephalus glaber*), which can live for over 30 years [73], some features of naked mole-rat immunology are intriguingly similar to what we have observed for *P. leucopus*. These include macrophages and blood myeloid cells with low to absent transcription of Nos2 or production of nitric oxide in response to LPS, even in the presence of added inteferon-gamma [31]. Like *P. leucopus* and in distinction to *M. musculus*, naked mole-rats showed an increase in the proportion of neutrophils in the blood 4 hours after intraperitoneal injection of LPS [39]. In another comparative study, the hematopoietic stem and progenitor cells of these rodents had a lower type 1 interferon response than mice to a TLR3 agonist [28].

In summary, if there is a vulnerability that *Peromyscus* accepts in return for relief from inflammation (and perhaps a longer life), it has not been identified yet. However, potential threats and stressors are many, and the number assessed either in the field or laboratory has been limited to date.

Implications for Lyme disease and other zoonoses

Our studies of *P. leucopus* began with a natural population and documented a >80% prevalence of infection and high incidence of re-infections by *B. burgdorferi* in the area's white-footed deermouse, the most abundant mammal there [16]. This was a Lyme disease endemic area [27], where residents frequently presented for medical care for a variety of clinical manifestations, from mild to serious, of *B. burgdorferi* infection [85]. Subclinical infections in humans occur, but most of those who become infected have a definable illness [86]. The localized or systemic presence of the microbe is a necessary condition for Lyme disease, but the majority of the symptoms and signs are attributable to inflammation elicited by the organism's presence and not from virulence properties per se or the hijacking of host cells [21]. Since humans are transmission dead-ends for *B. burgdorferi* and many other zoonotic agents in their life cycles, it is not surprising that human infections are generally more debilitating if not fatal than what adapted natural hosts experience.

It is in the space between the asymptomatic natural host and symptomatic inadvertent host where there may be insights with basic and translational application. With this goal, we consider the ways the results inform studies of the pathogenesis of Lyme disease, where "disease" includes lingering disorders akin to "long Covid" [71], and where "pathogenesis" includes both microbial and host contributions. Plausibly-germane deermouse-mouse differences identified in our studies are summarized in **Figure 11**. Two are highlighted here.

The first is macrophage polarization [68]. By the criteria summarized above, the response to LPS by *P. leucopus* is consistent with the alternatively-activated or M2 type, rather than the expected classical or M1 type. But it was not only LPS-treated deermice that had this attribute, the blood of untreated animals also displayed M2 type polarization features. This included a comparatively high Arg1 expression level and a Akt1/Akt2 ratio of more than 1 at baseline. This suggests that

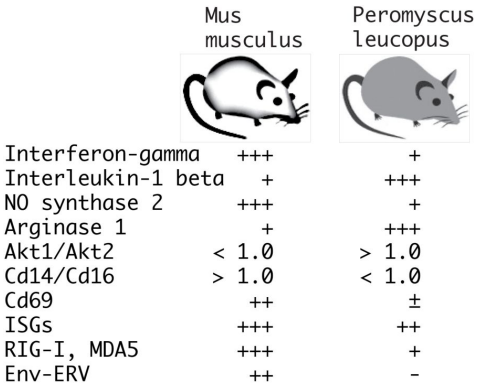

Figure 11.

Summary of distinguishing features of transcriptional responses in the blood between *P. leucopus* and *M. musculus* 4 h after treatment with LPS. There is semi-quantitative representation of relative transcription of selected coding sequences or ratios of transcription for selected pairs of genes in the blood.

studies of other mammals, including humans, need not administer LPS or other TLR agonist to assess disposition toward M1 or M2-type polarization. This reading could serve as a prognostic indicator of the inflammatory response to infection with *B. burgdorferi* or other pathogen and the long-term outcome.

The second difference we highlight is the activation of ERV transcription that was prominent in the LPS-treated mice and rats but not in similarly-treated deermice. A paradoxical enlistment of antiviral defenses, including type 1 and type 2 interferons, for an infection with an extracellular bacterium, like *B. burgdorferi*, may bring about more harm than benefit, especially if the resultant inflammation persists after antibiotic therapy. There are various ways to assess ERV activation in the blood, including assays for RNA, protein, and reverse transcriptase activity. A xenotropic MLV-related retrovirus has been discounted as a cause of chronic fatigue syndrome [60]. However, production of whole virions need not occur for there to be PRR signaling in response to cytoplasmic Env protein, single stranded RNA, or cDNA [78].

Methods

Animals

The study was carried out in accordance with the *Guide for the Care and Use of Laboratory Animals: Eighth Edition* of the National Academy of Sciences. The protocols AUP-18-020 and AUP-21-007 were approved by the Institutional Animal Care and Use Committee of the University of California Irvine.

Peromyscus leucopus, here also referred to as “deermice”, were of the outbred LL stock, which originated with 38 animals captured near Linville, NC and thereafter comprised a closed colony without sib-sib matings at the *Peromyscus* Genetic Stock Center at the University of South Carolina [45]. The LL stock animals for this study were bred and raised at the vivarium of University of California Irvine, an AAALAC approved facility. Outbred *Mus musculus* breed CD-1, specifically Crl:CD1(ICR) IGS, and here also referred to as “mice”, were obtained from Charles River. Fischer F344 strain inbred *Rattus norvegicus*, specifically F344/NHsd, and here also referred to as “rats”, were obtained from Charles River.

For the combined *P. leucopus-M. musculus* experiment the 20 *P. leucopus* were of a mean (95% confidence interval) 158 (156-159) days of age and had a mean 21 (19-22) g body mass. The 20 *M. musculus* were all 149 days of age and had a mean body mass of 47 (43-50) g. The ratio of average male to average female body mass was 1.04 for *P. leucopus* and 1.03 for *M. musculus*. The 6 female *P. leucopus* for the 12 h duration experiment were of a mean 401 (266-535) days of age and mean body mass of 20 (17-23) g. The 16 adult 10-12 week old female *R. norvegicus* had a mean 139 (137-141) g body mass. The 7 male *P. leucopus* for the infection study were of a mean 107 (80-134) days and mean body mass of 21 (18-24) g.

Animals were housed in Techniplast-ventilated cages in vivarium rooms with a 16 h-8 h light-dark cycle, an ambient temperature of 22 °C, and on ad libitum water and a diet of 2020X Teklad global soy protein-free extruded rodent chow with 6% fat content (Envigo, Placentia, CA).

For injections the rodents were anesthetized with inhaled isoflurane. The rodents were euthanized by carbon dioxide overdose and intracardiac exsanguination at the termination of the experiment. No animals died or became moribund before the 4 hour or 12 h termination time points in the LPS experiments or before the 5 d termination point of infection study.

LPS experiments

For the *P. leucopus* and *M. musculus* combined experiment, sample sizes replicated the specifications of the previous study, in which there were 20 *P. leucopus* and 20 *M. musculus*, equally divided between females and males and equally allotted between conditions [3]. The treatments were administered in the morning of a single day. At 15 min intervals and alternating between species, sex, and treatments, animals were intraperitoneally (ip) injected 50 µl volumes of either ion-exchange chromatography-purified *Escherichia coli* O111:B4 LPS (Sigma-Aldrich L3024) in a dose of 10 µg per g body mass or the diluent alone: sterile-filtered, endotoxin-tested, 0.9% sodium chloride (Sigma-Aldrich). The animals were visually monitored in separate cages continuously for the duration of the experiment. We recorded whether there was reduced activity by criterion of huddling with little or movement for > 5 min, ruffled fur or piloerection), or rapid respiration rate or tachypnea. At 4.0 h time after injection animals were euthanized as described above, and sterile dissection was carried out immediately.

Lower dose and longer duration experiment. In an experiment with 6 *P. leucopus*, the animals were administered the same single dose of LPS but at 1.0 µg/g and the same control solution. The animals were euthanized 12 h after the injection the following day.

Rat LPS experiment. The same experimental design was used for the rats as for the combined deermice-mice experiment, with the exception that the formulation of the *E. coli* O111:B4 LPS was "cell culture grade" (Sigma-Aldrich L4391), and the groups were sterile saline alone (n = 5), 5 µg LPS per g body mass (n = 6), or 20 µg LPS per g (n = 5).

Experimental infection

The infection of a group of *P. leucopus* LL stock with the relapsing fever agent *B. hermsii* and the processing of blood and tissues for RNA extraction 5 days into the infection were described previously [3]. In brief, animals were infected intraperitoneally on day 0 with either phosphate-buffered saline alone or 10^3 cells of *B. hermsii* MTW, a strain that is infectious for *Peromyscus* species [43]. Bacteremia was confirmed by microscopy on day 4, and the animals were euthanized on day 5. For that prior study the RNA-seq analysis was limited to the genome-wide transcript reference set. For the present study we used the original fastq format files for targeted RNA-seq as described below.

Hematology and plasma analyte assays

For the combined *P. leucopus-M. musculus* experiment automated complete blood counts with differentials were performed at Antech Diagnostics, Fountain Valley, CA on a Siemens ADVIA 2120i with Autoslide hematology instrument with manual review of blood smears by a veterinary pathologist. For the 12 h duration *P. leucopus* experiment hematologic parameters were analyzed on an ABCVet Hemalyzer automated cell counter instrument at U.C. Irvine. For the rat experiment complete blood counts with differentials were performed at the Comparative Pathology Laboratory of the University of California Davis. Multiplex bead-based cytokine protein assay of the plasma of the rats was performed at Charles River Laboratories using selected options of the Millipore MILLIPLEX MAP rat cytokine/chemokine panel.

RNA extraction of blood

After the chest cavity was exposed, cardiac puncture was performed through a 25 gauge needle into a sterile 1 ml polypropylene syringe. After the needle was removed, the blood was expelled into Becton-Dickinson K2E Microtainer Tubes, which contained potassium EDTA. Anticoagulated blood was split into a sample that was placed on ice for same-day delivery to the veterinary hematology laboratory and a sample intended for RNA extraction which was transferred to an Invitrogen RiboPure tube with DNA/RNA Later and this suspension was stored at −20 °C. RNA was

isolated using the Invitrogen Mouse RiboPure-Blood RNA Isolation Kit]. RNA concentration was determined on a NanoDrop microvolume spectrophotometer (ThermoFisher) and quality was assessed on an Agilent Bioanalyzer 2100.

RNA-seq of blood

The chosen sample sizes and coverage for the bulk RNA-seq were based on empirical data from the prior study [3], which indicated that with 10 animals per group and a two-sided two sample *t*-test we could detect with a power of ≥ 0.80 and at a significance level of 0.05 a ≥1.5 fold difference in transcription between groups for a given gene. We also were guided by the simulations calculations of Hart et al. [36], which indicated for a biological coefficient of variation of 0.4 within a group, a minimum depth of coverage of ≥ 10, and a target of ≥ 2x fold change that a sample size of 7-8 was sufficient for 80% power and type I error of 5%. For the *P. leucopus* and *M. musculus* samples production of cDNA libraries was with the Illumina TruSeq Stranded mRNA kit. After normalization and multiplexing, the libraries were sequenced at the University of California Irvine's Genomic High Throughput Facility on a Illumina NovaSeq 6000 instrument with paired-end chemistry and 150 cycles to achieve ~100 million reads per sample for the combined *P. leucopus-M. musculus* experiment. The same method for producing cDNA libraries was used for the *R. norvegicus* RNA and the *P. leucopus* in the infection study, but these were sequenced on a Illumina HiSeq 4000 instrument with paired-end chemistry and 100 cycles. The quality of sequencing reads was analyzed using FastQC (Babraham Bioinformatics). The reads were trimmed of low-quality reads (Phred score of <15) and adapter sequences, and corrected for poor-quality bases using Trimmomatic [12].

For the combined species experiment the mean (95% CI) number of PE150 reads per animal after trimming for quality was 1.1 (1.0-1.2) x 10^8 for *P. leucopus* and 1.1 (1.0-1.2) x 10^8 for *M. musculus* (p = 0.91). For *P. leucopus* of this experiment a mean of 83% of the reads mapped to the genome transcript reference set of 54,466; mean coverages for all transcripts and for the mean 62% of reference transcripts with ≥ 1x coverage were 97x and 157x, respectively. For *M. musculus* of this experiment a mean 91% of the reads mapped to the genome transcript reference set of 130,329; mean coverages for all transcripts and for the mean of 21% of reference transcripts with ≥ 1x coverage were 103x and 568x, respectively. For the lower dose-longer duration experiment with *P. leucopus* the mean number of PE150 reads was 2.5 (2.3-2.6) x 10^7. For the rat experiment the mean number of PE100 reads was 2.4 (2.2-2.5) x 10^7. For the *B. hermsii* infection experiment the mean number of PE100 reads was 4.9 (4.5-5.3) x 10^7.

Batched fastq files were subjected to analysis with CLC Genomics Workbench version 23 (Qiagen). Library size normalization was done by the TMM (trimmed mean of M values) method of Robinson and Oshlack [77]. The reference genome transcript sets on GenBank were the following: GCF_004664715.2 for *P. leucopus* LL stock, GCF_000001635.27_GRCm39 for *M. musculus* C57Bl/6, and GCF_015227675.2_mRatBN7.2 for *R. norvegicus*. The settings for PE150 reads were as follows for both strands: length fraction of 0.35, similarity fraction of 0.9, and costs for mismatch, insertion, or deletion of 3. For PE100 reads the settings were the same except for length fraction of 0.4. Principal Component Analysis was carried with the "PCA for RNA-Seq" module of the suite of programs.

For the *P. leucopus* RNA-seq analysis there were 54,466 reference transcripts, of which 48,164 (88%) were mRNAs with protein coding sequences, and 6,302 were identified as non-coding RNAs (ncRNA). Of the 48,164 coding sequences, 40,247 (84%) had matching reads for at least one of the samples. The five most highly represented *P. leucopus* coding sequences among the matched transcripts of whole blood among treated and control animals were hemoglobin subunits alpha and beta, the calprotectin subunits S100A8 and S100A9, and ferritin heavy chain. For the *M. musculus* analysis there were available 130,329 reference transcripts: 92,486 (71%) mRNAs with protein coding sequences and 37,843 ncRNAs. Of the coding sequences, 59,239 (64%) were detectably transcribed in one or both groups by the same criterion. The five most highly

represented coding sequences of mRNAs of identified genes for *M. musculus* were hemoglobin subunits alpha and beta, aminolevulinic synthase 2, ferritin light polypeptide 1, and thymosin beta. For *R. norvegicus* there were 99,126 reference transcripts, of which 74,742 (75%) were mRNAs. The five most highly represented coding sequences of mRNAs of identified genes for *R. norvegicus* were hemoglobin subunits alpha and beta, beta-2 microglobulin, ferritin heavy chain, and S100a9.

Genome-wide differential gene expression

Differential expression between experimental conditions was assessed with an assumption of a negative binomial distribution for expression level and a separate Generalized Linear Model for each [59]. Fold changes in TPM (transcripts per million) were log2-transformed. The False Discovery Rate (FDR) with corrected *p* value was estimated by the method of Benjamini and Hochberg [10]. To assess the limit of detection for differentially expressed genes between 10 animals treated with LPS and 10 with saline alone, we took the data for 4650 reference transcripts for which the mean TPM across 20 *P. leucopus* was >10 and randomly permuted the data to achieve another 9 sets and calculated the fold-change of sub-groups of 10 and 10 with one random group serving as the proxy of the experimental treatment and other second as the control for each of the sets. The expectation was that mean fold-change of the 9 permuted sets and the 4650 reference sequences would be ~1. The result was a mean and median of 1.08 with a 99.9% asymmetric confidence interval for the mean of 0.81-1.49. This was an indication that the choices for sample sizes were realistic for achieving detection of ≥ 1.5x fold changes.

Gene Ontology term analysis

M. musculus was selected as the closest reference for the *P. leucopus* data. The analysis was implemented for data for differentially expressed genes meeting the criteria of a FDR *p*-value ≤ 0.01 and fold-change of ≥ 1.5. The analysis was implemented with the tools of Metascape (*https://metascape.org*) [99]. Functional enrichment analysis was carried out first with the hypergeometric test and FDR *p*-value correction [10]. Then pairwise similarities between any two enriched terms were computed based on a Kappa-test score [22]. Similarity matrices were then hierarchically clustered and a 0.3 similarity threshold was applied to trim resultant trees into separate clusters. The lower the *p*-value, the less the likelihood the observed enrichment is due to randomness [98]. The lowest *p*-value term represented each cluster shown in the horizontal bar graph. Besides the terms beginning with “GO” and referring to the Gene Ontology resource (*http://geneontology.org*) [2], others refer to Kegg Pathway database (*https://www.kegg.jp*) for “mmu..” designations, WikiPathways database (*https://www.wikipathways.org*) for “WP...” designations, and Reactome database (*https://reactome.org*) for “R-MMU...” designations.

Targeted RNA-seq

RNA-seq of selected set of protein coding sequences (CDS), which are listed below, was carried out using CLC Genomics Workbench v. 23 (Qiagen). Paired-end reads were mapped with a length fraction of 0.35 for ~150 nt reads and 0.40 for ~100 nt reads, a similarity fraction of 0.9, and costs of 3 for mismatch, insertion, or deletion to the CDS of sets of corresponding orthologous mRNAs of *P. leucopus*, *M. musculus*, and *R. norvegicus*. Preliminary expression values were unique reads normalized for total reads across all the samples without adjustment for reference sequence length, as described [3]. Exceptions were the endogenous retrovirus coding sequences which differed in lengths between species. For within-and cross-species comparisons we initially normalized three different ways after quality filtering and removing vector and linker sequence: for total reads for the given sample, for unique reads for 12S ribosomal RNA for the mitochondria of nucleated cells in the blood, and for unique reads for the gene Ptprc, which encodes CD45, a marker for both granulocytes and mononuclear cells in the blood. This is described in more detail in Results. Following the recommendation of Hedges et al. we used the natural logarithm (*ln*) of ratios [37].

The target CDS were as follows: Acod1, Akt1, Akt2, Arg1, Bcl3, Camp, Ccl2, Ccl3, Ccl4, Cd14, Cd177, Cd3d, Cd4, Cd69, Cd8, Cfb, Cgas, Csf1, Csf1r, Csf2, Csf3, Csf3r, Cx3cr1, Cxcl1, Cxcl10, Cxcl2, Cxcl3, Dhx58, Fcer2, Fcgr2a, Fcgr2b, Fcgr3 (CD16), Fgr, Fos, Fpr2, Gapdh, Gbp4, Glrx, Gzmb, Hif1a, Hk3, Hmox1, Ibsp, Icam, Ifih1, Ifit1, Ifng, Il10, Il12, Il18, Il1b, Il1rn, Il2ra, Il4ra, Il6, Il7r, Irf7, Isg15, Itgam, Jak1, Jak2, Jun, Lcn2, Lpo, Lrg, Lrrk2, Ltf, Mapk1, Mmp8, Mmp9, Mpo, MT-Co1, Mt2, Mtor, Mx2, Myc, Myd88, Ncf4, Nfkb1, Ngp, Nos2, Nox1, Nr3c1, Oas1, Olfm4, Padi4, Pbib, Pkm, Ptx, Ptprc (CD45), Retn, Rigi (Ddx58), S100a9, Saa3, Serpine1, Slc11a1 (Nramp), Slpi, Socs1, Socs3, Sod2, Stat1, Stat2, Stat4, Steap1, Sting, Tgfb, Thy1, Timp1, Tlr1, Tlr2, Tlr4, Tnf, Tnfrsf1a, and Tnfrsf9. The sources for these coding sequences were the reference genome transcript sets for *P. leucopus*, *M. musculus*, and *R. norvegicus* listed above. If there were two or more isoforms of the mRNAs and the amino acid sequences differed, the default selection for the coding sequence was the first listed isoform. The lengths of the orthologous pairs of *P. leucopus* and *M. musculus* coding sequences were either identical or within 2% of the other. Fcgr1, the gene for high affinity Fc gamma receptor I or CD64, was not included in the comparison, because in *P. leucopus* it is an untranscribed pseudogene (7). For the targeted RNA-seq of blood of deermice infected with *B. hermsii* (**Table 6**), the reference sequence was the cp6.5 plasmid of *B. hermsii* (NZ_CP015335).

Quantitative PCR assays

Reverse transcriptase (RT)-qPCR assays and the corresponding primers for measurement of transcripts of genes for nitric oxide synthase 2 (Nos2) and glyceraldehyde 3-phosphate dehydrogenase (Gapdh) were those described previously (3). These primers worked for *M. musculus* as well as *P. leucopus* using modified cycling conditions. For the arginase 1 transcript assays different primer sets were used for each species. The forward and reverse primer sets for the 352 bp Arg1 product for *P. leucopus* were 5'-TCCGCTGACAACCAACTCTG and 5'-GACAGGTGTGCCAGTAGATG, respectively. The corresponding primer pairs for a 348 bp Arg1 of *M. musculus* were 5'-TGTGAAGAACCCACGGTCTG and 5'-ACGTCTCGCAAGCCAATGTA. cDNA synthesis and qPCR were achieved with a Power Sybr Green RNA-to-Ct 1-Step Kit (Applied Biosystems) in 96 MicroAmp Fast Reaction Tubes using an Applied Biosystems StepOne Plus real-time PCR instrument. The initial steps for all assays were 48 °C for 30 min and 95 °C for 10 min. For Arg1 and Nos2 assays, this was followed by 40 cycles of a 2-step PCR of, first, 95°C for 15 s and then, second, annealing and extension at 60 °C for 1 min. The cycling conditions for Gapdh were 40 cycles of 95 °C for 15 s followed by 60 °C for 30 s. Quantitation of genome copies of *B. hermsii* in extracted DNA was carried out by probe-based qPCR as described (6).

Additional statistics

Means are presented with asymmetrical 95% confidence intervals (CI) to accommodate data that was not normally distributed. Parametric (*t* test) and non-parametric (Mann-Whitney) tests of significance were 2-tailed. Unless otherwise stated, the *t* test *p* value is given. Categorical variables were assessed by 2-tailed Fisher's exact test. FDR correction of *p* values for multiple testing was by the Benjamini-Hochberg method (10), as implemented in CLC Genomics Workbench (see above), or False Discovery Rate Online Calculator (*https://tools.carbocation.com/FDR*). Discriminant Analysis, linear regression, correlation, coefficient of determination, and General Linear Model analyses were performed with SYSTAT v. 13.1 software (Systat Software, Inc.). Box plots with whiskers display the minimum, first quartile, median, third quartile, and maximum.

Data availability

The experiments reported here are associated with National Center for Biotechnology Information (*https://ncbi.nlm.nih.gov*) BioProjects PRJNA975149 for the combined *P. leucopus* and *M. musculus* experiment, PRJNA874306 for the 12 h-lower dose *P. leucopus* experiment, PRJNA973677 for the *R. norvegicus* LPS experiment, and PRJNA508222 for the *B. hermsii* infection of *P. leucopus*

experiment. PRJNA975149 includes 40 BioSamples (SAMN35347136-SAMN35347175) and 40 sets of Sequence Read Archive (SRA) fastq files of Illumina paired-end chemistry reads 1 and 2 (SRR24733648-SRR24733687). PRJNA874306 includes 6 BioSamples (SAMN30561752-SAMN30561757) and the corresponding SRA files SRR24451178-SRR24451180, SRR24451183, SRR24451194, and SRR24451195. PRJNA973677 includes 16 BioSamples (SAMN35351370-SAMN35351385) and the corresponding SRA files SRR24731678-SRR24731693. PRJNA508222 includes 7 BioSamples (SAMN10522571-SAMN10522573; SAMN10522575-SAMN10522578) and SRA files (SRR8283809; SRR8283811-SRR8283816). The datasets of the genome-wide RNA-seq and the differentially-expressed gene analysis of RNA-seq data for the 4 h LPS experiments for *P. leucopus*, *M. musculus*, and *R. norvegicus* are available under the title "Differentially-expressed genes in blood in response to lipopolysaccharide in three rodent species" at the public data repository Dryad (*https://datadryad.org*). The link for Dryad Tables D1-D6 and their descriptions is *https://doi.org/10.5061/dryad.zw3r228dh*. A Transcriptome Shotgun Assembly (TSA) for stranded mRNA of blood of the 20 female and male *P. leucopus* of the 4 h LPS experiment comprised 14,982 contigs of ≥ 400 nt and ≥ 20x coverage and have TSA accession number GKOE00000000.

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Reviewer #1 (Public Review):

Summary:

o A well-executed series of experiments that will likely be of immense interest to (a) vector-borne disease researchers and (b) gram-negative sepsis/bacteremia researchers. The study uses comparative transcriptomics to begin probing what makes Peromyscus leucopus a unique host for numerous pathogens across the tree of life. Authors responded well to concerns raised in peer review and have produced an excellent second version of the manuscript.

Strengths:

o Use of outbred M. musculus is a commendable choice for the studies here.
o Use of both LPS and B. hermsii allows analysis of multiple different signaling pathways that may differ between the species.
o Upload of analyzed data onto Dryad is appreciated.

Weaknesses:

o None noted beyond the authors own limitation discussion section

Reviewer #2 (Public Review):

Milovic, Duong, and Barbour investigate the inflammatory response of three species of small mammals (P. leucopus, M. musculus, and R. norvegicus) to endotoxin lipopolysaccharide (LPS) injection via genome-wide transcriptomics from blood samples. Understanding the inflammation response of P. leucopus is of importance as they are a reservoir for several pathogens. The study is a thorough, controlled, well researched analysis that will be valuable for designing and interpreting future studies. The authors discuss the limitations of the data and the potential directions. Clearly P. leucopus respond differently to the LPS exposure which is very interesting and opens the door for numerous other comparative studies.

The conclusions of the manuscript are thoughtful and supported by the data. The authors addressed my questions about mouse numbers, sex differences, and the presentation of Nos2 and Arg1 data.

Author Response

The following is the authors' response to the original reviews.

Re: Revised author response for eLife-RP-RA-2023-90135 ("The white-footed deermouse, an infection-tolerant reservoir for several zoonotic agents, tempers interferon responses to endotoxin in comparison to the mouse and rat" by Milovic, Duong, and Barbour")

The revised manuscript has taken into account all the comments and questions of the two reviewers. Our responses to each of the comments are detailed below. In brief, the modifications or additional materials for the revision each specifically address a reviewer comment. These modifcations or materials include the following….

• a more in-depth consideration of sample sizes

• a better explanation of what p values signify for a GO term analysis

• a more detailed account of the selection of the normalization procedure for cross-species targeted RNA-seq (including a new supplemental figure)

• several more box plots in supplementary materials to complement the scatterplots and linear regressions of the figures of the primary text

• provision in a public access repository of the complete data for the RNA-seq analyses as well as primary data for figures and tables as new supplementary tables

• the expansion of description of the analysis done for the revision of Borrelia hermsii infection of P. leucopus. This included a new table (Table 10 of the revision) • development of the possible relevance of finding for longevity studies by citing similarities of the findings in P. leucopus with those in the naked mole-rat

• what we think is a better assessment of differences between female and male P. leucopus for this particular study, while still keeping focus on DEGs in common for females and males. This included a new figure (Figure 4 of the revision).

• removal of reference to a “inverse” relationship between Nos2 and Arg1 while still retaining ratios of informative value

We note that in the interval between uploading the original bioRxiv preprint and now we learned of the paper of Gozashti, Feschotte, and Hoekstra (reference 32), which supports our conception of the important place of endogenous retroviruses in the biology and ecology of deermice. This is the only addition or modification that was not a direct response to a reviewer comment or question, but it was germane to one of Reviewer #1’s comments (“Regarding..”).

> ***Reviewer #1:***
>
> *Supplemental Table 1 only lists genes that passed the authors statistical thresholds. The full list of genes detected in their analysis should be included with read counts, statistics, etc. as supplemental information.*

We agree that provision of the entire lists of reference transcripts and the RNA-seq results for each of the 40 animals is merited. These datasets are too large for what the journal’s supplementary materials resource was intended for, so we have deposited them at the Dryad public access repository.

> *While P. leucopus is a critical reservoir for B. burgdorferi, caution should be taken in directly connecting the data presented here and the Lyme disease spirochete. While it's possible that P. leucopus have a universal mechanism for limiting inflammation in response to PAMPs, B. burgdorferi lack LPS and so it is also possible the mechanisms that enable LPS tolerance and B. burgdorferi tolerance may be highly divergent.*

The impetus for the study was the phenomenon of tolerance of infection of P. leucopus by a number of different kinds of pathogens, not just B. burgdorferi. We take the reviewer’s point, though. Certainly, the white-footed deermouse is probably most notable at-large for its role as a reservoir for the Lyme disease agent. We doubt that the species responses to LPS and to the principal agonists of B. burgdorferi are “highly divergent”, though. Other than the TLR itself-TLR4 for LPS vs the heterodimer TLR2/TLR1 for the lipoproteins of these spirochetes--the downstream signaling is generally similar for amounts comparable in their agonist potency.

We had thought that we had addressed this distinction for B. burgdorferi and other Borreliaceae members by referring to the earlier study. But we agree with the reviewer that what was provided on this point was insufficient in the context of the present work.

Accordingly, for the revision we have added a new analysis of the data on experimental infection of P. leucopus with Borrelia hermsii, which lacks LPS and for which the TLR agonists eliciting inflammation are lipoproteins. We do this in a format (new Table 6) that aids comparison with the LPS experimental data elsewhere in the article. As the manuscript references, B. burgdorferi infection of P. leucopus elicits comparatively little inflammation in blood even at the height of infection. While this phenomenon with the Lyme disease agent was part of the rationale driving these studies, the better comparison with LPS was 5 days into B. hermsii infection when the animals are spirochetemic.

> *Statistical significance is binary and p-values should not be used as the primary comparator of groups (e.g. once a p-value crosses the deigned threshold for significance, the magnitude of that p-value no longer provides biological information). For instance, in comparing GO-terms, the reason for using of high p-value cutoffs ("None of these were up-regulated gene GO terms with p values < 1011 for M. musculus.") to compare species is unclear. If the authors wish to compare effect sizes, comparing enrichment between terms that pass a cutoff would likely be the better choice. Similarly, comparing DEG expression by p-value cutoff and effect size is more meaningful than analyses based on exclusively on p-value: "Of the top 100 DEGs for each species by ascending FDR p value." Description in later figures (e.g. Figure 4) is favored.*

Effect sizes--in this case, fold-changes--were taken into account for GO term analysis and were specified in the settings that are described. So, any gene that was “counted” for consideration for a particular GO term would have passed that threshold and with a falsediscovery corrected p value of a specified minimum. There is no further scoring of the “hit” based upon the magnitude of the p value beyond that point. It is, as the reviewer writes, binary at that point. We are in agreement on those principles.

As we understand the comment above, though, the p-values referred to are in regard to the GO term analysis itself. The objective was discovery followed by inference. The situation was more like a genome-wide association study (GWAS) study. This is not strictly speaking a hypothesis test, because there was no stated hypothesis ahead of time or one driving the design. The “p value” for something like GO term analysis or GWAS provides an estimate of the strength of the association. It is not binary in that sense. The lower the p value, the greater confidence about the association. In a GWAS of a human population an association of a trait with a particular SNP or indel is usually not taken seriously unless the p value is less than 10^-7 or 10^-8. In the case of GO terms, the p value approximates (but is not equivalent to) the number of genes that are differentially expressed that belong to a GO cluster out of the total number of genes that define that cluster. The higher the proportion of the genes in the cluster that are associated with a treatment (LPS vs. saline), the lower the p value. Thus, it provides information beyond the point at which it would be rightly deemed of little additional value in many hypothesis testing circumstances.

That said, we agree that the original manuscript could have been clearer on this point and have for the revision expanded the description of the GO term analysis in the Methods, including some explanation for a reader on what the p value signifies here. We also refrain from specifying a certain p value for special attention and merely list 20 by ascending p value.

> *The ability to use of CD45 to normalize data is unclear. Authors should elaborate both on the use of the method and provide some data how the data change when they are normalized. For instance, do correlations between untreated Mus and Peromyscus gene expression improve? The authors seem to imply this should be a standard for interspecies comparison and so it would be helpful to either provide data to support that or, if applicable, use of the technique in literature should be referenced.*

The reviewer brings up an important point that we considered addressing in more depth for the original manuscript but in the end deferred to considerations about length and left it out.

But we are glad to address this here, as well as in the revised manuscript.

We did not intend to imply either that this particular normalization approach had been done before by others or that it “should” be a standard. We are not aware of another report on this, and it would be up to others whether it would be useful or not for them. We made no claim about its utility in another model or circumstance. The challenge before us was to do a comparative analysis of transcription in the blood not just for animals of one species under different conditions but animals of two different genera under different conditions. A notable difference between the animals was in their white blood cell counts, as this study documents. White cells would be the source of a majority of transcripts of potential relevance here, but there would also be mRNA for globins, from reticulocytes, from megakaryocytes, and likely cell-free RNA with origins in various tissues. If the white cell numbers differed, but the non-white cell sources of RNA did not, then there could be unacknowledged biases.

It would be like comparing two different kinds of tissues and assuming them to be the same in the types and numbers of cells they contained. Four hours after a dose of LPS the liver cells (or brain cells) would differ in their transcriptional profiles from untreated the livers (or brains) of untreated animals for sure, but there would not be much if any change in the numbers of different kinds of cells in the liver (or brain) within 4 hours. The blood can change a lot in composition within that time frame under these same conditions. Some sort of accounting for differing white cell numbers in the blood in different outbred animals of two species seemed to be called for.

The normalization that was done for the genome-wide analysis was not based on a particular transcript, but instead was based on the total number of reads, the lengths of the reference transcripts, and the distributions of reads matching to the tens of thousands of references for each sample. This was done according to what are standard procedures by now for bulk RNAseq analyses. Because the reference transcript sets for P. leucopus and M. musculus differed in their numbers and completeness of annotation, we did not attempt any cross-species comparison for the same set of genes at that point. That would not be possible because they were not entirely commensurate.

The GO term analysis of those results provided the leads for the more targeted approach, which was roughly analogous to RT-qPCR. For a targeted assay of this sort, it is common to have a “housekeeping gene” or some other presumably stably transcribed gene for normalization. A commonly used one is Gapdh, but we had previously found that Gapdh was a DEG itself in the blood in P. leucopus and M. musculus at the four hour mark after LPS. The aim was to provide for some adjustment so datasets for blood samples differing in white blood cell counts could be compared. Two options were the 12S ribosomal RNA of the mitochondria, which would be in white cells but not mature erythrocytes, and CD45, which has served an approximately similar function for flow cytometry of the blood. As described in what has been added for the revision and the supplementary materials, we compared these different approaches to normalization. Ptprc and 12S rRNA were effectively interchangeable as the denominator with identifying DEGs of P. leucopus and M. musculus and cross-species comparisons.

> *Regarding the ISG data-is a possible conclusion not that Peromyscus don't upregulate the antiviral response because it's already so high in untreated rodents? It seems untreated Peromyscus have ISG expression roughly equivalent to the LPS mice for some of the genes. This could be compared more clearly if genes were displayed as bar plots/box and whisker plots rather than in scatter plots. It is unclear why the linear regression is the key point here rather than normalized differences in expression.*

In answer to the question: yes, that is possible. In the interval between uploading of the manuscript and this revision, we became aware of a study by Gozashti and Hoekstra published this year in Molecular Biology and Evolution (reference 32) and reporting on the "massive invasion" of endogenous retroviruses in P. maniculatus and the defenses deployed in response to achieve silencing. We cite this work and discuss it, including related findings for P. leucopus, in the revision.

We had originally intended to include box plots as well as scatterplots with regressions for the data, but thought it would be too much and possibly considered redundant. But with this encouragement from the reviewer we provide additional box plots in supplementary materials for the revision.

> *Some sections of the discussion are under supported:*
>
> *The claim that low inflammation contributes to increased lifespan is stated both in the introduction and discussion. Is there justification to support this? Do aged pathogen-free mice show more inflammation than aged Peromyscus?*

We respectively point out that there was not a claim of this sort. We stated a fact about P. leucopus' longevity. We made no statement connecting longevity and inflammation beyond the suggestion in the introduction that the explanation(s) for infection tolerance might have some bearing for studies on determinants of life span.

But the reviewer's comment prompted further consideration of this aspect of Peromyscus biology. This led eventually to the literature on the naked mole-rat, which seems to be the rodent with the longest known life span and the subject of considerable study. The discussion section of the revision has an added paragraph on some of the similarities of P. leucopus and the naked mole-rat in terms of neutrophils, expression of nitric oxide synthase 2 in response to LPS, and type 1 interferon responses. While this is far from decisive, it does serve to connect some of the dots and, hopefully, is considered at least partially responsive to the reviewer's question.

> *The claim that reduced Peromyscus responsiveness could lead to increased susceptibility to infection is prominently proposed but not supported by any of the literature cited.*

There was not this claim. In fact, it was framed as a question, not a statement. Nevertheless, we think we understand what the comment is getting at and acknowledge in the revision that there may be unexamined circumstances in which P. leucopus may be more vulnerable.

> *References to B. burgdorferi, which do not have LPS, in the discussion need to ensure that the reader understands this and the potential that responses could be very different.*

We think we addressed this comment in a response above.

> ***Reviewer #2:***
>
> 1. *How were the number of animals for each experiment selected? Was a power analysis conducted?*

A power analysis of any meaning for bulk RNA-seq with tens of thousands of reference transcripts, each with their own variance, and a comparison of animals of two different genera is not straight forward. Furthermore, a specific hypothesis was not being tested. This was a broad, forward screen. But the question about sample sizes is one that deserves more

attention than the original manuscript provided. This now provided in added text in two places in Methods (“RNA-seq” and “Genome-wide different gene expression”) in the revision.

> 1. *The authors conducted a cursory evaluation of sex differences of P. leucopus and reported no difference in response except for Il6 and Il10 expression being higher in the males than the females in the exposed group. The data was not presented in the manuscript. Nor was sex considered for the other two species. A further discussion of the role that sex could play and future studies would be appreciated.*

We agree that the limited analysis of sex differences and the undocumented remark about Il6 and Il10 expression in females and males warranted correction. For the revision we removed that analysis of targeted RNA-seq of P. leucopus from the two different studies. For this study we were looking for differences that applied to both species. This was the reason that there were equal numbers of females and males in the samples. We agree that further investigation of differences between sexes in their responses is of interest but is probably best left for “future studies”.

But in revision we do not entirely ignore the question of sex of the animal and provide an additional analysis of the bulk RNA-seq for P. leucopus with regard to differences between females and males. This basically demonstarted an overall commensurability between sexes, at least for the purposes of the GO term analysis and subsequent targeted RNA-seq, but did reveal some exceptions that are candidate genes for those future studies.

In the revision, we also add for the discussion and its “study limitations” section a disclaimer about possibly missing sex associated differences because the groups were mixed sexes.

> 1. *The ratio of Nos2 and Arg1 copies for LPS treated and control P. leucopus and M.musculus in Table 3 show that in P. leucopus there is not a significant difference but in M.musculus there is an increase in Nos2 copies with LPS treatment. The authors then used a targeted RNA-seq analysis to show that in P. leucopus the number of Arg1 reads after LPS treatment is significantly higher than the controls. These results are over oversimplified in the text as an inverse relationship for Nos2/Arg1 in the two species.*

We agree. In addition to providing box plots for Arg1 and Nos2, as suggested by Reviewer #1, we also replaced “ratio” in commenting on Arg1 and Nos2, with “differences in Nos2 and Arg1 expresssion” replacing “ratio of Nos2 to Arg1 expression” at one place. At another place we have removed “inverse” with regard to Nos2 and Arg1. But we respectfully decline to remove Nos2/Arg1 from Figure 5 (now Figure 6) or inclusion of Nos2/Arg1 ratios elsewhere. According to our understanding there need not be an inverse relationship for a ratio to have informative value.

We thank the two reviewers for their constructive recommendations and suggestions, in some case pointing out errors we totally missed. For the great majority, the recommendations were followed. Where we decline or disagree we explain this in the response.

> ***Reviewer #1 (Recommendations For The Authors):***
>
> *• How was the FDR < 0.003 cutoff chosen for DEG? All cutoffs are arbitrary but there should be some justification.*

We agree and have provided the rationale at that point in the paper (before Figure 3) in R2: "For GO term analysis the absolute fold-change criterion was ≥ 2. Because of the ~3-fold

Recommendations For the Authors

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> *• How was the FDR < 0.003 cutoff chosen for DEG? All cutoffs are arbitrary but there should be some justification.*

We agree and have provided the rationale at that point in the paper (before Figure 3) in R2: "For GO term analysis the absolute fold-change criterion was ≥ 2. Because of the ~3-fold greater number of transcripts for the M. musculus reference set than the P. leucopus reference set, application of the same false-discovery rate (FDR) threshold for both datasets would favor the labeling of transcripts as DEGs in P. leucopus. Accordingly, the FDR p values were arbitrarily set at <5 x 10-5 for P. leucopus and <3 x 10-3 for M. musculus to provide approximately the same number of DEGs for P. leucopus (1154 DEGs) and M. musculus (1266 DEGs) for the GO term comparison."

> *• It would be helpful to include a figure demonstrating the correlation between CD45 and WBC ("Pearson's continuous and Spearman's ranked correlations between log-transformed total white blood cell counts and normalized reads for Ptprc across 40 animals representing both species, sexes, and treatments were 0.40 (p = 0.01) and 0.34 (p = 0.03), respectively.")*

In both the first version of the revision (R1) and in R2 we provide a fuller explanation of the choice of CD45 (Ptprc) for normalization as detailed in the response to Reviewer #1's public comment. In the revision only Pearson's correlation and p value is given. We did not think another figure was justified after there was additional space devoted to this in both R1 and R2.

> *• Unclear what the following paragraph is referring to-is this from the previous paper? Was this experiment introduced somewhere? "Low transcription of Nos2 and high transcription of Arg1 both in controls and LPS-treated P. leucopus was also observed in the experiment where the dose of LPS was 1 μg/g body mass instead of 10 μg/g and the interval between injection and assessment was 12 h instead of 4 h (Table 4)."*

This experiment is described in the Methods in the original and subsequent versions, but we agree that it is not clear whether it was from present study or previous one. Here is the revised text for R2: "Low transcription of Nos2 in both in controls and LPS-treated P. leucopus and an increase in Arg1 with LPS was also observed in another experiment for the present study where the dose of LPS was 1 μg/g body mass instead of 10 μg/g and the interval between injection and assessment was 12 h instead of 4 h (Table 4)."

> *• Regarding the differences in IFNy between outbred and BALB/c mice-are there any other RNA-seq datasets you can mine where other inbred mice (B/6, C3H, etc) have been injected with LPS and probed roughly the same amount of time later? Do they look like BALB/c or the outbreds?*

In both the original and R1 and R2 we cite two papers on the difference of BALB/c mice. While this is of interest for follow-up in the future, we did not think additional content on a subject that mainly pertains to M. musculus was warranted here, where the main focus is Peromyscus.

• Figure 8 and its legend are difficult to follow. The top half of the figure is not well explained and it's unclear what species this is. Decreased use of abbreviations would help. Consider marking each R2 value as Mus or Peromyscus (As done in Fig 9). There are some typographical errors in the legend ("gree," incomplete sentence missing the words LPS or treatment AND Mus: "Co-variation between transcripts for selected PRRs (yellow) and ISGs (gree) in the blood of P. leucopus (P) or (M) with (L") or without (C)."

This is now Figure 9 in both R1 and R2. We revised it for R1 to include references to the box plots in supplementary materials, but agree with Reviewer #1's recommendation to correct the typos and make the legend less confusing. We did not think that further labeling of the R2 values in the scatterplots with the species names was necessary. The data points are not just colors but also different symbols, so it should be fairly easy for readers to distinguish the regression lines by species. For R2 this is the revised legend with additions in response to the recommendation underlined:

"Figure 9. Co-variation between transcripts for selected PRRs and ISGs in the blood of P. leucopus (P) or M. musculus (M) with (L) or without (C) LPS treatment. Top panel: matrix of coefficients of determination (R2) for combined P. leucopus and M. musculus data. PRRs are indicated by yellow fill and ISGs by blue fill on horizontal and vertical axes. Shades of green of the matrix cells correspond to R2 values, where cells with values less than 0.30 have white fill and those of 0.90-1.00 have deepest green fill. Bottom panels: scatter plots of log-transformed normalized Mx2 transcripts on Rigi (left), Ifih1 (center), and Gbp4 (right). The linear regression curves are for each species. For the right-lower graph the result from the General Linear Model (GLM) estimate is also given. Values for analysis are in Table S4; box plots for Gbp4, Irf7, Isg15, Mx2, and Oas1 are provided in Figure S6."

> *• Discussion section could benefit from editing for clarity. Examples listed: o Unclear what effect is described here "The bacterial infection experiment indicated that the observed effect in P. leucopus was not limited to a TLR4 agonist; the lipoproteins of B. hermsii are agonists for TLR2 (Salazar et al. 2009)."*

Both R1 and R2 include the new section on the B. hermsii infection model. This was added in response to Reviewer #1 public comment. So the expanded consideration of this aspect should address the reviewer's recommendation for more clarity and context here. For R2 we modified the text in the discussion of R1:

"The analysis here of the B. hermsii infection experiment also indicated that the phenomenon observed in P. leucopus was not limited to a TLR4 agonist."

> *o Unclear what the takeaway from this paragraph is: "Reducing the differences between P. leucopus and the murids M. musculus and R. norvegicus to a single all-embracing attribute may be fruitless. But from a perspective that also takes in the 2-3x longer life span of the whitefooted deer mouse compared to the house mouse and the capacity of P. leucopus to serve as disease agent reservoir while maintaining if not increasing its distribution (Moscarella et al. 2019), the feature that seems to best distinguish the deer mouse from either the mouse or rat is its predominantly anti-inflammatory quality. The presentation of this trait likely has a complex, polygenic basis, with environmental (including microbiota) and epigenetic influences. An individual's placement is on a spectrum or, more likely, a landscape rather than in one or another binary or Mendelian category."*

We agree that modification, simplication, and clarification was called for. In response to a public comment of Reviewer #1 we had changed that section, leaving out reference to longevity here. Here is the revised text in both R1 and R2:

"Reducing differences between P. leucopus and murids M. musculus and R. norvegicus to a single attribute, such as the documented inactivation of the Fcgr1 gene in P. leucopus (7), may be fruitless. But the feature that may best distinguish the deermouse from the mouse and rat is its predominantly anti-inflammatory quality. This characteristic likely has a complex, polygenic basis, with environmental (including microbiota) and epigenetic influences. An individual's placement is on a spectrum or, more likely, a landscape rather than in one or another binary or Mendelian category."

> *Minor comments:*
>
> *• Use of blue and red in figures as the -only- way to easily distinguish between groups is a poor choice-both in terms of how inclusivity of color-blind researchers and enabling grayscale printing. Most detrimental in Figure 2, but also slightly problematic in Figure 1. Use of color and shape (as done in other figures) is a much better alternative.*

We agree. Both figures have been modified to include an additional characteristic for denoting the data point. For Figure 1 it is a black filling, and for Figure 2 it is the size of symbol in additon to the color. This should enable accurate visualization by color blind individuals and printing in gray scale. We have added definitions for the symbols within the graph itself, so there is no need to refer to the legend to interpret what they mean.

> *• Note the typo where it should read P leucopus: "The differences between P. musculus and M. musculus in the ratios of Nos2/Arg1 and IL12/IL10 were reported before (BalderramaGutierrez et al. 2021),"*

We thank the reviewer for pointing this typo out, which also carried over to R1. It has been corrected for R2.

> *• Optional: Can the relationship between the ratios in figure 5 and macrophage "types" be displayed graphically alongside the graphs? It's a little challenging to go back and forth between the text and the figure to try to understand the biological implication.*

We considered something like this but in the end decided that we were not yet comfortable assigning "types" in this fashion for Peromyscus.

> ***Reviewer #2 (Recommendations For The Authors):***
>
> *• Be consistent with nomenclature for your species/treatment groups in the text, figures, and tables. For example, you go back and forth between "P. leucopus" and "deermouse" in the text. And in figures you use "P," "Peromyscus", or "Pero".*

In the Methods section of the original and revisions R1 and R2 we indicate that "deermouse" is synonymous with "Peromyscus leucopus" and "mouse" is synonymous with "Mus musculus" in the context of this paper. We think that some alternation in the terms relieves the text of some of its repetitiveness and that readers should not have a problem with equating one with the other. The use of "deermouse" also reinforces for readers that Peromyscus is not a mouse. With regard to the abbreviations for P. leucopus, those were used to accommodate design and space issues of the figures or tables. In all cases, the abbreviations referred to are defined in the legends of the figures. So, we respectfully decline to follow this recommendation.

> *• Often the sentence structure and/or word choice is irregular and makes quick/easy comprehension difficult. Several examples are:*

> *o The third paragraph of the introduction*

We agree that the first and second sentences are unclear. Here is the revision for R2:

“As a species native to North America, P. leucopus is an advantageous alternative to the Eurasian-origin house mouse for study of natural variation in populations that are readily accessible (9, 53). A disadvantage for the study of any Peromyscus species is the limited reagents and genetic tools of the sorts that are applied for mouse studies.”

> *o The first line after Figure 5 on page 9.*

We agree. The long sentence which we think the reviewer is referring to has been in split into two sentences for R2.

“An ortholog of Ly6C (13), a protein used for typing mouse monocytes and other white cells, has not been identified in Peromyscus or other Cricetidae family members. Therefore, for this study the comparison with Cd14 is with Cd16 or Fcgr3, which deermice and other cricetines do have.”

> *o The sentence that starts "Our attention was drawn to..." on page 14.*

We agree that the sentence was awkward and split into two sentences.

“Our attention was drawn to ERVs by finding in the genome-wide RNA-seq of LPS-treated and control rats. Two of the three highest scoring DEGs by FDR p value and fold-change were a gagpol polyprotein of a leukemia virus with 131x fold-change from controls and a mouse leukmia virus (MLV) envelope (Env) protein with 62x fold-change (Dryad Table D5).”

> *• For figures with multiple panels, use A), B) etc then indicate which panel you are discussing in your text. This is a very data heavy study and your readers can easily get lost.*

We agree and have added pointers in the text to the panels we are referring to. But we prefer to use easily understood descriptors like “left” and “upper” over assigned letters.

> *• For all the figures, where are the stats from the t-tests? Why didn't you do a two-way ANOVA? Instead of multiple t-tests?*

Where we are not hypothesis testing and we are able to show all the data points in box-whisker plots with distributions fully revealed, our default position is not to apply significance tests in a post hoc fashion. If a reader or other investigator wants to do this for other purposes, e.g. a meta-analysis, the data is provided in public repository for them to do this. We are not sure what the reviewer means by "multiple t-tests" for "all figures". Where we do 2-tailed t-tests for presentation of data for many genes in a table for the targeted RNA (where individual values cannot shown in the table), there is always correction for multiple testing, as indicated in Methods. The p values shown as "FDR" are after correction.

> *• Results paragraph "LPS experiment and hematology studies"*
>
> *o List the two species for the first description to orient the reader since you eventually include rat data.*

We agree that this is warranted and followed this recommendation for R2.

> *o Not all the mice experienced tachypnea, but the text makes it seem like 100% did.*

We are not sure what the reviewer is referring to here. This is what is in the text on tachypnea: "By the experiment's termination at 4 h, 8 of 10 M. musculus treated with LPS had tachypnea, while only one of ten LPS-treated P. leucopus displayed this sign of the sepsis state (p = 0.005)." The only other mention of "tachypnea" was in Methods.

> *• Figure 1: Why was the M. musculus outlier excluded? Where any other outliers excluded?*

That data point for the mouse was not "excluded" from the graph. It is identified (MM17) for reference with Table 1, and there is the graph for all to see where it is. It was only excluded from the regression curve for control mice. There was no significance testing. There were no other outliers excluded.

> *• Figure 3: explain the colors and make the scales the same for all the panels or at least for the upregulated DEGs and the downregulated DEGs.*

We have modified the legend for Figure 3 to include fuller definitions of the x-axes and a description of the color spectrum. We decline to make the x-axis scale the same for all the panels because the horizontal bars in "transcription down" panels would take up only a small fraction of the space. The x-axes are clearly defined and the colors of the bars also indicate the differences in p-values. We doubt that readers will be misled. Here is the revised legend: "Figure 3. Gene Ontology (GO) term clusters associated with up-regulated genes (upper panels) and down-regulated genes (lower panels) of P. leucopus (left panels) and M. musculus (right panels) treated with LPS in comparison with untreated controls of each species. The scale for the x-axes for the panels was determined by the highest -log10 p values in each of the 4 sets. The horizontal bar color, which ranges from white to dark brown through shades of yellow through orange in between, is a schematic representation of the -log10 p values."

> *• Results paragraph "Targeted RNA seq analysis"*
>
> *o In the third paragraph, an R2 of 0.75 is not close enough to 1 to call it "~1"*

What the reviewer is referring to is no longer in either R1 and R2, as detailed in the authors' response to public comments.

> *o In the 4th paragraph, where are your stats?*

We have replaced terms like "substantially" and "marginally" with simple descriptions of relationships in the graphs.

"For the LPS-treated animals there was, as expected for this selected set, higher expression of the majority genes and greater heterogeneity among P. leucopus and M. musculus animals in their responses for represented genes. In contrast to the findings with controls, Ifng and Nos2 had higher transcription in treated mice. In deermice the magnitude of difference in the transcription between controls and LPS-treated was less."

> *• Figure 4: The colors are hard to see, I suggest making all the up regulated reads one color, the down regulated reads a different color, and the reads that aren't different black or gray.*

This is now Figure 5 in R1 and R2. The selected genes that are highlighted in the panels are denoted not only by color but also by type of symbol. We do not think that readers will have a problem telling one from another even if color blind. The purpose of this figure was to provide an overview and a visual representation with calling out of selected genes, some of

which will be evaluated in more detail later. We thought that this was necessary before diving deeper into the data of Table 2. We do not think further discriminating between transcripts in the categorical way that the reviewer suggests is warranted at this point. So, we respectfully decline to follow this suggestion.

> *• Results paragraph " Alternatively- activated macrophages...."*
>
> *o Include a brief description of Nos2 and Arg1*

We have defined what enzymes these are genes for in R2.

> *o How do you explain the lack of a difference in P. leucopus Arg1? Your text says the RT-qPCR confirms the RNA-seq findings.*

There was a difference in P. leucopus Arg1 by RT-qPCR between control and LPS treated by about 3-fold. By both RNA-seq and RT-qPCR Arg1 transcription is higher in P. leucopus than in M. musculus under both conditions. But we have modified the sentence so that does not imply more than what the data and analysis of the table reveal.

"While we could not type single cells using protein markers, we could assess relative transcription of established indicators of different white cell subpopulations in whole blood. The present study, which incorporated outbred M. musculus instead of an inbred strain, confirmed the previous finding of differences in Nos2 and Arg1 expression between M. musculus and P. leucopus (Figure 5; Table 2). Results similar to the RNA-seq findings were obtained with specific RT-qPCR assays for Nos2 and Arg1 transcripts for P. musculus and M. musculus (Table 3)."

> *• Figure 5: reorganize the panels to make the text description and label with letters, where are the stats?*

We thought the figure (now Figure 6) was self-explanatory, but agree that further explanation in the legend was indicated. We prefer to use descriptions of locations (“upper left”) over labels, like “panel C”, which do not obviously indicate the location of the panel. Of course, if the journal’s style mandates the other format we will do so. Our response about “stats” for boxplot figures is the same as what we provided above.

> *• Results paragraph "Interferon-gamma and interleukin-1 beta..."*
>
> *o Either add the numbers or direct the viewer to where Ifng is in Table 2. The table is very big and Ifng is all the way at the bottom!*

We agree that this table is large, but we thought it better to err on the side of inclusiveness by having a single table, rather than have some genes in the main article and other results in a supplementary table. We thought that it would make it easier for reviewers and readers to find a gene of interest, but we also acknowledge the challenge to locate the genes we highlight. We follow for R2 that reviewer's recommendation to provide some guidance for readers trying to locate a featured gene by pointing relative locations. While adding a column of numbers to already complex table seems more than what is called for, we are depositing an Excel spreadsheet of the table at the Dryad repository to facilitate searching by an interested reader for a particular gene.

> *• Figure 6: stats? The pink and red are hard to easily distinguish from each other. I also suggest not using red and green together for color blind readers.*

With regard to the box-plots and significance testing, please see response above to an earlier recommendation. We have removed an interpretative adjective (i.e. "marked") from the description of the graph. Different symbols as well as colors are used, so we do not think that this will pose a problem for readers, even those with complete red-green color blindness. For what it's worth, with regard to the "red" and "pink" issue, according to the figure on our displays the colors of the two symbols appear to be red and purple. They are also applied to different species and different conditions for those species.

> *• Figure 8: In the legend it says "... PRRs (yellow) and ISGs (gree)" which is a typo, but don't you mean blue not green anyways?*

See response above to Reviewer #1's recommendation. This has been corrected.