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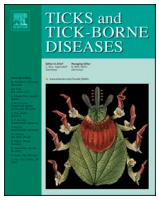

Short communication

Microbial genetic variation impacts host eco-immunological strategies and microparasite fitness in Lyme borreliae-reptile system

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ABSTRACT

Tolerance and resistance are two host eco-immunological strategies in response to microparasite invasion. In the strategy of "resistance", host responses are induced to decrease microparasite replication while the "tolerance" strategy allows hosts coexistence with microparasites by minimizing responses to avoid immune-mediated damage. The causative agent of Lyme disease is a group of genotypically diverse bacterial species, *Borrelia burgdorferi* sensu lato (*Bb*), which is transmitted by *Ixodes* ticks and persists in different reservoir animals. In North America, eastern fence lizards (*Sceloporus undulatus*) can be fed on by *Ixodes* ticks but are incompetent to one genotype of *Bb* (i.e., ospC type A). However, field-collected lizards showed evidence of previous infection by *Bb* strains with undefined genotypes. Supporting this evidence, we introduced three genotypically different *Bb* strains individually to eastern fence lizards and found a *Bb* genotype-dependent manner of infectivity. We compared liver transcriptomics and observed elevated immune responses triggered by a lizard-incompetent *Bb* strain (strain B31). We showed two lizard-competent strains with one having no immunomodulation (strain B379) but the other developing upregulated immune responses (strain 297). These results suggest that genetic variation in microparasites both induces different host strategies for dealing with infection and determines microparasite fitness in the hosts. These findings demonstrate that *Bb* and eastern fence lizards can serve as a model to investigate the mechanisms underlying eco-immunological strategies of tolerance vs. resistance during host-microparasite interaction.

1. Introduction

The evolution of host-microparasite interactions favors hosts developing divergent eco-immunological strategies to combat microparasite invasion (Kilpatrick, 2010). Hosts can mount "resistance" responses to reduce microparasite replication and virulence, resulting in enhanced microparasite clearance (Boots and Bowers, 1999). Alternatively, some hosts develop "tolerance" that minimizes the harmful responses of microparasites (McCarville and Ayres, 2018). The occurrence of tolerance was thought to decrease the host efforts in maintaining resistance mechanisms (e.g., producing functional immune proteins) and/or preventing the trade-off of other physiologically important phenotypes for the maintenance of resistance (Boots and Haraguchi, 1999). Theory predicts a trade-off between resistance and tolerance during host-microparasite interactions (Singh and Best, 2021), and host genetic polymorphisms have been investigated as a determining factor for the occurrence of these different eco-immunological strategies (Best et al., 2008). However, whether pathogen genetic variation contributes to eliciting different host responses remains unclear.

Lyme disease is one of the most common vector-borne diseases in the

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Northern hemisphere and is caused by the genospecies of spirochete bacteria, *Borrelia burgdorferi* sensu lato (also known as *Borrelia burgdorferi* s.l., *Borreliella burgdorferi*, or Lyme borreliae) (Steere et al., 2016) (Adeolu and Gupta, 2014; Margos et al., 2020, 2018). Lyme borreliae has diversified into multiple species. Each species of the Lyme borreliae has evolved into many genetically distinct strains (defined by a series of neutral genes, such as multi-locus sequence types (MLST) or the polymorphic loci, such as *ospC* and RNA intergenic spacer type (RST)) (Cerar et al., 2016; Jones et al., 2006; Lemieux et al., 2023; Margos et al., 2008; Strle et al., 2009, 2011; Wang et al., 1999). These species and strains of Lyme borreliae can be transmitted by *Ixodes* ticks as vectors and maintained in different vertebrate hosts in nature (Tufts et al., 2019; Wolcott et al., 2021). In Europe, the Lacertidae family of lizards coexist with *Ixodes ricinus* ticks that commonly carry Lyme borreliae and were observed as reservoir hosts of a *B. burgdorferi* s.l. species, *Borrelia lusitaniae* ((Amore et al., 2007; Foldvari et al., 2009; Majlathova et al., 2006; Mendoza-Roldan et al., 2019; Musilova et al., 2022; Norte et al., 2015; Richter and Matuschka, 2006; Richter et al., 2013; Taragelova et al., 2016) for the review, please see (Nowak et al., 2024)). In Lyme disease prevalent regions of North America, eastern fence lizards (*Sceloporus undulatus*) colocalize with *Ixodes scapularis* ticks that are competent to *B. burgdorferi* sensu stricto (hereafter *B. burgdorferi*, the main causative agent of Lyme disease in North America) (Eisen et al., 2016; Nowak et al., 2024; Sites, 1992). One genotype of *B. burgdorferi* (*ospC* type A) was demonstrated to not persist in eastern fence lizards (Rulison et al., 2014), but unidentified genotypes of *B. burgdorferi* were observed in eastern fence lizards collected from Lyme prevalent regions or were found to colonize these lizards in laboratory infections (Levin et al., 1996; Nowak et al., 2023; Swanson and Norris, 2007). These results raise the possibility that *B. burgdorferi* has a genotype-specific survivability in reptile hosts and support an understudied concept that genetically distinct Lyme borreliae selectively associate with different vertebrate hosts (Tufts et al., 2019). In this study, we aimed to use *B. burgdorferi* and eastern fence lizards as a model to define the eco-immunological strategies and microparasite fitness impacted by microbial genetic variation during host-microparasite interactions.

2. Materials and methods

2.1. Bacterial strains

The *Borrelia burgdorferi* strains B31–5A4, B379, and 297 utilized in this study are described in Table S1. These strains were used because they are genetically variable and have been documented showing distinct survivability in rodents (e.g., mouse and/or white-footed mice) and birds (e.g., quail and American robins)(Table S1). All *Borrelia* strains were grown in BSK-II completed medium at 33 °C (Barbour et al., 1983). Cultures of *B. burgdorferi* B31–5A4 were tested with PCR to ensure a full plasmid profile before use (Bunikis et al., 2011; Purser and Norris, 2000). As no plasmid profiling approaches for *B. burgdorferi* 297 or B379 have been established, the cultures of these strains were maintained <10 passages to reduce the risks of plasmid loss.

2.2. Lizard collection and husbandry

The eastern fence lizards were captured at the Wharton State Forest at Batsto Village in New Jersey, USA (N39.64275, W74.65147) on August 26th, 2022, between 07:30–15:30. Twenty-five juvenile lizards with estimated ages of 7-week-old were captured. Eastern fence lizards do not develop sexual dimorphisms to be differentiated for sexes until adult stages. Therefore, we were unable to differentiate the sexes of the captured juvenile lizards. At intake, lizards were assigned accession numbers on their ventral scales, between the sternum and anal pore with indelible marker, and weighed for health monitoring. The sera from those lizards were collected to confirm Lyme borreliae seronegative as described in the section "ELISA." Additionally, tails were removed by caudal autotomy. Captive lizard habitats were developed based on previous reports on green anole (*Anolis carolinensis*) husbandry and care, as it has been frequently used as a reptilian model for laboratory studies of morphology and behavior (Lovern et al., 2004). Four 110-liter glass aquaria (76 × 30 × 48 cm) were modeled with identical metal screens, water bowl, plastic trees and leaf litter, wooden hideouts, opaque adsorbent paper between units (to decrease visual interactions between groups), and corn cob bedding. A 14-watt light bulb and UV light bulb with respective reflectors were mounted on the mesh cage tops with transparent indoor-outdoor caulk. Lights were synchronized on a power bar with on-off capacity to maintain a 14:10 day light:dark cycle. Temperature was maintained in a dedicated vivarium suite between 25 and 30 °C, relative humidity at 40–60 %, monitored by internal controls and Bluetooth hygrometers placed in worse case corner of terraria. Crickets, mealworms, and waxworms were maintained in a separate guinea pig cage. Crickets were maintained with high calcium quencher and food particulate over the duration of the study. The weights of each lizard were monitored weekly throughout the experiments and none of the lizards lost weight.

2.3. ELISA

The seropositivity of the lizards after infection with *B. burgdorferi* was determined by detecting the presence or absence of the IgY that recognize C6 peptides, commonly used for Lyme disease diagnosis in humans and other animals and as described in our previous work (Chen et al., 2022; Liang et al., 1999). The goat anti-alligator IgY antibody was used as the secondary antibody (Bethyl lab, Montgomery, TX). The seropositive lizards were defined as the lizards with the serum samples yielding a value greater than the threshold, the mean plus three-fold standard deviation of the IgY values derived from the previously defined Lyme seronegative lizards (Nowak et al., 2023).

2.4. B burgdorferi positivity determination by qPCR and cultivation after infection

Lizards were inoculated subcutaneously under the right axilla (armpit) with *B. burgdorferi* strains (10^6 bacteria in 100 μl of BSK-II media per lizard; seven, six, and seven lizards for B31–5A4-, 297-, and B379-infected groups, respectively), as described (Levin et al., 1996). Five lizards per group were inoculated with BSK-II media only (Control, 100 μl of BSK-II media per lizard). Cohorts were monitored carefully, and checked at least twice daily for location and activity, and weighed weekly. Crickets were added ad libitum, dusted with 2:1 RepCal: Herptivite supplements, replenished at least every other day. Euthanasia was performed on day 30 post infection by injecting lizards subcutaneously with 100ul of 390mg/mL pentobarbital sodium (barbituric acid derivative) with 50 mg phenytoin sodium, followed by decapitation as the secondary confirmatory method. Pentobarbital sodium has been approved by American Veterinary Medical Association for euthanasia of reptiles (AVMA Guidelines for the Euthanasia of Animals, 2020) and was used in this study as no evidence supports that pentobarbital sodium impacts the viability of *Borrelia* spirochetes. We then collected blood, liver, kidney, tail post cloaca (tail), proximal leg tissue/humeroulnar-radial right leg (right leg), and brain. Among these tissues, the liver, kidney, and tail were immediately split into half. Half of each tissue were incubated at 33 °C in the *Borrelia* antibiotics (rifampicin at 45.4 mg/mL, fosfomycin at 193 mg/mL and amphotericin at 0.25 mg/mL; Sigma-Aldrich) mixed with BSK-II media at the ratio of 1:30 for *B. burgdorferi* cultivation. This ratio was determined based on prior experience using different concentrations of antibiotic cocktails, with this ratio shown to prevent contamination (data not shown). Cultures were checked daily until four weeks post incubation. When live or inactive bacteria with spirochetal morphology were observed by dark-field microscopy. The other half of liver, kidney, and tail, as well as the whole right leg and brain were store at −20 °C freezer until the day of

DNA extraction. At the day of DNA extraction, we extracted DNA from these tissues by permitting tissues to be homogenized with a power drill using disposable polyproylene pestles (Fisherbrand™ RNase-Free Disposable pellet pestles). The rest procedure of DNA extraction from tissues and the determination of *B. burgdorferi* burdens in tissues using qPCR based on the amplification of *B. burgdorferi recA* were described previously using the primers indicated in Table S2 (Lin et al., 2014). The number of *recA* copies was calculated by establishing cycle threshold values of a standard curve prepared by serial dilution with a known copy number of *recA* previously extracted from cultures (*B. burgdorferi* B379, 297, B31–5A4). Bacterial burdens were presented as the copy of *recA* in 100 ng of total DNA.

2.5. mRNA Extractions for RNASeq analyses

The right lobe of the liver was placed immediately on dry ice and stored at −80C° until processing completed within 12-h. Zymo Research Direct-zol RNA Miniprep Plus R2072 kit (ThermoFisher Scientific) was used for total RNA extraction of 50 mg of tissues. A drill and polypropylene pestles were used to lyse TRIzol® suspended tissues in Eppendorf Tubes, followed by the treatment of DNAse I as described in the vendor's manuals. Elutions were prepared in DNAse and RNAse free water, and the quality of RNA provided by Nanodrop Eight Spectrophotometer (ThermoFisher Scientific), 260/280 targeting 1.95–2.04. RINe (RNA Integrity Number) values greater than 6.5 with strong indications of robust 18S and 28S (28S/18S) peaks, with minimal evidence of degraded small RNA at the lower threshold range, were submitted for further processing. The RNA samples were processed for mRNA enrichment using Dynabeads mRNA Purification Kit per vendor's manual (ThermoFisher) and then submitted for next generation sequencing with the Illumina MiSeq benchtop system in the Wadsworth Advanced Genomic Technologies Core and Bioinformatics Core.

2.6. RNASeq analysis

Fastq files were processed using the Nextflow workflow management system in the Google Cloud Project. Adapter removal and reads quality filtering were performed with trim_galore v0.6.7 with a quality value cutoff of 20 and a minimum read length requirement of 100 bp. Reads were quantified by Salmon v1.10.2 with the validate mapping argument and the coding sequences of *Sceloporus undulatus* (assembly GCF_019175285.1) serving as the reference transcriptome. The results from Salmon were imported into R v.4.2.3 with tximport and a DESeq2 object was created with the DESeq dataset from tximport function. Differential expression analyses were performed by DESeq2 with minreplicates for replace set to 30. RNASeq results from the 297-, B31–5A4-, and B379-infected lizards were compared to the control.

VolcaNoseR was used to plot and explore differentially expressed genes (DEGs) in volcano plot format (Goedhart and Luijsterburg, 2020). Adjusted p-values ≤ 0.05 and log2 fold changes $\geq$ abs(0.58) were used as the initial cutoffs defining DEGs. Additional analyses defined the cut-offs as adjusted p-value ≤ 0.05 and log2 fold changes $\geq$ abs(2). The number shared and unique DEGs among B31–5A4-, 297-, or B379- vs. mock-infected lizards was visualized as Venn diagram with Venny 2.1 (Oliveros, 2007–2015). The identification of DEGs was shown in Dataset S1.

2.7. Verification of differential expression of mRNA by qRT-PCR

For qRT-PCR, cDNA was synthesized from 1 μg of RNA extracted from the section "The mRNA Extractions for RNASeq analyses" as described previously using qScript cDNA SuperMix (Quanta Bioscience, Beverly, MA) (Lin et al., 2022). These cDNA products were then used to amplify the DNA products of selected genes of lizard proteins, including TNF, NLRX1, TGFβR2, IL1, NFκB2, IL17, IL27, and β-actin using the primers listed (Table S2). Applied Biosystems 7500 Real-Time PCR System (ThermoFisher) in conjunction with PowerUp SYBR Green Master Mix (ThermoFisher) to detect the expression levels of the above-mentioned genes. The cycling parameters were 50 °C for two min, 95 °C for 10 min, and 45 cycles of 95 °C for 15 s, and 60 °C for one min. The resulting amplifications of Ct values for each gene were normalized to respective Ct values from β-actin to obtain the relative expression levels. The relative expression of the genes encoding the above-mentioned proteins, or β-actin was presented by normalizing the Ct-derived from each of these genes to that of β-actin from respective animals through the following equation (Equation 1).

$$\textit{Gene expression relative to } \beta\textit{-actin} = 2^{-(Ct(genes)-Ct(actin))} \quad \text{(Equation 1)}$$

2.8. Statistical analyses

Significant differences were determined with a Kruskal-Wallis test with the two-stage step-up method of Benjamini, Krieger, and Yekutieli (Benjamini, 2006) or two-tailed Fisher test (Fisher, 1934), using GraphPad Prism 9.3.1. A p-value < 0.05 was used to determine significance.

3. Results and discussion

3.1. B burgdorferi displayed a strain-specific survivability in eastern fence lizards

We subcutaneously inoculated wild-caught, Lyme borreliae-seronegative eastern fence lizards with bacterial culture media, BSK-II (mock), or each of three previously tested, *in vitro* phenotypically distinct *B. burgdorferi* strains (Fig. 1A, S1). These strains include B31–5A4 (low competent strain for lizards, control (Rulison et al., 2014)) and competency unknown strains, 297 and B379 (Table S1). We measured *B. burgdorferi* burdens by quantitative PCR (qPCR) at 30 days post infection (dpi) and found that *B. burgdorferi* B31–5A4 colonized brains less efficiently than 297 and B379 and colonized the other tissues less robustly than B379 (Fig. 1B-F). Additionally, strain B379 colonized more efficiently than 297 at tails (Fig. 1C). These results are consistent with our observations when measuring IgY titers against Lyme borreliae C6 peptides: All seven B31–5A4-inoculated lizards turned Lyme borreliae seronegative whereas all tested B379- or 297-infected lizards were seropositive (Fig. 1G). We also cultivated *B. burgdorferi* from these lizard tissues and found no B31–5A4-infected lizards yielding spirochetes cultivated from any tested tissues (Table S3), consistent with reported incompetence of eastern fence lizards to this genotype (*B. burgdorferi ospC* type A) (Rulison et al., 2014). Every 297- or B379-infected lizard had at least one tissue in which spirochetes could be cultivated (Table S3). The results thus grouped tested spirochetes into competent strains (297 and B379 (*ospC* type K) vs. incompetent strains (B31–5A4 (*ospC* type A)), showing a *B. burgdorferi* genotype-specific survival in eastern fence lizards.

3.2. Eastern fence lizards mounted distinct levels of immune responses to B. burgdorferi strains

We next examined the host responses to cope with the inoculation of different *B. burgdorferi* strains by comparing tissue transcriptomics from different infected groups of lizards at 30dpi (Fig. 1A and 2A-C). Liver has a role in producing many immune-related proteins and contains large populations of resident immune cells (Crispe, 2009). Therefore, the livers from these lizards were utilized in this study. Compared to mock-infected lizards, infection with B31–5A4 and 297 resulted in a robust increase differentially expressed genes (DEGs) (Fig. 2A-B): In B31–5A4-inoculated lizards, 942 genes were up- and 804 genes were down-regulated, whereas in 297-inoculated lizards, 2146 genes were up- and 1872 were down-regulated (Fig. 2C-D, adjusted $P < 0.05$ and absolute ($\log_2$fold change) > 0.58). The majority of the upregulated (89 %) and downregulated (92 %) genes were shared between B31–5A4 and

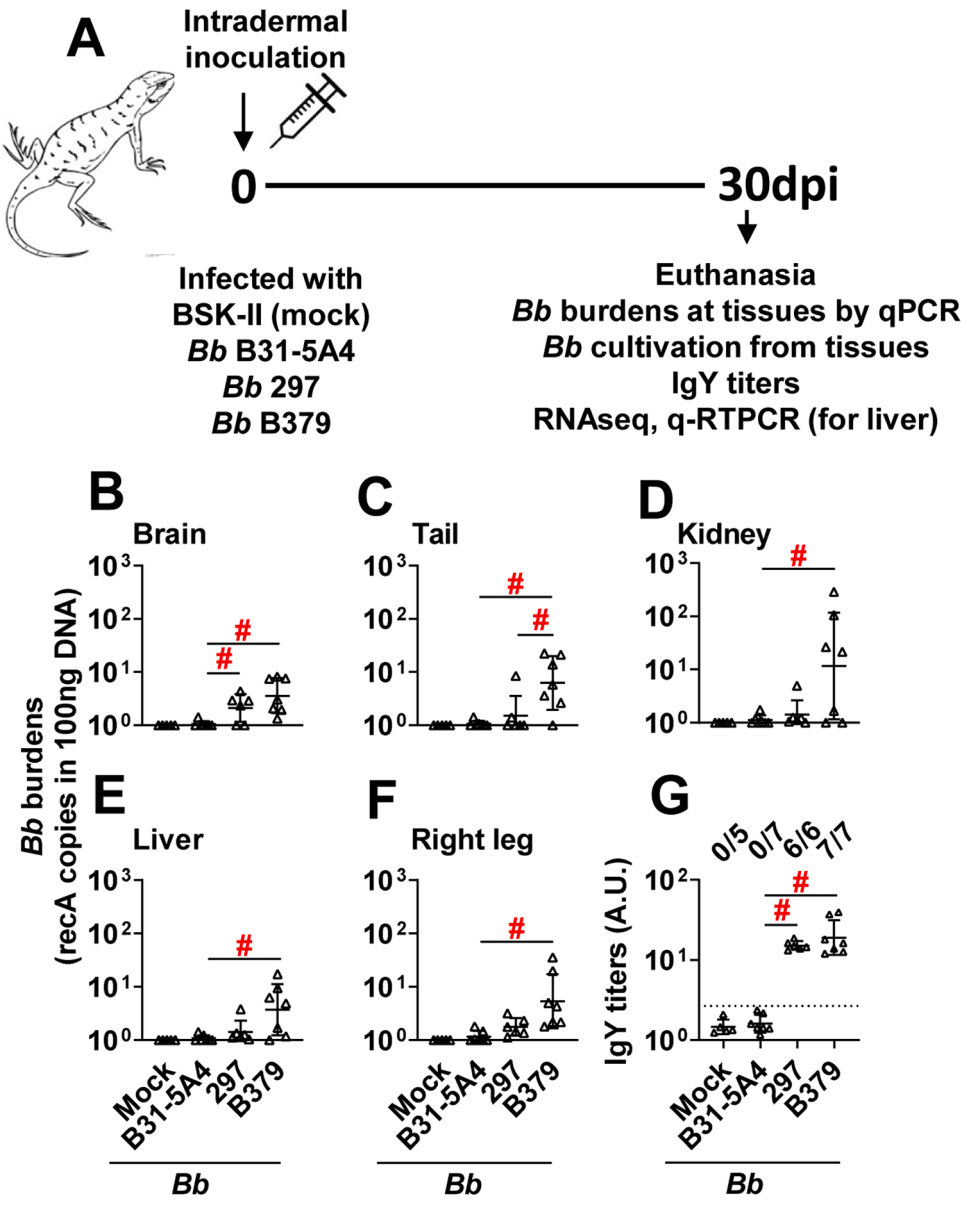

Fig. 1. *B. burgdorferi* displayed a strain-to-strain different levels of colonization in eastern fence lizards. (A) Schematic diagram showing the infection timelines. Eastern fence lizards were subcutaneously inoculated with BSK-II media (Mock; control; five lizards per group) or *B. burgdorferi* (*Bb*) B31–5A4, 297, or B379 (seven, six, and seven lizards for B31–5A4-, 297-, and B379-infected groups, respectively). Indicated tissues were collected at 30 days post inoculation. (B-F) The bacterial loads at (B) brain, (C) tail, (D) kidney, (E) liver, and (F) right leg collected immediately after euthanasia were determined by qPCR. The bacterial loads in tissues were normalized to 100 ng total DNA. Shown are the geometric mean of bacterial loads ± geometric standard deviation of bacterial burdens in five, seven, six, and seven lizards per group for lizards with BSK-II, the strains B31–5A4, 297, and B379, respectively. (G) Lizard sera were applied to determine their levels of recognition to C6 peptides using ELISA as described in the section "ELISA" in Materials and Methods. Data shown are the geometric mean ± geometric standard deviation of IgY titers in five, seven, six, and seven lizards per group for lizards inoculated with BSK-II, the strains B31–5A4, 297, and B379, respectively. The seropositive lizards were defined as lizards with serum samples yielding a value greater than the threshold, the mean plus three-fold standard deviation of the IgY values derived from the seronegative lizards in Fig. S1. Significant differences ($p < 0.05$, the Kruskal-Wallis test followed by the two-stage step-up method of Benjamini, Krieger and Yekutieli.) in the (B-F) spirochete burdens or (G) IgY titers between two strains relative to each other ("#'').

297-infected lizards (Fig. 2C-D). The majority of the B31–5A4-triggered DEGs (89 % and 92 % for up- and down-regulated DEGs respectively) were shared by 297-infected lizards (Fig. 2C-D). This trend was also observed when a more stringent threshold (absolute ($\log_2$fold change) > 1) was used to define DEGs (Fig. S2). Annotation of the DEGs shared by B31–5A4- and 297-infected lizards revealed many immune-related genes, including several inflammatory cytokines (e.g., IL1, IL27, TNF, TGFβ) and receptors and the signaling proteins to confer immunomodulation (e.g., NLRX1, NFkB) (Table S4–5). Cytokines and associated receptors/signaling proteins are often used as proxy to determine inflammatory levels. Using quantitative RT-PCR, we further showed that each of these immune-related genes was significantly up-regulated in the livers of B31–5A4- or 297-infected lizards, compared to those from mock-infected lizards (Fig. 2F-K). Moreover, comparison of B379- and mock-infected lizards identified only one up- and five down-regulated DEGs, respectively (Fig. 2C-E, Table S6), suggesting that lizards did not mount responses to cope with B379 infection. Overall, these findings showed lizards used different eco-immunological strategies in response to the introduction of genetically distinct *B. burgdorferi* strains: resistance for B31–5A4 and 297 and tolerance for B379.

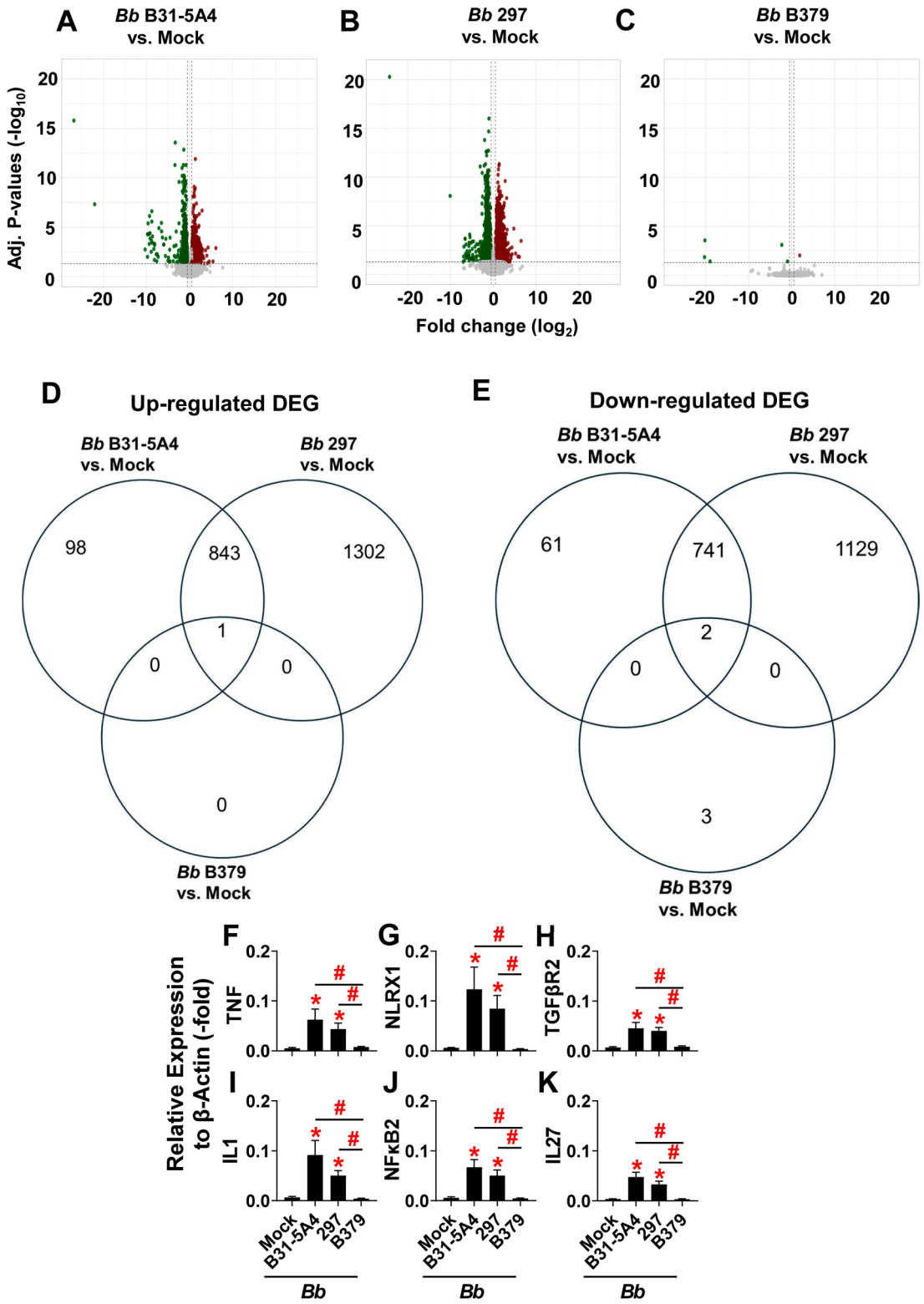

Fig. 2. *B. burgdorferi* infection resulted in strain-to-strain different levels of immunomodulation in eastern fence lizards. Eastern fence lizards were subcutaneously inoculated with BSK-II media (Mock; control; five lizards per group) or *B. burgdorferi* B31–5A4, 297, or B379 (seven, six, and seven lizards animals for B31–5A4-, 297-, and B379-infected groups, respectively). (A-C) At 30 days post inoculation (dpi), the expression levels of lizard genes in the liver from the lizards infected with (A) B31–5A4 (B) 297 or (C) B379 were compared with those from mock-infected lizards and shown in a Volcano plot. Shown are fold changes of each of these genes vs. their adjusted P values. Differentially expressed genes (DEGs) are defined by an adjusted P values <0.05 and absolute ($\log_2$fold change) > 0.58. DEGs are highlighted in red and green, representing significantly up- and down-regulated genes, respectively. (D-E) Venn diagram comparing the number of (D) up- or (E) down-regulated DEGs identified in B31–5A4-, 297-, or B379- vs. Mock-infected lizards from (A-C). (F-K) Ten immune-related DEGs in the livers of B31–5A4- or 297-infected lizards were plotted to compare with their expression levels in indicated groups of the lizards determined by qRT-PCR. Significant differences ($p < 0.05$, the Kruskal-Wallis test followed by the two-stage step-up method of Benjamini, Krieger and Yekutieli.) in the expression levels of the genes in the indicated group, compared to the mock-infeted groups ("*"), or between two infection groups of lizards relative to each other ("#'').

3.3. Microparasite genetic diversity impacts B. burgdorferi fitness under lizard tolerance vs. resistance strategies

When comparing the survivability of B379 vs. B31–5A4 with these strains-triggered immune responses in lizards, our results are consistent with the concept of negative correlation between immune responses and *B. burgdorferi* fitness as reported previously in avian and mammalian hosts (Barbour, 2017; Lin et al., 2022; Milovic et al., 2024). The fact that these two strains have distinct infection and immune response outcomes in the same host lizard species highlights the role of microparasite genetic variation on host eco-immunology strategy. In this study, we did not identify the pathogen determinants that link microbial genetic variation to host eco-immunology strategy. However, previous work showing the host inflammatory responses can be triggered by Lyme borreliae outer surface proteins, some of which are polymorphic (e.g., OspC), suggesting the possibility that polymorphic microbial proteins contribute to microparasite genetic variation-influenced host tolerance vs. resistance strategies, warranting further investigation (Antonara et al., 2010; Salazar et al., 2005). It is noteworthy that the strains 297 and B379 survived in all inoculated eastern fence lizards but triggered distinct levels of immune response although they are the same *ospC* genotype (*ospC* type K). In fact, in addition to OspC, Lyme borreliae produce other allelically variable proteins, some of which confers immune evasion and thus can impact microbial fitness in vertebrate hosts (Lin et al., 2020). One example is that *B. burgdorferi* OspE variants from the strains B379 and 297 facilitate spirochete evasion to lizard complement-mediated killing whereas the OspE variant from B31–5A4 did not (Nowak et al., 2023). Nonetheless, the *in vivo* survivability in lizards for B379 and 297 in this study significantly differed. Thus, complement evasion ability may not be sufficient to support bacterial *in vivo* viability, and the functions conferred by other genomic variation between B379 and 297 may also be involved in the spirochete survivability. In fact, our observations of notable genomic differences between 297 and B379 (Fig. S3) support this concept, particularly within some loci encoding outer surface lipoproteins with immune evasion or virulence association functions (Table S7, Dataset S2). These results thus would build the paradigm in testing the role of these loci to contribute to the fitness differences of B379 and 297 in lizards in the future study.

4. Conclusion

In this study, we used *B. burgdorferi* and eastern fence lizards as a model showing how microbial genetic variation and host eco-immunological strategies play in concert to modulate microparasite fitness. This model can further be used as a platform to investigate the immunological and ecological mechanisms underlying diverse host eco-immunological strategies during host-microparasite interactions.

Abbreviations

Bb: Borrelia burgdorferi sensu lato; Differentially expressed genes (DEGs); quantitative PCR (qPCR); Multi-locus sequence types (MLST); RNA intergenic spacer type (RST).

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Ethics statement

All lizard experiments were performed in strict accordance with all provisions of the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the PHS Policy on Humane Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Wadsworth Center, New York State Department of Health (Protocol docket number 22–451) and Hofstra University (Protocol 19/20–9). The lizard collecting permit was also approved by New Jersey Department of Environmental Protection (Permit number SC 2,020,106) and New York State Department of Conservation (Permit number NYSDEC #609). All efforts were made to minimize animal suffering.

CRediT authorship contribution statement

Tristan A. Nowak: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carly Fernandes:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Data curation. **Jill Malfetano:** Writing – review & editing, Methodology, Investigation, Data curation. **Erica Lasek-Nesselquist:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Matthew Combs:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Klemen Strle:** Writing – review & editing, Validation. **Russell L. Burke:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Yi-Pin Lin:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declarations of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2024.102410.

Data availability

Data will be made available on request.

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