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Effects of cyanobacteria *Oscillatoria* sp. lipopolysaccharide on B cell activation and Toll-like receptor 4 signaling

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Abstract

Cyanobacteria (“blue-green algae”), such as *Oscillatoria* sp., are a ubiquitous group of bacteria found in freshwater systems worldwide that are linked to illness and in some cases, death among humans and animals. Exposure to cyanobacteria occurs via ingestion of contaminated water or food-products. Exposure of the gut to these bacteria also exposes their toxins, such as lipopolysaccharide (LPS), to B cells in the gut associated lymphoid tissue. However, the effect of *Oscillatoria* sp. LPS on B cell activation is unknown. To test the hypothesis that *Oscillatoria* sp. LPS exposure to murine B cells would result in B cell activation, murine B cells were incubated in the absence or presence of *Oscillatoria* sp. LPS or *E. coli* LPS as a positive control. The data indicate that *Oscillatoria* sp. LPS induces B cells to proliferate, upregulate MHC II and CD86, enhance antigen uptake and induce IgM production at low levels. Additional studies demonstrate that this low level of stimulation may be due to incomplete TLR4 signaling induced by *Oscillatoria* sp. LPS, since IRF-3 is not induced in B cells after stimulation with *Oscillatoria* sp. LPS. These findings have important implications for the mechanisms of toxicity of cyanobacteria in both humans and animals.

Keywords

Cyanobacteria; *Oscillatoria* sp; Lipopolysaccharide (LPS); B cells; Toll-like receptor 4 signaling

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Conflict of interest

None.

1. Introduction

Cyanobacteria (“blue-green algae”) are a ubiquitous group of Gram-negative bacteria found in freshwater systems worldwide (Kagalou et al., 2008; Palus et al., 2007; Stewart et al., 2006) that negatively impact human, animal, and environmental health. In particular, marine and freshwater strains belonging to the genera *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodularia*, *Oscillatoria*, and *Planktothrix* are linked to illness and, in some cases, death among humans and other animals (*i.e.* livestock, wildlife) (Bernardova et al., 2008; Mayer et al., 2011; Notch et al., 2011; Stewart et al., 2006). Exposure to cyanobacteria is via absorption through skin, inhalation, ingestion of drinking water, and food-products contaminated through bioaccumulation (Lang-Yona et al., 2014; Stewart et al., 2006). Exposure of the gut to these bacteria also exposes their toxins to the gut associated lymphoid tissue (GALT). Within the GALT resides a significant proportion of the body’s B cells (Pabst et al., 2008) of the immune system that respond to pathogen invasion by proliferating, serving to activate other cells of the immune system, and producing antibodies that neutralize pathogen invasion and toxins (LeBien and Tedder, 2008). Lipopolysaccharides (LPS), widely recognized as toxins in Gram-negative bacteria, are implicated in the toxicity of cyanobacteria (Stewart et al., 2006). Cyanobacteria LPS is suggested to cause illnesses in humans, ranging from headache, fever, allergy, respiratory disease, and gastro-intestinal illness (Carmichael, 1993; Codd et al., 1999, 2005). Cyanobacteria LPS is thought to contribute to these symptoms through innate immune cells such as monocytes and macrophages (Ohkouchi et al., 2015). However, B cells of the adaptive immune system are known to become activated after exposure to LPS through the stimulation of Toll Like Receptor 4 (TLR4) (Mikheyskaya et al., 1977; Minguet et al., 2008) and despite the exposure of GALT B cells to cyanobacteria LPS, the ability of cyanobacteria to modulate their activation is unknown.

Previous studies have assessed the ability of *Oscillatoria* cyanobacteria LPS to activate cells of the immune system (Mayer et al., 2011, 2016; Ohkouchi et al., 2015). One strain of *Oscillatoria*, *Oscillatoria planktothrix* FP1, produces an LPS-like molecule (CyP) that acts as a TLR4 antagonist that blocks the toxicity associated with other Gram-negative bacteria (Carillo et al., 2014; Jemmett et al., 2008). Additional studies using CyP indicate that CyP also antagonizes microglial cytokine release *in vitro* (De Paola et al., 2012). In contrast, a different strain of *Oscillatoria*, *Oscillatoria* sp., produces an LPS molecule that activates rat microglia to produce cytokines and chemokines *in vitro* (Mayer et al., 2016). However, none of the previous studies have addressed the ability of *Oscillatoria* LPS to activate B cells, which are likely exposed to *Oscillatoria* LPS after ingestion of contaminated water or food.

In the current study, we tested whether *Oscillatoria* sp. LPS directly activates B cells. Our data indicate that purified murine B cells proliferate, upregulate activation markers, increase antigen uptake and produce IgM in response to *Oscillatoria* sp. LPS at low levels. Additional studies demonstrate that this low level of stimulation may be due to incomplete TLR4 signaling induced by *Oscillatoria* sp. LPS, as IRF-3 is not induced in B cells after stimulation with *Oscillatoria* sp. LPS. Understanding the potential of LPS from different species of Gram-negative bacteria to activate immune cells will help us to understand the mechanism by which these bacteria induce disease in both animals and humans.

2. Materials and methods

2.1. B cell isolation and culture conditions

Murine B cells were purified from the spleens of forty 6–8 week old male and female c57Bl/6 mice using Stemcell “EasySepTM” (STEMCELL Technologies Inc., Vancouver, Canada) murine B cell isolation kit following the manufacturer’s instructions. B cells purification reached > 97% purity as determined by flow cytometry and yielded approximately 20×10^6 B cells per spleen (data not shown). Purified B cells were then cultured in the absence or presence of increasing concentrations of *Oscillatoria* sp. LPS or *E. coli* LPS Stain 0111:B4 (Sigma) in cRPMI (10% Fetal Calf Serum (FCS), penicillin/streptomycin, and L-glutamine) at 37 °C, 5% CO₂. *Oscillatoria* sp. LPS was prepared from *Oscillatoria* sp. strain HCC-1097 by hot phenol/water extraction followed by further extraction to remove RNA and protein impurities as described previously (Mayer et al., 2016). The *Oscillatoria* sp. LPS endotoxin activity was quantified using Toxinsensor chromogenic endotoxin assay kit (Genscript, Piscataway, NJ), according to the manufacturer’s instructions, which uses *E. coli* LPS as a reference standard. *E. coli* LPS purchased from Sigma is also purified by phenol/water extraction.

2.2. Proliferation assay

Purified B cells (5×10^5) were incubated in quadruplicate in 96 well plates as described above for 72 h before proliferation was assessed using an MTT assay. Briefly, MTT 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (Sigma, St. Louis, MO) was added at a final concentration of 2.5 mg/ml to each well and incubated for 1 h at 37 °C in 5% CO₂. The absorbance was determined at 562 nm using a Multiskan FC microplate reader (Thermo Fisher Scientific, Waltham, MA). The average of the individual wells from each experiment was used to combine three independent experiments to calculate the level of proliferation and perform statistical analysis.

2.3. Immunofluorescence staining for B cell activation markers

Purified B cells (5×10^5) were incubated in individual wells of a 12 well plate as described above for 48 h. Cells were harvested, washed three times in FACS buffer (PBS/1% FCS) and stained with either PE-conjugated rat anti-mouse MHC II (IA^b) or PE-conjugated rat anti-mouse CD86 (Biolegend, Sand Diego, CA) for 30 min in the dark on ice. The cells were subsequently washed three times in PBS and analyzed using a FACS Calibur followed by Cell Quest Software (BD Biosciences, San Jose, CA). The Mean Fluorescence Intensity (MFI) for each individual culture from each experiment was used to combine three independent experiments to calculate the mean fold change in MFI and perform statistical analysis.

2.4. Antigen internalization assay

Purified B cells (2×10^6) were incubated as described above in individual wells of a 12 well plate and harvested 48 h later. The cells were washed three times in cRPMI before plating 5×10^5 B cells with FITC-Dextran (Thermo Fisher Scientific, Waltham, MA) for 1 h at 37 °C in 5% CO₂, as described previously (Xu et al., 2008). Alternatively, cells were incubated

with FITC-Dextran at 4 °C for 1 h to serve as a basis for non-specific binding. All cells were then incubated for five minutes with Trypan Blue to quench any autofluorescence, washed, and analyzed using the Amnis Flowsight (EMD Millipore, Billerica, MA) to determine intracellular mean fluorescence. Not only were cells compared to controls incubated with FITC-Dextran at 4 °C, but the membrane of cells analyzed were also “masked” using the Amnis Flowsight IDEAS software to remove any membrane-specific fluorescence (Wang et al., 2009). The Mean Fluorescence Intensity for each individual culture from each experiment was used to combine three independent experiments to calculate the level of antigen internalization (MFI) and perform statistical analysis.

2.5. IgM ELISA

Purified B cells (1×10^5) were incubated in quadruplicate in 200 μ l of cRPMI in 96 well plates as described above for 5 days before 100 μ l of supernatants were harvested and analyzed for IgM production using the Mouse IgM Ready Set Go[®] ELISA kit (eBioscience Inc., San Diego, CA), following the manufacturer’s instructions. The average of the individual wells from each experiment was used to combine three independent experiments to calculate the level of IgM and perform statistical analysis.

2.6. Western blot analysis

After incubation with either *E. coli* or *Oscillatoria* sp. LPS, murine B cells were washed twice with PBS and resuspended in RIPA buffer (Thermo Fisher Scientific, Waltham, MA) at a concentration of 10×10^6 cells/50 μ L along with Halt[™] Protease and Phosphatase inhibitors (Thermo Fisher Scientific, Waltham, MA). Protein lysates were boiled with LDS Sample Buffer and Reducing Agent (Thermo Fisher Scientific, Waltham, MA) at 95 °C for 5 min. Samples were then loaded into 4–12% Bolt Bis-Tris Plus Gels (Thermo Fisher Scientific, Waltham, MA) and run for 20 min at 180 V. The gel was then transferred to a PVDF membrane using Bolt Transfer (Thermo Fisher Scientific, Waltham, MA) and run for 1 h at 20 V. The membrane was coated with blocking buffer containing milk and rocked for 1 h at room temperature. Antibodies were then added against Phospho-I κ B α (Ser32/36), Total-I κ B α , Phospho-p38 MAPK (Thr180/Tyr182), or Total-p38 MAPK, (Cell Signaling Technology, Danvers, MA) in blocking buffer containing milk and rocked at 4 °C overnight. After washing the blot in TBS-T, secondary anti-rabbit IgG-HRP (Pierce) or anti-mouse IgG-HRP (Cell Signaling Technology, Danvers, MA) conjugated antibodies were diluted in blocking buffer and added to membrane to rock for 1 h at room temperature. Following additional washes with TBS-T, blots were incubated for 5 min at room temperature with Amersham[™] ECL Select[™] (GE Healthcare, Buckinghamshire, UK) and chemiluminescent signals were measured using a Bio-Rad imager. Image analysis and quantification was done using ImageJ software (National Institutes of Health, Bethesda, MD). Each experiment was performed three times independently with similar results.

2.7. Statistics

Experiments with three or more groups were initially analyzed using a one-way analysis of variance (ANOVA) to determine if there were statistically significant differences within the experiment. Subsequently, a Bonferroni post-hoc test was used to compare individual

groups. A finding is determined to be significant when the p value was equal to or less than 0.05. Data were analyzed with Prism Software Version 7.00.

3. Results

The ability of cyanobacteria LPS to activate human and animal B cells is unknown. LPS from other Gram-negative bacteria, such as *E. coli*, induces B cell proliferation, activation, and differentiation into plasma cells that produce high levels of antibody. To investigate if LPS from cyanobacteria *Oscillatoria* sp. induces the proliferation of murine B cells, purified B cells were incubated with increasing concentrations of either *Oscillatoria* sp. LPS or *E. coli* LPS (as a positive control) for 72 h. As shown in Fig. 1, *E. coli* LPS at concentrations of 1000 ng/ml–100,000 ng/ml induces murine B cells to proliferate 10- to 12-fold greater when compared to B cells not exposed to any LPS ($p < 0.05$). In contrast, *Oscillatoria* sp. LPS induces a 3-fold increase in proliferation of B cells at 100,000 ng/ml when compared to unexposed B cells (Fig. 1, $p < 0.05$). These data indicate that *Oscillatoria* sp. LPS has agonistic effects on B cells, but only at high concentrations.

In addition to inducing B cell proliferation, LPS enhances the ability of B cells to serve as antigen presenting cells to activate T cells by increasing MHC Class II and CD86 expression (Krieger et al., 1985; Snyder et al., 2002; Van Gool et al., 1996). Therefore, we tested whether *Oscillatoria* sp. induced the expression of these two critical molecules in antigen presentation. Purified B cells were exposed to either *Oscillatoria* sp. or *E. coli* LPS for 48 h. As shown in Fig. 2A, the addition of *E. coli* or *Oscillatoria* sp. LPS resulted in an increase in the mean fluorescence intensity (MFI) for MHC Class II as indicated by a shift of the shaded histogram to the right when compared to the non-shaded histogram. When the fold change in MFI of MHC Class II expression was combined from three independent experiments, it was determined that *Oscillatoria* sp. LPS could only significantly increase the level of MHC Class II expression at the highest concentration of LPS used (100,000 ng/ml) ($\text{MFI}_{\text{Osc. sp.}} = 1.45$ -fold increase, $p < 0.05$ when compared to non-LPS exposed B cells) (Fig. 2B). In contrast, *E. coli* LPS significantly increased MHC Class II expression at 10,000–100,000 ng/ml ($\text{MFI}_{\text{E.coli}} = 2.2$ –2.4-fold increase, respectively, $p < 0.05$ when compared to non-LPS exposed B cells) (Fig. 2B). Similarly, LPS from both strains of gram-negative bacteria significantly increase the level of CD86 expression (Fig. 2C–D: $\text{MFI}_{\text{E.coli}} = 2.1$ –8.5-fold increase at 100–100,000 ng/ml; $\text{MFI}_{\text{Osc. sp.}} = 2.0$ -fold increase at 100,000 ng/ml, $p < 0.05$ when compared to non-LPS exposed B cells for both groups), suggesting that *Oscillatoria* sp. LPS at high concentrations is capable of increasing cell surface markers critical for T cell activation.

However, for B cells to present peptide in the context of MHC II, the B cells must first uptake antigen. Previous data indicate that LPS from *E. coli* increases the ability of B cells to uptake antigen through pinocytosis (Xu et al., 2008). To determine if *Oscillatoria* sp. LPS increases antigen uptake, purified murine B cells were incubated in the absence or presence of *Oscillatoria* sp. LPS or *E. coli* LPS for 48 h. At the end of this incubation, the cells were washed to remove any residual LPS and incubated with fluoresceinated-dextran (FITC-Dextran-MW 40,000) particles. Antigen internalization was visualized manually and automatically by the use of the Flowsight flow cytometer. As shown in Fig. 3A,

unstimulated B cells do not demonstrate any detectable fluorescence, which is in contrast to murine B cells exposed to either *E. coli* or *Oscillatoria* sp. LPS. When the fluorescence intensity from multiple independent experiments are combined, both *E. coli* and *Oscillatoria* sp. LPS induce significant increase in MFI when compared to unstimulated B cells ($\text{MFI}_{E.coli} = 2340$; $\text{MFI}_{Osc. sp.} = 787$, $p < 0.05$ when compared to non-LPS exposed B cells for both groups), indicating that *Oscillatoria* sp. LPS increases antigen internalization by B cells.

Another outcome of LPS engagement on B cells is the production of IgM (Lu and Munford, 2016). To test whether *Oscillatoria* sp. LPS activates B cells to produce IgM, murine B cells were activated in the absence or presence of increasing concentrations of *Oscillatoria* sp. or *E. coli* LPS for 5 days and analyzed by ELISA for IgM production. As shown in Fig. 4, at concentrations of *E. coli* LPS of 10,000 ng/ml–100,000 ng/ml, murine B cells produce significantly more IgM (14- to 33-fold increase) when compared to B cells not exposed to any LPS ($p < 0.05$). In contrast, *Oscillatoria* sp. LPS induces a 7-fold increase in IgM production by B cells at 100,000 ng/ml when compared to unexposed B cells (Fig. 4, $p < 0.05$). These data indicate that *Oscillatoria* sp. LPS is agonistic to induce IgM production by murine B cells at high concentrations.

A striking finding among all of the data is that *Oscillatoria* sp. LPS activates murine B cells, but only at high concentrations. This low level of *Oscillatoria* sp. LPS-induced B cell activation could be due to alterations in the level of TLR4 engagement after exposure to *Oscillatoria* sp. LPS vs *E. coli* LPS. LPS-mediated activation of TLR4 induces the MYD88-dependent activation of both p38 and NF- κ B, through phosphorylation of IKK β (Carpenter and O'Neill, 2009; Carter et al., 1999; Kawai and Akira, 2007). To test if *Oscillatoria* sp. LPS induced TLR4 signaling, murine B cells were incubated in the absence or presence of either *Oscillatoria* sp. or *E. coli* LPS (as a positive control) for 10–30 min and the levels of IKK β phosphorylation were analyzed by Western blot analysis. As shown in Fig. 5A, the data confirm that exposure of murine B cells to *E. coli* LPS results in a significant increase in phosphorylation of IKK β at 10 min, with the levels decreasing at 30 min. This decrease at 30 min in the *E. coli* LPS-treated murine B cells is in part due to the degradation of IKK β , as indicated by decreased levels of total IKK β (Fig. 5A). This decrease in total IKK β is not due to changes in protein levels loaded into each well, since the levels of immunoglobulin light chain were consistent between all groups within the experiment. When the levels of IKK β -phosphorylation are analyzed after exposure to *Oscillatoria* sp. LPS, there is a slight increase in phosphorylated IKK β at 10 min with levels returning to normal by 30 min (Fig. 5A). Additionally, *E. coli* LPS induced strong and sustained phosphorylation of p38K at both 10 and 30 min (Fig. 5B), which is in contrast to *Oscillatoria* sp. LPS in which p38K phosphorylation is not detected until after 30 min of exposure. Finally, TLR4 signaling activates IRF-3 in addition to the MYD88 pathway (Kawai and Akira, 2006; McCoy et al., 2008). When the levels of phosphorylated IRF-3 were analyzed in B cells exposed to either *E. coli* or *Oscillatoria* sp. LPS, *E. coli* LPS induced significant phosphorylation of IRF-3 by 60 min, which increased through 180 min. In contrast, in multiple experiments, *Oscillatoria* sp. LPS did not significantly enhance IRF-3 phosphorylation through 180 min (Fig. 5C). Taken together, these data suggest that the low level of B cell activation induced by *Oscillatoria* sp. LPS, as seen in Figs. 1–5, are due to incomplete signaling through TLR4.

4. Discussion

Multiple studies have analyzed the ability of cyanobacteria LPS to influence the function of innate immune cells (Jemmett et al., 2008; Macagno et al., 2006; Mayer et al., 2016). However, LPS also has significant effects on B cells of the adaptive immune system. Since it is highly likely that the B cells found within the GALT will be exposed to LPS from cyanobacteria after the ingestion of contaminated food and/or water, the current study investigated whether cyanobacteria *Oscillatoria* LPS could activate murine B cells. Our findings indicate that *Oscillatoria* sp. LPS significantly activates B cells to proliferate, serve as an antigen-presenting cells and produce antibody, albeit at low levels.

LPS typically acts as a B cell mitogen that induces the proliferation and differentiation of B cells into plasma cells that produce high levels of antibody, irrespective of their specificity (Lu and Munford, 2016). Our data indicate that at high concentrations of *Oscillatoria* sp. LPS, B cells proliferate and differentiate into IgM-producing plasma cells. The mucosal lymphoid tissue that surrounds the gut has an extremely high concentration of B cells (Pabst et al., 2008), including B cells with specificities that result in pathology (Arnaboldi et al., 2005; Cardoso et al., 2008). The inappropriate expansion of B cells increases the likelihood the *Oscillatoria* sp. LPS-mediated activation could contribute to disease, such as allergic responses seen after *Oscillatoria* sp. exposure (Stewart et al., 2006). Future experiments that analyze the ability of cytokines to promote B cell switch to IgE in the presence of *Oscillatoria* sp. LPS may provide insight into a possible mechanism of allergic responses seen after *Oscillatoria* exposure.

One of the most striking findings is that *Oscillatoria* sp. LPS only induced B cell activation at the highest concentrations of *Oscillatoria* sp. LPS and with incomplete TLR4 signal transduction. There are multiple reasons that could be responsible for this finding. First, LPS must first interact with LPS binding protein (LBP) and subsequently with CD14. This complex can then interact with TLR4 to activate B cells (Maeshima and Fernandez, 2013). It is possible that *Oscillatoria* sp. LPS binds to these subunits and/or TLR4 with low affinity to generate a weak and incomplete TLR4 signal. While we have not determined the affinity of *Oscillatoria* sp. LPS for TLR4, our data indicate that TLR4 engagement by *Oscillatoria* sp. LPS does not signal in a conventional manner (Fig. 5).

Alternatively, differences in the Lipid A structure between *E. coli* and *Oscillatoria* sp. LPS could yield the findings in this study. The number of lipid chains directly impacts the functional activity of LPS (Maeshima and Fernandez, 2013). For example, lipid A variants with six lipid chains results in full TLR stimulation and activation, while lipid A variants with five lipid side chains have approximately 100-fold less activity (Rhee, 2014). Unfortunately, the composition of *Oscillatoria* sp. LPS is not known and therefore, it is impossible to currently compare the structure between the LPS of these two species. However, preliminary studies suggest *Oscillatoria* sp. LPS is comprised of different lipids than *E. coli* LPS (Philip Williams, unpublished data). Additional experiments analyzing the lipid content and structural aspects of *Oscillatoria* sp. LPS are currently under investigation.

It is likely that there are structural differences between not only *Oscillatoria* sp. LPS and *E. coli* LPS, but also with the LPS-like molecule, CyP from *Oscillatoria planktothrix* FP1. CyP acts as an MD2-TLR4 antagonist, and inhibits the ability of LPS from other Gram-negative bacteria to induce cytokine production by innate immune cells (Jemmett et al., 2008; Macagno et al., 2006; Maeshima and Fernandez, 2013). Structural information regarding CyP was recently determined and it identified differences in the lipid A portion and a much higher molecular mass of the O-chain of LPS, when compared to another cyanobacteria LPS from *Synechococcus* (Carillo et al., 2014). Therefore, it seems as if differences in the LPS structure among species of cyanobacterium may dictate their ability to induce disease through activation or antagonism of TLR4.

The data presented here are the first to show that *Oscillatoria* sp. LPS acts as an agonist to stimulate B cell activation and differentiation into antibody-producing cells, which may contribute to cyanobacterial disease. Future studies that analyze the effects of *Oscillatoria* sp. LPS on other TLR4-expressing immune cells, such as monocytes, will help us to understand the interplay of immune cells that is induced *in vivo* after exposure to *Oscillatoria* sp. LPS. Furthermore, the determination of the structure of *Oscillatoria* sp. LPS and its interaction with TLR4 may give further insight into the mechanisms by which this cyanobacteria contributes to disease in humans and animals.

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Fig. 1.

E. coli or *Oscillatoria* LPS activate purified murine B cells to proliferate. Murine B cells were isolated from c57Bl/6 mice and incubated with increasing concentrations of LPS for 72 h. Proliferation was assessed using the MTT assay as described in the Materials and methods. The data are a combination of three experiments. The error bars represent the standard error of the mean (SEM). * Indicates $p < 0.05$ from B cells not exposed to any LPS. The F value for the data in Fig. 1A is 29 with 5 degrees of freedom. The F value for Fig. 1B is 19 with 5 degrees of freedom.

Fig. 2.

E. coli or *Oscillatoria* LPS increases MHC II expression and CD86 expression. B cells were isolated from c57Bl/6 mice and incubated in the absence or presence of *E. coli* or *Oscillatoria* LPS (10^5 ng/ml) for 48 h. B cells were then washed and stained either with a (A–B) PE-rat anti-mouse anti-MHC II antibody (IA^b) (top panels) or (C–D) PE-rat anti-mouse CD86 antibody before analysis by flow cytometry. The data in (A) and (C) are representative of three experiments with similar results. Unshaded histograms represent unstimulated B cells, while the shaded histograms represent LPS-stimulated B cells. The data in (B) and (D) are a combination of the MFI of each group from three individual experiments and are expressed as a fold change in MFI. The error bars represent the standard error of the mean (SEM). *Indicates $p < 0.05$ from B cells not exposed to any LPS. The F

value for the data in Fig. 2B is 17.4 with 2 degrees of freedom. The F value for Fig. 2D is 6.1 with 2 degrees of freedom.

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Fig. 3.

E. coli and *Oscillatoria* sp. LPS increases B cell antigen internalization. B cells were isolated from c57Bl/6 mice and incubated in the absence or presence of *E. coli* or *Oscillatoria* LPS (10^5 ng/ml) for 48 h. B cells were then washed and incubated with FITC-dextran for 1 h before analysis by flow cytometry using the Amnis Flowsight flow cytometer. (A) Flowsight analysis of antigen internalization of FITC-dextran by individual cells as described in the Material and Methods. The numbers next to the bright field image refers to the sample number of 10,000 cells that were analyzed. (B) The increase in MFI as an indication of antigen internalization of FITC-Dextran was calculated from three independent experiments and combined. The error bars represent the standard error of the mean (SEM). *Indicates $p < 0.05$ when compared to B cells not exposed to any LPS (-LPS). The F value for the data in Fig. 2B is 6.1 with 2 degrees of freedom.

Fig. 4.

E. coli or *Oscillatoria* sp. LPS induces IgM production. B cells were isolated from c57Bl/6 mice and incubated in the absence or presence of increasing concentrations of *E. coli* or *Oscillatoria* LPS for 5 days. Supernatants were harvested before analysis by ELISA. The data are a representative of three experiments with similar results. The error bars represent the standard error of the mean (SEM). *Indicates $p < 0.05$ from B cells not exposed to any LPS. The F value for the data in Fig. 4A is 5.5 with 5 degrees of freedom. The F value for Fig. 4B is 3.6 with 5 degrees of freedom.

Fig. 5. *E. coli* or *Oscillatoria* LPS induces altered Toll like receptor 4 (TLR4) signaling. B cells were isolated from c57Bl/6 mice and incubated in the absence or presence of *E. coli* or *Oscillatoria* LPS (10^5 ng/ml) for 10 or 30 min. Protein lysates were obtained and analyzed by Western blot analysis for (A) phospho-IKK β , total IKK β , or mouse light chain (as a control for protein loading), (B) phospho-p38K and total p38K, (C) phospho-IRF-3 and total IRF-3. Data are representative of 3 experiments with similar results.