HDAC6 Inhibition Aggravates the Lyme Disease-Associated Inflammation

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Abstract

Histones are basic proteins that wrap around DNA for conditional accessibility of DNA to their transcription; however, HDAC6 is a unique histone deacetylase capable of deacetylating both histone and non-histone proteins. Recently HDAC6 has been shown to play an inflammatory role by limiting p38-MAPK activation in an LPS sepsis model. Here, we tested the ability of HDAC6 inhibition to control inflammation initiated by *B. burgdorferi* both *in vitro* and *in vivo*. The data indicate inhibition of HDAC6 leads to an increase in pro-inflammatory cytokines in macrophages and mice. HDAC6 deficient macrophages revealed similar results when co-incubated with *B. burgdorferi*. Inhibition of HDAC6 also resulted in diverse responses when exposed to different TLR ligands, suggesting a more complex role of HDAC6 in the inflammatory process. Additionally, inhibition of HDAC6 led to an increased acetylation of tubulin; however, there was minimal impact on p38-MAPK phosphorylation.

Keywords: HDAC6; Tubastatin A; Borrelia; Inflammation; TLR

Highlights:

1. HDAC6 inhibition leads to more pro-inflammatory cytokine production in response to *B. burgdorferi.*

2. HDAC6-deficient macrophage transcribe more pro- and anti-inflammatory mediators in response to *B. burgdorferi.*

3. HDAC6 inhibition impacts differently on various TLR ligands recognition by the macrophage.

4. TBSA treatment of mice does not reduce the *Borrelia*-induced inflammation.

Introduction

Lyme disease is caused by a spirochetal pathogen transmitted to the mammalian host by tick bite [1]. The bacterium does not have any known toxin to cause the disease symptoms; however, it contains several ligands for Toll-like receptor(s) (TLRs), recognition of which by the immune cells triggers a formidable inflammatory process [1]. The initiation of the inflammatory process is required for the clearance of the *Borrelia burgdorferi*; however, a timely resolution of the inflammation is needed for regression of the symptoms [2-4]. Inflammation initiated by the bacterium is a convoluted process involving several receptors, signaling molecules and gene functions. Recently inhibition of histone deacetylase 6 (HDAC6) was shown to be helpful in inflammatory conditions by inhibiting the pro-inflammatory cytokine production by macrophages [5-7]. Additionally, inhibition of HDAC6 increased p38-MAPK activation in macrophages treated with a TLR4 ligand LPS that has been shown to be essential for the production of anti-inflammatory cytokine IL-10 [7]. We and others showed a protec-

tive role of p38-MAPK in bacterial clearance and resolution of cytokine production during Lyme borreliosis [4,8,9]. Therefore, we tested inflammatory cytokine production by the primary macrophages in response to *B. burgdorferi.*

Herein we found an increase in tubulin acetylation upon HDAC6 inhibitor treatment; however, there was no impact on the activation of p38-MAPK upon exposure to live *B. burgdorferi*. Furthermore, drug treatment led to an increase in pro-inflammatory cytokines and chemokine transcription both *in vitro* and *in vivo*. Similar data were obtained using HDAC6 deficient macrophages. Further investigation also revealed a dichotomous role of HDAC6 in pro-inflammatory cytokine production based upon the TLR ligand used, shedding light on a few contradictory findings.

Materials and Methods

Materials

The following antibodies were used for Western blot analysis: phosphor-p38 (Cat # 4511, Cell signaling, Denver MA), acetylated alpha-tubulin (Cat # 3971, Cell Signaling, Denver MA) and GAPDH (Cat # G8795, Sigma Adrich,). To stimulate macrophages, Lipopolysaccharide (LPS) (Cat # L9641, Sigma Aldrich), Monophosphoryl Lipid A (MPL) (Cat # L6895, Sigma Aldrich), CpG DNA (Cat # ODN 2007, Invivogen USA), poly IC (Cat # tlrl-pau, Invivogen USA), Pam3Cysk4 (Cat # tlrl-pam) and Flagellin (Cat # SRP8029, Sigma Aldrich) were used. HDAC6 inhibitor Tubastatin A (PubChem CID: 57336514) was purchased from LC Laboratories.

Cultivation of *Borrelia burgdorferi* and animal infection

Low passage *Borrelia burgdorferi* 297 were maintained at 23°C in Complete Barbour-Stonner_kelly medium (BSK-H) (Cat # B8291; Sigma Aldrich). A 12 ml BSK-H media were inoculated at the final concentration of 5×10^4 spirochetes/ml and kept at 37°C. When the culture concentration reached upto 5×10^7/ml OspC expression was determined by silver staining of the whole cell lysate. After confirming the expression of OspC, bacteria were washed twice with phosphate-buffered saline lacking Ca^{++} and Mg^{++}, counted on dark field microscope, and finally resuspended in PBS with a final concentration of 2×10^6/ml. 50 μl of this bacterial suspension were injected at sternum via interdermal route to inject 1×10^5 spirochetes in each mouse.

Bone marrow-derived macrophage culture and treatment with the inhibitor

Bone marrow-derived macrophages (BMDMs) were generated from the C3H/HeN mice obtained from Taconic Farms as described earlier [4]. The BMDM cells were pretreated with 1μM Tubastatin A (TBSA) for 18 h, and then co-incubated with the bacteria at MOI=10. Femurs isolated from HDAC6 KO (Hdac6-/-) mice gifted from Dr. Alejandro Villagra, Moffitt Cancer Center were used to generate Hdac6-/- macrophages; whereas femurs of C57BL/6 mice purchased from Jackson Laboratories were used to generate Hdac6 competent macrophages for the comparison.

RNA isolation and quantitative real-time PCR

Total RNA were isolated using Aurum RNA isolation kit (Bio-Rad, Hercules, California) and the isolated RNA (0.5 μg) were converted to cDNA using iScript reverse transcriptase kit (Bio-Rad, Hercules, California) as per manufacturer instructions. The generated cDNA (0.1 μl/reaction) were used to measure the relative amount of RNA in the sample. The data were normalized with 18S rRNA (endogenous control), and fold change was calculated by the 2^dCT method as described earlier [4]. All primer sequences used in this study are listed in table 1.

Names	Sequence	Reference
Tnf For	AGTGGTGCCAGCCGATGGGTTGT	[25]
Tnf Rev	GCTGAGTTGGTCCCCCTTCTCCAG	
Il1a For	AGGGAGTCAACTCATTGGCG	This study
Il1a Rev	CTTCCCGTTGCTTGACGTTG	
Il23 For	GCTGTGCCTAGGAGTAGCAG	This study
Il23 Rev	CACTGGATACGGGGCACATT	
Cxcl1 For	ACTCAAGAATGGTCGCGAGG	This study
Cxcl1 Rev	GTGCCATCAGAGCAGTCTGT	
Cxcl2 For	GCTGTCCCTCAACGGAAGAA	This study
Cxcl2 Rev	CAGGTACGATCCAGGCTTCC	
Cxcl16 For	GCACTCAGCACTCCACTCTT	[11]
Cxcl16 Rev	AGTTCTGCTGTGTCGCTCTC	
Socs1 For	AGGATGGTAGCACGCAAC	[4]
Socs1 Rev	GAAGACGAGGACGAGGAG	
Socs3 For	AGGAGAGCGGATTCTACTG	[4]
Socs3 Rev	TCACACTGGATGCGTAGG	
Ccl3 For	TCTGCGCTGACTCCAAAGAG	This study
Ccl3 Rev	GTGGCTACTTGGCAGCAGCAAAC	
Iigp For	GTAGTGTGCTCAATGTTGCTGTCAC	[11]
Iigp Rev	TACCTCCACCACCCCAGTTTTAGC	
Igtp For	TAGAGCAGACCCACAGAGTTCAGG	[11]
Igtp Rev	CAGCAGTCATAGATTTAGACCACGG	
Cxcl3 For	GAAAGGAGGAAGCCCCTCAC	This study
Cxcl3 Rev	ACACATCCAGACACCGTTGG	
vα14i-jα18 For	CACCCTGCTGGATGACACTGCC	[8]
vα14i-jα18 Rev	CTCCAAAATGCAGCCTCCCTAAG	
Bb For	GGTCAAGACTGACGCTGAGTCA	[11]
Bb Rev	GGCGGTCCACTTAACACGTTAG	
Csfr1 For	CCTCTTCCTCTGTTCCCTTTCA	This study
Csfr1 Rev	GATGCAGGTTGGAGAGTCGG	
18S rRNA For	ATAGCGTATATTAAAGTTG	[4]
18S rRNA Rev	GTCCTATTCCATTATTCC	

Table 1

In vivo experiment

C3H/HeN mice were obtained from Taconic Farms and kept in the Animal Facility at the University of Florida under an approved IACUC. TBSA dissolved in DMSO (3 mg/mL) was delivered daily by intraperitoneal (i.p.) injection at 0.5 mg per kg (diluted in PBS) a day

before the introduction of *B. burgdorferi* and then daily for two weeks. The mice were infected subcutaneously with 1 x 10^5 live *B. burgdorferi,* as described earlier [4]. Mice described as infection only also received equivalent amount of the vehicle including DMSO. At two-week post-infection, mice were euthanized, the joint thickness measured by digital calipers, and total joint RNA isolated for transcript analysis, as mentioned earlier [4].

Statistics

A two-way Analysis of Variance (ANOVA) followed by a Fisher LSD posthoc test (Prism 8, GraphPad Software Inc., La Jolla, Ca) was used to analyze data presented in figure 4A. Other data were analyzed for statistical significance using parametric two-way ANOVA with Bonferroni correction if data fitted a Gaussian distribution (tested using the method Kolmogorov and Smirnov). The data were analyzed for statistical significance using a non-parametric two-way ANOVA with Mann-Whitney U test when the data were not distributed normally. An $\alpha = 0.05$ was used to determine whether data were significant.

Results

Inhibition of HDAC6 increases tubulin acetylation but does not increase p38-MAPK phosphorylation in response to *B. burgdorferi*

Immune responses initiated by the recognition of *B. burgdorferi* determines the overall One of the primary known targets of HDAC6 is tubulin [10]; therefore, we tested the capacity of the HDAC6 inhibitor to acetylate alpha-tubulin. BMDMs isolated from the Lyme susceptible C3H/HeN mice were treated with 1 µM Tubastatin A (TBSA) for 18h and the cells were either co-incubated or left alone with *B. burgdorferi* at MOI of 10. The total protein was subjected to Western blot for the evaluation of tubulin acetylation (Figure 1A). The data showed a robust increase in tubulin acetylation in the cells treated with TBSA. Earlier, tubulin acetylation was linked to an increase in p38-MAPK phosphorylation upon LPS treatment [7]; however, macrophages pretreated with TBSA and co-incubated with *B. burgdorferi* at 10 MOI, did not show increased p38 activation (Figure 1A).

Inhibition of HDAC6 leads to robust inflammatory cytokine production in response to *B. burgdorferi*

There are several conflicting reports on cytokine production by the macrophages in response to bacterial cell wall constituents, e.g. LPS [6,7]. We blocked the HDAC6 activity with 18h incubation using 1 µM TBSA and then co-incubated the cells with *B. burgdorferi* (10 MOI). Total RNA was isolated and tested for gene activity of inflammatory and disease susceptibility genes; all the inflammatory indicator genes (*Tnf, Il1a, Il23, Ccl3, Cxcl1, Cxcl2, Cxcl3, Cxcl16*) were significantly elevated, whereas the known anti-inflammatory genes (*socs1* and *socs3*) remained unchanged (Figure 1B).
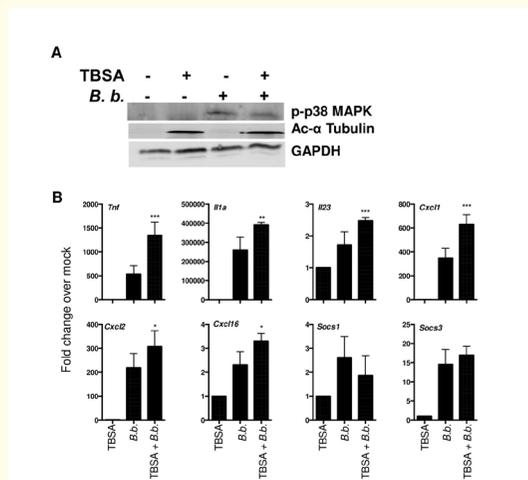

Figure 1: *HDAC6 inhibition increases the pro-inflammatory cytokine transcription in response to Borrelia burgdorferi. (A) Macrophages from C3H/HeN mice were either pretreated with 1uM TBSA for 18 h and then exposed with B. burgdorferi for 30 mins. Total proteins were isolated and subjected for Western blot analysis using anti phospho-p38, acetylated alpha tubulin and GAPDH. (B) C3H/HeN macrophages were pretreated with 1uM TBSA for 18h and then they were coincubated with 10 MOI B. burgdorferi for 6 h. Total RNA were isolated and quantitative real-time PCR was used to evaluate the transcription. 18S rRNA was used as endogenous control and the uninfected, untreated C3H/HeN cells were used as control. Results represent mean +/- SEM from three independent experiments. *P < 0.05, **P < 0.005, ***P < 0.001.*

HDAC6 deficient cells transcribe more inflammatory mediators in response to *B. burgdorferi*

Previous studies have shown that sometimes, pharmacological inhibition leads to a different outcome due to non-specific inhibition of related enzymes. TBSA is a well-studied inhibitor of HDAC6 that has been used extensively in the field; however, to evaluate the role of HDAC6 in *B. burgdorferi* induced inflammation, we co-incubated wild-type and *Hdac6* deficient macrophages with *B. burgdorferi* at MOI=10 for 6 h. *Hdac6*^{-/-} macrophages transcribed significantly higher levels of pro-inflammatory cytokines (*Tnf, Il23, Il1a*) and chemokines (*Ccl3, Cxcl1, Cxcl2*). The anti-inflammatory mediators *Socs1* and *Socs3* were also elevated in absence of HDAC6, possibly in response to excessive pro-inflammatory cytokines; however, the increase was not statistically significant due to higher variability (Figure 2).
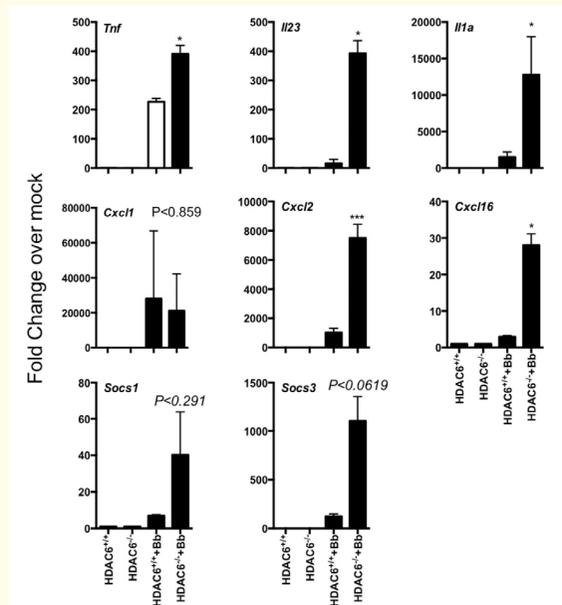

*Figure 2: HDAC6 deficiency leads to higher pro-inflammatory and anti-inflammatory mediators in response to B. burgdorferi. HDAC6 competent and deficient macrophages were coincubated with 10 MOI B. burgdorferi for 6 h. Total RNA was isolated and quantitative real-time PCR was used to evaluate the transcription. 18S rRNA was used as endogenous control and the uninfected cells of respective genotype were used as control. Results represent mean +/- SEM from three independent experiments. *P < 0.05, ***P < 0.001.*

Differential impact of TBSA on the response to different TLR ligands

TLRs recognize different microbial signatures with 11 different receptors on various immune cells. Depending upon the ligand(s) present on the pathogens, different TLRs are engaged to provide a complex outcome in their recognition. To test whether TBSA influences all the TLRs in the same fashion, we incubated macrophages generated from C3H/HeN mice with PamCys4K (1µg/ml), PolyI:C (1 µg/ml), LPS isolated from *E. coli* (1 µg/ml), MPL (1 µg/ml), Flagellin (100 ng/ml) and CpG DNA (1µg/ml) for 6h in the presence or absence of HDAC6 inhibition as mentioned above. Transcription of *Tnf* as a representative of inflammatory genes was evaluated. In response to different TLR ligands macrophages transcribed *Tnf* at various levels; macrophages pretreated with TBSA showed a diverse influence on *Tnf* transcription with varying ligands of TLR. TLR2 ligand (Pam3Cys4K) and TLR9 ligand (CpG DNA) response increased in the presence of TBSA, while TLR3 ligand (poly IC) and TLR5 ligand (Flagellin) responses decreased with HDAC6 inhibition by TBSA. We have used two different TLR4 ligands, LPS from *Escherichia coli* and monophosphoryl lipopolysaccharide (MPL), and they responded differently in

the presence of TBSA. Conventional LPS responses increased, whereas MPL responses muted in the presence of TBSA, suggesting more a complex role of TBSA during this signaling (Figure 3).
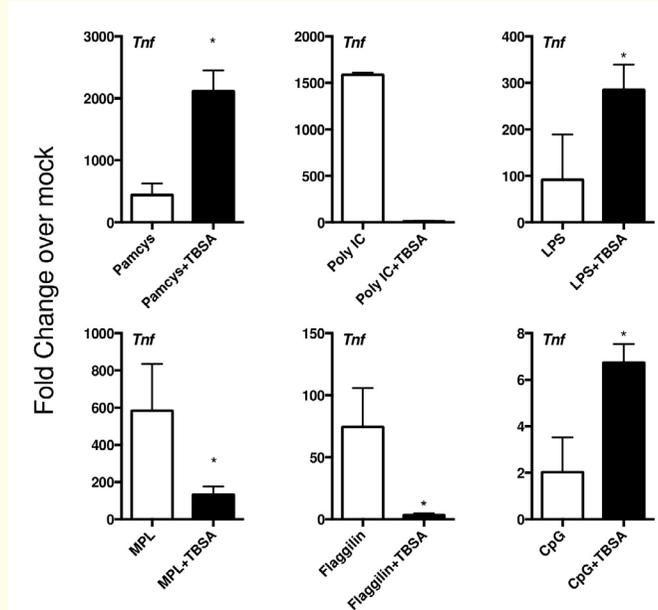

*Figure 3: HDAC6 inhibition leads to a differential transcription of Tnf in response to different TLR ligands. Macrophages from C3H/HeN mice were pretreated with 1 uM TBSA for 18h and then exposed with Pam3CysK4 (1 μg/ml), Poly IC (1 μg/ml), LPS (1 μg/ml), MPL, (1 μg/ml) Flaggelin (100 μg/ml) or CpG DNA (1 μg/ml) for 6h. Total RNA was isolated, and quantitative real-time PCR was used to evaluate the transcription. 18S rRNA was used as endogenous control and the uninfected, untreated C3H/HeN cells were used as control. Results represent mean +/- SEM from three independent experiments. *P < 0.05.*

TBSA increases inflammatory signatures *in vivo*

To test the capacity of the TBSA to modulate Lyme disease pathology susceptible C3H mice were injected with TBSA (0.5 mg per kg) one day before the infection with *B. burgdorferi* and then daily until the completion of the experiment. Two weeks post-infection, mice were euthanized to measure tibiotarsal joint thickness and joints were excised as described earlier for isolation of RNA [4,11]. The total RNA was isolated, and inflammatory gene signatures were measured. The *B. burgdorferi* specific 16S rRNA were used to determine the presence of bacteria in the infected joints [11]. Mice inoculated subcutaneously with 1 x 10^5 live temperature shifted *B. burgdorferi* 297 strain showed a significant increase in tibiotarsal joint thickness measured by digital calipers (Figure 4A). However, no significant difference was found between the TBSA treated and untreated mice at two-week post-infection. The isolated RNA showed increase in inflammatory signatures in the mice treated with TBSA compared to untreated mice with infection. TBSA treatment increased the expression of leukocyte chemo-attractants (*Ccl3, Cxcl2, Cxcl1, Cxcl3, Cxcl16*) to bring more immune cells at the inflammatory locus. Despite the increase of the chemokines above, there was a ten-fold increase in the presence of Borrelia-specific 16S rRNA [11] in the joints of the mice treated with TBSA. Additionally, in the TBSA treated mice joints, greater accumulation of invariant chain natural killer T cells (iNKT cells) was found, determined by the PCR primers detecting the *vα14i-jα18* junction as mentioned earlier [8] (Figure 4B).
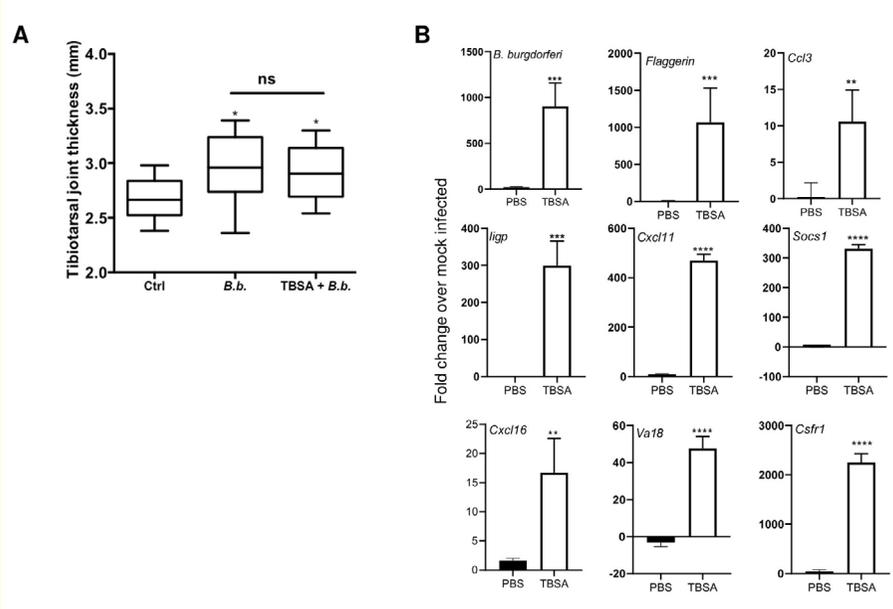
*Figure 4: HDAC6 inhibition does not reduce the inflammation in murine Lyme Borreliosis. C3H/HeN mice were infected with 1 x 105 B. burgdorferi. Infected mice were treated either with 0.5 mg/Kg TBSA or equivalent volume of solvent. At two-week post-infection mice were euthanized and (A) tibiotarsal joint thickness were measured using digital calipers. (B) Total RNA isolated and quantitative real-time PCR was used to evaluate the transcription. 18S rRNA was used as endogenous control and the RNA isolated from the uninfected, untreated C3H/HeN mice joints were used as control. Results represent mean +/- SEM from three independent experiments. *P < 0.05.*

Discussion

Borrelia burgdorferi is the etiology of Lyme disease, the most common vector-borne disease in the United States. There is a general understanding that the symptoms arise from bacterial recognition by innate immune cells [1]. Therefore, a better understanding of the signaling events and unveiling different signaling partners will help in the development of next-generation interventions. Recently, several published conflicting reports [5-7] regarding the role of HDAC6 in macrophages and dendritic cells intrigued us to test this molecule in the murine model of Lyme disease. HDAC6 is the only Histone deacetylase that contains two functional N-terminal catalytic domains along with a C-terminal ubiquitin-binding area [12]. The catalytic domains of the enzyme deacetylate tubulin, cortactin, and Hsp90 to regulate cell motility, cilium assembly, cell adhesion, immune synapses, macropinocytosis and maturation of the glucocorticoid receptor [13-15]. Depending on its availability in the nucleus, HDAC6 can also deacetylate histones [16]. In addition to its deacetylase domains, HDAC6 possesses a ZnF-UBP finger that binds to ubiquitin and is involved in ubiquitin-dependent aggresome formation and cellular clearance of misfolded proteins by autophagy [17]. HDAC6 also interacts with several nuclear factors, HDAC11, sumoylated p300 transcriptional core-pressor LCoR and transcription factors NF-kB and Runx2 [12]. Based upon the different substrates of HDAC6, one can conclude that inhi-bition of a multifunctional protein, such as HDAC6 would have a broader impact on cell behavior and overall outcome of the phenotype. Earlier, in a few reports using specific pharmacological inhibitors or knockout mice, a complex role of HDAC6 came out. A few published reports suggested HDAC6 inhibitors could be used as an anti-inflammatory drug against LPS. Wang., *et al*. showed that the increase in IL-10 production by the cells during TBSA treatment is due to increased p38 activation [7]. However, others showed no change in IL-10 production in macrophages upon treatment with HDAC inhibitor, but there was an effect on TNF production in response to LPS, suggest-

ing some other pathway involved in the process [6]. Contrary to these reports, others reported macrophages and dendritic cells deficient in HDAC6 released less IL-10 and more pro-inflammatory cytokines [18,19]. The ability of HDAC6 to contribute to IL-10 transcription was associated with its ability to recruit HDAC11 and SP1 at the IL-10 locus of chromatin [20].

We showed the anti-inflammatory properties of p38-MAPK in Lyme disease, where p38 played a critical role in the induction of anti-inflammatory mediators, *Socs1*, *Socs3* and *Il10* in mice and isolated macrophages in response to live *B. burgdorferi* [4,9]. Additionally, the limited activity of p38-MAPK in myeloid cells showed a decrease in iNKT cell-mediated bacterial clearance [8]. Inhibition of HDAC6 using TBSA did not impact the activity of p38-MAPK when macrophages were exposed to *B. burgdorferi*; however, TBSA treatment increased the tubulin acetylation. The inhibition of HDAC6 also increased the pro-inflammatory cytokine transcription, such as *Tnf*, *Il1a,* and *Il23*, along with the increase in the critical chemokines (*Ccl3, Cxcl1, Cxcl3, Cxcl16*). Earlier, we reported p38-MAPK-mediated elevation in the transcription of *Socs1, Socs3* and *Il10* controls the proinflammatory cytokine release during the recognition of *B. burgdorferi* by the macrophages, which remained unchanged in the macrophages with HDAC6 inhibition. To confirm the role of HDAC6 on the proinflammatory cytokines and chemokine transcription, we exposed HDAC6-deficient macrophages to *B. burgdorferi* that showed similar responses. In several reports where TBSA showed an anti-inflammatory role, the source of the LPS was not clear [6-8]; LPS from different origins engages different signaling cascades [21-24]. We found the opposite influence of TBSA on *Tnf* transcription upon recognition of LPS isolated from *E. coli* and a synthetic ligand MPL. Both, LPS and MPL are recognized by TLR4; however, the coreceptors' engagement during their signaling differs subtly. We further expanded the study to understand the differential impact of TBSA on the different TLR ligands. We tested the influence of TBSA on TLR2, 3, 5 and 9 signalings, which showed diverse effects. Pretreatment of TBSA led to an increase in *Tnf* transcription by synthetic TLR2 ligand Pam3Cys4K and CpG; however, *Tnf* transcription in response to TLR3 ligand polyIC and TLR5 ligand flagellin was reduced in the macrophages pretreated with TBSA. These diverse effects of TBSA on TLR signaling suggest a more intricate influence of TBSA on inflammation. Therefore, it is speculated that the inflammation induced by a pathogen is diverse due to simultaneous engagement of different TLR ligands.

Resolution of bacteria-induced inflammation hinges on cellular signaling and clearance of instigating bacteria that can only be studied *in vivo*. We treated infected C3H mice for two weeks, and at the end of the experiments, joint thickness and gene transcripts were evaluated. TBSA treatment did not change the joint thickness; however, TBSA treated mice exhibited higher proinflammatory gene signatures along with the increase in bacterial specific 16S rRNA. Often elevated proinflammatory cytokine and chemokine production leads to the accumulation of immune cell to facilitate bacterial removal. However, despite the robust inflammatory response, there was more bacterial RNA in the joints suggesting impaired phagocytosis and/or cell migration. Both phagocytosis and cell migration are dependent upon the tubulins and actins. Earlier reports indicated that there is no difference in the phagocytosis of the bacteria during HDAC6 inhibition [5,6] however, impaired cell migration has been implicated with HDAC6 inhibition [10]. Thereby, we speculate that the strong inflammatory response is not the pivotal factor and there may be incapacitated cell migration resulting in decreased bacterial clearance.

Author Contributions

MM, LP, CV and BS did experimentation and BS wrote the manuscript.

Conflicts of Interest

There is no conflict of interest disclosed.

Bibliography

1. Radolf JD., *et al.* "Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes". *Nature Reviews Micro-biology* 10.2 (2012): 87-99.

2. Lasky CE., *et al.* "Macrophage Polarization during Murine Lyme Borreliosis". *Infection and Immunity* 83.7 (2015): 2627-2635.

3. Sonderegger FL., *et al.* "Localized production of IL-10 suppresses early inflammatory cell infiltration and subsequent development of IFN-gamma-mediated Lyme arthritis". *Journal of Immunology* 188.3 (2012): 1381-1393.

4. Sahay B., *et al.* "CD14 signaling restrains chronic inflammation through induction of p38-MAPK/SOCS-dependent tolerance". *PLOS Pathogens* 5.12 (2009): e1000687.

5. Li Y., *et al.* "Inhibition of histone deacetylase 6 improves long-term survival in a lethal septic model". *The Journal of Trauma and Acute Care Surgery* 78.2 (2015): 378-385.

6. Yan B., *et al.* "HDAC6 deacetylase activity is critical for lipopolysaccharide-induced activation of macrophages". *PLoS One* 9.10 (2014): e110718.

7. Wang B., *et al.* "Microtubule acetylation amplifies p38 kinase signalling and anti-inflammatory IL-10 production". *Nature Communications* 5 (2014): 3479.

8. Hawley K., *et al.* "Macrophage p38 mitogen-activated protein kinase activity regulates invariant natural killer T-cell responses during Borrelia burgdorferi infection". *The Journal of Infectious Diseases* 206.2 (2012): 283-291.

9. Sahay B., *et al.* "Induction of Interleukin 10 by Borrelia burgdorferi Is Regulated by the Action of CD14-Dependent p38 Mitogen-Activated Protein Kinase and cAMP-Mediated Chromatin Remodeling". *Infection and Immunity* 86.4 (2018): e00781.

10. Hubbert C., *et al.* "HDAC6 is a microtubule-associated deacetylase". *Nature* 417.6887 (2002): 455-458.

11. Crandall H., *et al.* "Gene expression profiling reveals unique pathways associated with differential severity of lyme arthritis". *Journal of Immunology* 177.11 (2006): 7930-7942.

12. Li Y., *et al.* "Histone deacetylase 6 plays a role as a distinct regulator of diverse cellular processes". *The FEBS Journal* 280.3 (2013): 775-793.

13. Bertos NR., *et al.* "Role of the tetradecapeptide repeat domain of human histone deacetylase 6 in cytoplasmic retention". *Journal of Biological Chemistry* 279.46 (2004): 48246-48254.

14. Verdel A., *et al.* "Active maintenance of mHDA2/mHDAC6 histone-deacetylase in the cytoplasm". *Current Biology: CB* 10.12 (2000): 747-749.

15. Boyault C., *et al.* "HDAC6-p97/VCP controlled polyubiquitin chain turnover". *The EMBO Journal* 25.14 (2006): 3357-3366.

16. Liu Y., *et al.* "Modulation of histone deacetylase 6 (HDAC6) nuclear import and tubulin deacetylase activity through acetylation". *Journal of Biological Chemistry* 287.34 (2012): 29168-29174.

17. Fusco C., *et al.* "The E3-ubiquitin ligase TRIM50 interacts with HDAC6 and p62, and promotes the sequestration and clearance of ubiquitinated proteins into the aggresome". *PLoS One* 7.7 (2012): e40440.

18. Cheng F., *et al.* "A novel role for histone deacetylase 6 in the regulation of the tolerogenic STAT3/IL-10 pathway in APCs". *Journal of Immunology* 193.6 (2014): 2850-2862.

19. Villagra A., *et al.* "The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance". *Nature Immunology* 10.1 (2009): 92-100.

20. Cheng F., *et al.* "Divergent roles of histone deacetylase 6 (HDAC6) and histone deacetylase 11 (HDAC11) on the transcriptional regulation of IL10 in antigen presenting cells". *Molecular Immunology* 60.1 (2014): 44-53.

21. Zenk SF., *et al.* "Role of Salmonella enterica Lipopolysaccharide in Activation of Dendritic Cell Functions and Bacterial Containment". *The Journal of Immunology* 183.4 (2009): 2697-2707.

22. Pupo E., *et al.* "Intact rough- and smooth-form lipopolysaccharides from Escherichia coli separated by preparative gel electrophoresis exhibit differential biologic activity in human macrophages". *The FEBS Journal* 280.4 (2013): 1095-1111.

23. Rittig MG., *et al.* "Smooth and rough lipopolysaccharide phenotypes of Brucella induce different intracellular trafficking and cytokine/chemokine release in human monocytes". *Journal of Leukocyte Biology* 74.6 (2003): 1045-1055.

24. Godowski PJ. "A smooth operator for LPS responses". *Nature Immunology* 6.6 (2005): 544-546.

25. YL Lightfoot., *et al*. "Colonic Immune Suppression, Barrier Dysfunction, and Dysbiosis by Gastrointestinal *Bacillus anthracis* Infection". *PLoS One* 9 (2014): e100532.