INDUCTION OF INNATE IMMUNE RESPONSES BY COMMENSAL AND PATHOGENIC *NEISSERIA* SPECIES

By

LAURA KATHERINE WADE

A Thesis Submitted to the Honors College

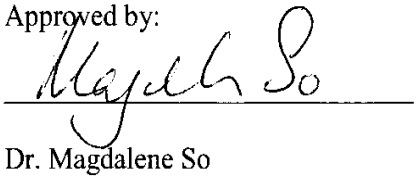
In Partial Fulfillment of the of the Bachelors Degree
With Honors in

Physiology

THE UNIVERSITY OF ARIZONA

M A Y 2 0 1 2

Approved by:



Dr. Magdalene So
Department of Immunobiology

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for a degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Signed: Laura Wade

Abstract

Neisseria gonorrhoeae, the etiological agent of gonorrhea, infects over 62 million people annually worldwide. Many of these infections occur asymptomatically, demonstrating that *N. gonorrhoeae* can infect mucosal epithelia without causing overt disease. The *Neisseria* genus also contains over eight commensal species, which by definition, are capable of colonizing host cells without causing disease. These findings indicate that *Neisseria* species have developed strategies for minimizing the host immune response to infection. To investigate these strategies we infected endocervical and nasopharyngeal epithelial cells with *N. gonorrhoeae*, a pathogen, or *N. elongata*, a commensal. We then compared protein levels of two different transcriptional regulators, NFκB and ATF3. These represent two different signaling pathways involved in regulating the innate immune response. We found that both species were able to increase levels of both NFκB and ATF3. In addition, we tested the role of type IV pilus (Tfp) retraction in the upregulation of ATF3 during infection with both species. We found little evidence that Tfp retraction plays a role in ATF3 upregulation. Responses to infection with the pathogen and commensal varied by cell type. We conclude that both the pathogen and commensal alter host cell signaling pathways in order to establish asymptomatic infections.

Introduction

The gram-negative bacterium *Neisseria gonorrhoeae* is the etiological agent of gonorrhea. Worldwide, there are over 62 million people infected annually with gonorrhea [1]. Although gonorrhea is easily treated with antibiotics many gonorrhea infections are asymptomatic, particularly in women. The World Health Organization estimates that up to 60% of cervical gonorrhea cases are asymptomatic [2]. Left untreated, gonorrhea can lead to complications such as pelvic inflammatory disease (PID) with subsequent risk of ectopic pregnancies [1]. The large number of asymptomatic gonorrhea infections poses challenges for treating and preventing the spread of the disease.

The *Neisseria* genus includes ten species that are known to colonize human epithelial cells [3, 4]. Of these, only *N. gonorrhoeae* and *N. meningitidis*, a cause of bacterial meningitis, are classified as pathogenic. The remaining eight species of human *Neisseria* are commensal organisms and are thus capable of colonizing the host without causing disease. Commensal species of *Neisseria* normally inhabit the oro- and nasopharynx [5]. *N. gonorrhoeae* can also colonize the nasopharynx and rectal mucosa, usually asymptomatically [1]. These observations suggest that *N.gonorrhoeae* is able to mimic commensal species by colonizing human epithelial cells without causing overt symptoms.

The innate immune system is the body's first line of defense against pathogens. Inflammation is a common strategy used by the innate immune system to contain a foreign antigen to a localized area and to recruit professional immune cells to the site of infection. This inflammation is mediated by cytokines and chemokines that are secreted by infected cells [6]. Since *Neisseria* species colonize various mucous membranes, the host's first defenses will be through activation of the innate immune system by epithelial cells. *N. gonorrhoeae* infections in

men and women have been shown to upregulate the expression of various pro-inflammatory cytokines. In an experimental model of male urethral gonorrhea, IL-1β, IL-6, IL-8, and TNFα can be detected at elevated levels in the urine and serum of volunteers [7]. Additionally, women with natural gonorrhea infections have increased serum IL-6 levels [8]. Tissue cultures of cervical epithelial cells infected with *N. gonorrhoeae* also show elevated levels of several cytokines, including IL-1β, IL-6, IL-8 and TNFα [9]. These findings indicate that *N. gonorrhoeae* induces the expression of multiple pro-inflammatory cytokines in patients and that these results can be reproduced with in vitro tissue culture models of infection.

The transcriptional regulator nuclear factor-kappa B (NFκB) is known to target genes involved in the immune response, including those encoding cytokines [10]. In macrophages and T cells, NFκB plays a role in activating transcription of pro-inflammatory cytokines [11]. It has also been shown that the NFκB pathway is activated in *N. gonorrhoeae*-infected epithelial cells, leading to activation of the IL-6 promoter [11]. In contrast to NFκB, activating transcription factor 3 (ATF3) negatively regulates transcription of pro-inflammatory cytokines [12].

ATF3 plays an important role in preventing an unnecessary or prolonged inflammatory response. Our lab has previously shown that ATF3 protein levels are upregulated in epithelial cells infected with *N. gonorrhoeae,* and that knocking down ATF3 levels leads to increased IL-6 expression during infection [13]. The upregulation of ATF3 during *N. gonorrhoeae* infection therefore leads to a downregulation of the innate immune response. This in turn may lengthen the time of infection and increase survival of the pathogen. Given that commensals can colonize their hosts without causing overt disease, it is likely that commensal *Neisseria* have also developed strategies to downregulate the host immune response.

The type IV pilus (Tfp) is a fimbriate structure found on the outer membrane of *N. gonorrhoeae*. General functions of Tfp include adhesion, twitching motility, DNA uptake, and quorum sensing. Tfp also mediate important interactions between the bacteria and host epithelial cells. Pili are required for the formation of bacterial microcolonies on the host cell surface, and for the recruitment of host cell proteins that are important for infection [14]. Multiple gonococcal genes are involved in the synthesis, extension and retraction of Tfp. The gene *pilE* encodes pilin monomers [15] which are transported to the periplasmic space to be processed by the PilD peptidase/transmethylase [16]. Once processed, the mature pilin monomers are assembled into a pilus fiber by the ATPase PilF[16], and the PilQ pore complex then extrudes and anchors the pilus fiber [17-19]. Retraction of the pilus and disassembly requires the function of a second ATPase, PilT [20, 21]. All of the human *Neisseria* encode a complete set of Tfp biogenesis genes [22]. Recent work demonstrated that *Neisseria elongata*, which is the most ancestral *Neisseria* species, expresses functional Tfp that can mediate adherence to human epithelial cells and microcolony formation [23].

Gonoccocal infection causes profound changes in host cell signaling and gene expression, and many of these changes are enhanced by Tfp retraction. For example, *N. gonorrhoeae* infection can activate multiple host cell signaling pathways, including the mitogen activated protein kinase (MAPK) and phosphoinositide-3 kinase (PI3K) pathways. Activation of both MAPK and PI3K pathways is enhanced in cells infected with wild type *N. gonorrhoeae* compared to cells infected with a *pilT* mutant [24 -26]. Bacteria that lack PilT still adhere to epithelial cells at the same rate as wild type bacteria, but cannot retract their pili, and do not form microcolonies [14, 20]. It has also been shown that *N. gonorrhoeae pilT* mutants activate NFκB in epithelial cells at reduced levels [27]. As NFκB regulates the expression of several cytokines,

this finding provides a link between pilus retraction and activation of the innate immune response. Pilus retraction also leads to enhanced expression of many epithelial cell genes [26]. Among the host cell genes whose expression is enhanced by pilus retraction is ATF3, which has been shown to negatively regulate the innate immune response to *N. gonorrhoeae* infection [13]. The importance of Tfp retraction during *N. gonorrhoeae* infection has been well characterized; however, the role of pilus retraction in commensal *Neisseria* infection remains unknown.

In this thesis, I compared epithelial cells infected with pathogenic or commensal *Neisseria* to elucidate the strategies used by these bacteria to dampen the host's innate immune response. Tissue cultures of endocervical cells (End1) and nasopharyngeal cells (Detroit 562) are used to represent two common locations for *Neisseria* colonization. *N. gonorrhoeae* was used as a representative pathogenic species, and *N. elongata* as representative of the commensals. I hypothesized that both pathogenic and commensal *Neisseria* are able to influence the host cell immune response by altering signaling pathways. I further hypothesized that Tfp retraction is an important component for dampening the innate immune response. I found that both the pathogen and commensal alter the protein levels of NFκB and ATF3 in both cells lines, indicating that infection has an impact on these signaling pathways. The importance of Tfp retraction varies by cell type but is not strictly required for dampening the innate immune response, suggesting that *Neisseria* is using additional strategies to influence the host cell immune response.

Results

NFκB is activated by *Neisseria* infection

Phosphorylation of the transcription factor NFκB is elevated in cells infected with either pathogenic or commensal *Neisseria* species compared to uninfected cells. This activation was seen in both End1 (endocervical) cells and Detroit (nasopharyngeal) cells (Figure 1). The amount of activation was measured by comparing relative levels of phosphorylated NFκB to the total amount of NFκB protein present in whole cell lysates. Prior to relocation to the nucleus, NFκB in the cytosol is bound to IκB, an inhibitory molecule. When the signaling pathway is activated IκB is degraded and NFκB is phosphorylated. Therefore, a larger proportion of phospho-NFκB indicates that the signaling pathway has been activated. NFκB then goes on to regulate a variety of immune related genes [11]. Our results show that *N. elongata* and *N. gonorrho*eae both activate this pathway (Figure 1) and therefore are capable of activating the innate immune response in epithelial cells.

Although upregulation was seen in both cell lines, there appears to be a tissue-type component to the amount of activation. End1 cells represent a tissue common for gonorrhea infection. In End1 cells, *N. gonorrhoeae* induces a slightly higher level of phosphorylation than *N. elongata* until the last time point at three hours where they are approximately equal. The greatest difference between the pathogen and commensal occurs at 90 minutes post-infection. Detroit cells represent a tissue common for commensal *Neisseria* colonization. In Detroit cells, there is no distinct difference between *N. gonorrhoeae* and *N. elongata* throughout the course of the infection. These results suggest that both commensal and pathogenic *Neisseria* activate NFκB.

ATF3 is upregulated by both commensal and pathogenic *Neisseria*

It has been previously shown that pathogenic *Neisseria* upregulates ATF3 as a mechanism to dampen the host immune response. ATF3 negatively regulates the expression of pro-inflammatory cytokines such as IL-6 [13]. We were interested in determining whether the commensal *N. elongata* also upregulates AFT3 as a means to avoid causing disease in the host. Our results show that *N. elongata* does upregulate ATF3 protein levels in both End1 and Detroit cells compared to uninfected cells (Figure 2). The level of ATF3 induced by *N. elongata* is almost identical to the level induced by *N. gonorrhoeae* in End1 cells for the eight hour duration of infection. Interestingly, this same pattern is not observed in Detroit cells. Instead, *N. elongata* induces lower levels of ATF3 compared to *N. gonorrhoeae*, particularly at six hours post-infection. Detroit cells represent a tissue location where *N. elongata* is commonly found and where *N. gonorrhoeae* infections are usually asymptomatic so both the commensal and pathogen are able to colonize the epithelial cells without causing symptoms. This is where we would expect the two species to interact with host cells the most similarly. Once again, the data indicates that there are tissue-specific factors involved that are important in determining how the bacteria and host cells will interact. Despite the differences across cell types, the data demonstrates the ability of the commensal *N. elongata* to activate ATF3.

Role of pilus retraction in ATF3 induction

We know that Tfp retraction plays a role in enhancing the expression of many different genes during *N. gonorrhoeae* infections [25]. It has been demonstrated that ATF3 expression is enhanced by Tfp retraction [13]. However, Tfp tetraction is not required for ATF3 induction, since Δ*pilE* mutants, which lack Tfp, and Δ*pilT* mutants, which are piliated but retraction deficient, both induce ATF3 in T84 colorectal cells to a certain extent [13]. Given that *N.*

elongata is able to induce ATF3, we investigated whether the commensal also uses Tfp retraction as the pathogen does. To test this, the retraction deficient mutant *N. elongata* Δ*pilT* and triple knockout Δ*pilT1*, Δ*pilT2*, Δ*pilU* were used to infect End1 and Detroit cells. As is in the pathogen, the *N. elongata* retraction deficient mutants were still able to induce ATF3 in both cells lines (Figure 3a). The mutants appear to induce slightly higher levels early in infection before dropping slightly below the level induced by the wild type strain. This suggests that the *pilT* mutants behave differently from wild type at 3 and 6 hours post-infection; however, these differences are not statistically significant. Early induction of ATF3 may be accomplished through a *pilT* independent pathway and induction at later time points may be enhanced by pilus retraction, but the current data is too weak to form a definite conclusion. A similar trend can be seen in *N. gonorrhoeae* infection of End 1 cells (Figure 3b). The Δ*pilT* mutant induces higher levels of ATF3 than the wild type at the outset of infection but then falls behind as the infection continues. In contrast, the *N. gonorrhoeae* wild type and Δ*pilT* mutant induce very similar levels of ATF3 throughout the infection in Detroit cells (Figure 3b). Overall, Figure 3 shows that pilus retraction does not cause a statistically significant difference in ATF3 levels in either End1 or Detroit cells.

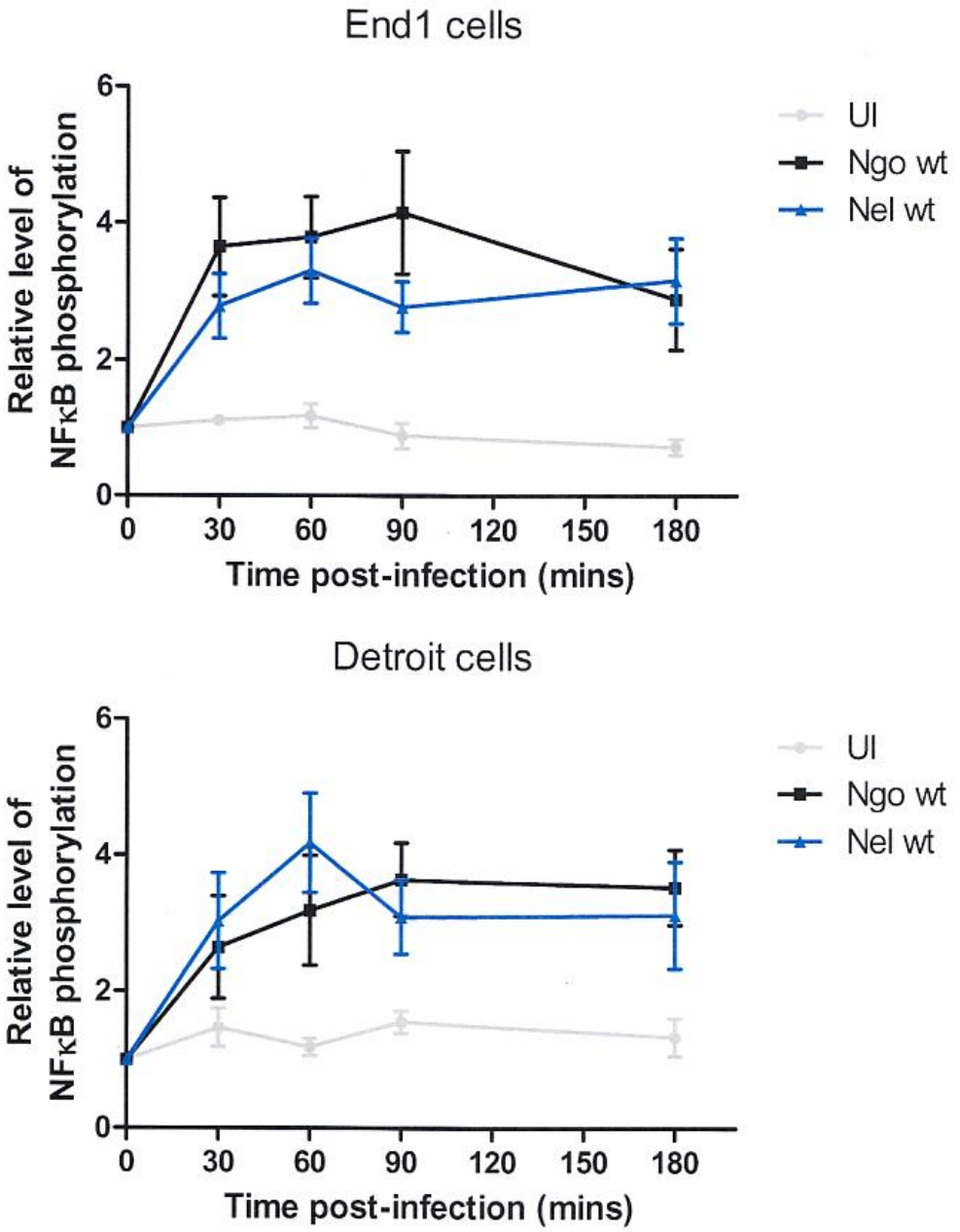


Figure 1: ***N. gonorrhoeae*** **and** ***N. elongata*** **induce NFκB phosphorylation. End1 or Detroit cells were infected with wild type** ***N. gonorrhoeae*** **(Ngo wt) or** ***N. elongata*** **(Nel wt) at an MOI of 50 for varying lengths of time. Cell lysates separated by 10% SDS-PAGE and Western blotted using mAb against p-NFκB and total NFκB. Protein levels were quantified by densitometry. Mean phospho-NFκB values are relative to time zero, which is set to one, and normalized to the loading control of total NFκB (± SEM, n=4).**

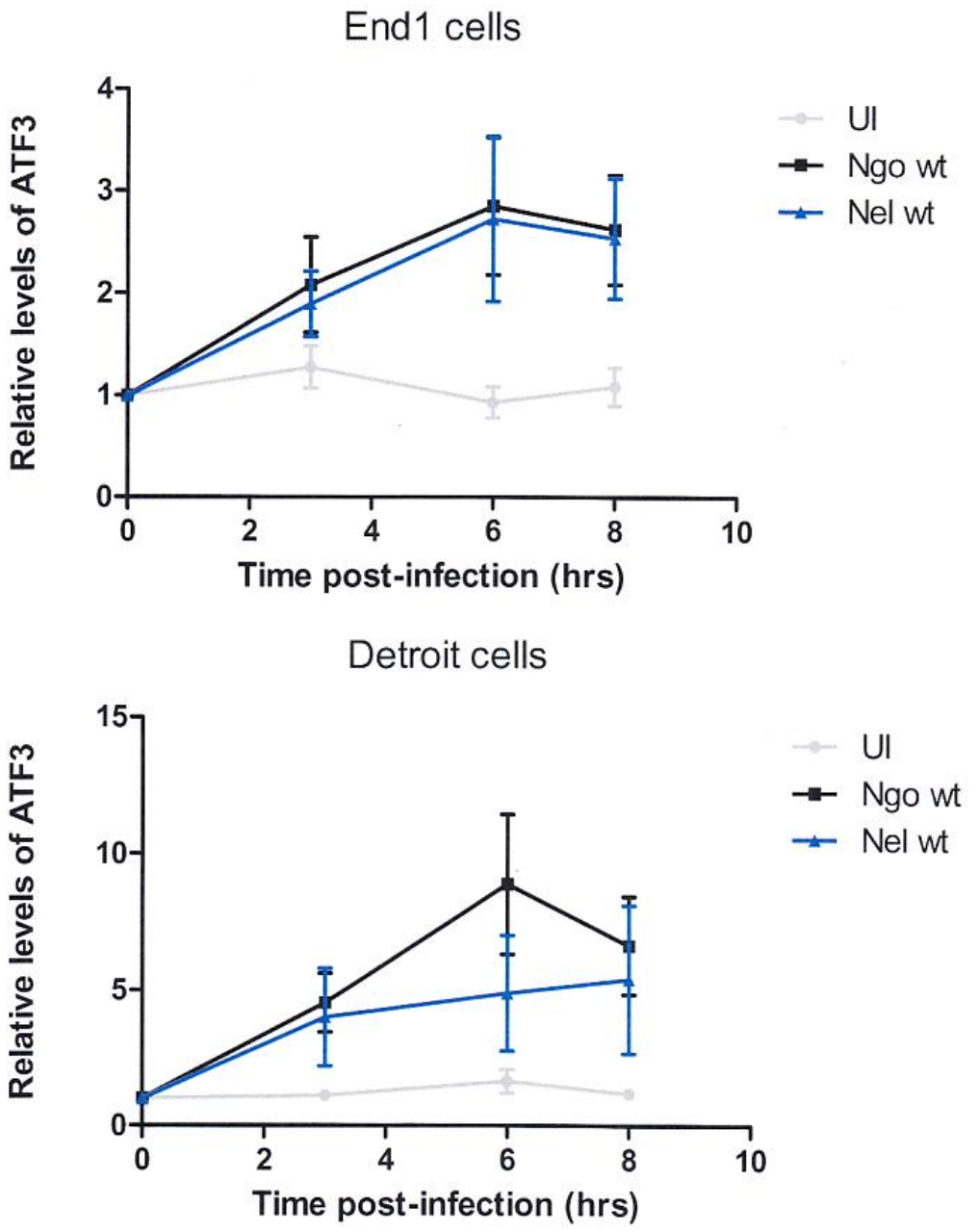


Figure 2: Upregulation of ATF3 by pathogenic and commensal *Neisseria*. End1 or Detroit cells were infected with wild type type *N. gonorrhoeae* (Ngo wt) or *N. elongata* (Nel wt) at an MOI of 50 for varying lengths of time. Cell lysates separated by 13.5% SDS-PAGE and Western blotted using mAb against ATF3 and β-tubulin. Protein levels were quantified by densitometry and normalized to the β-tubulin loading control. Mean values of ATF3 protein relative to zero hour time point, which is set at one (±SEM, n=6).

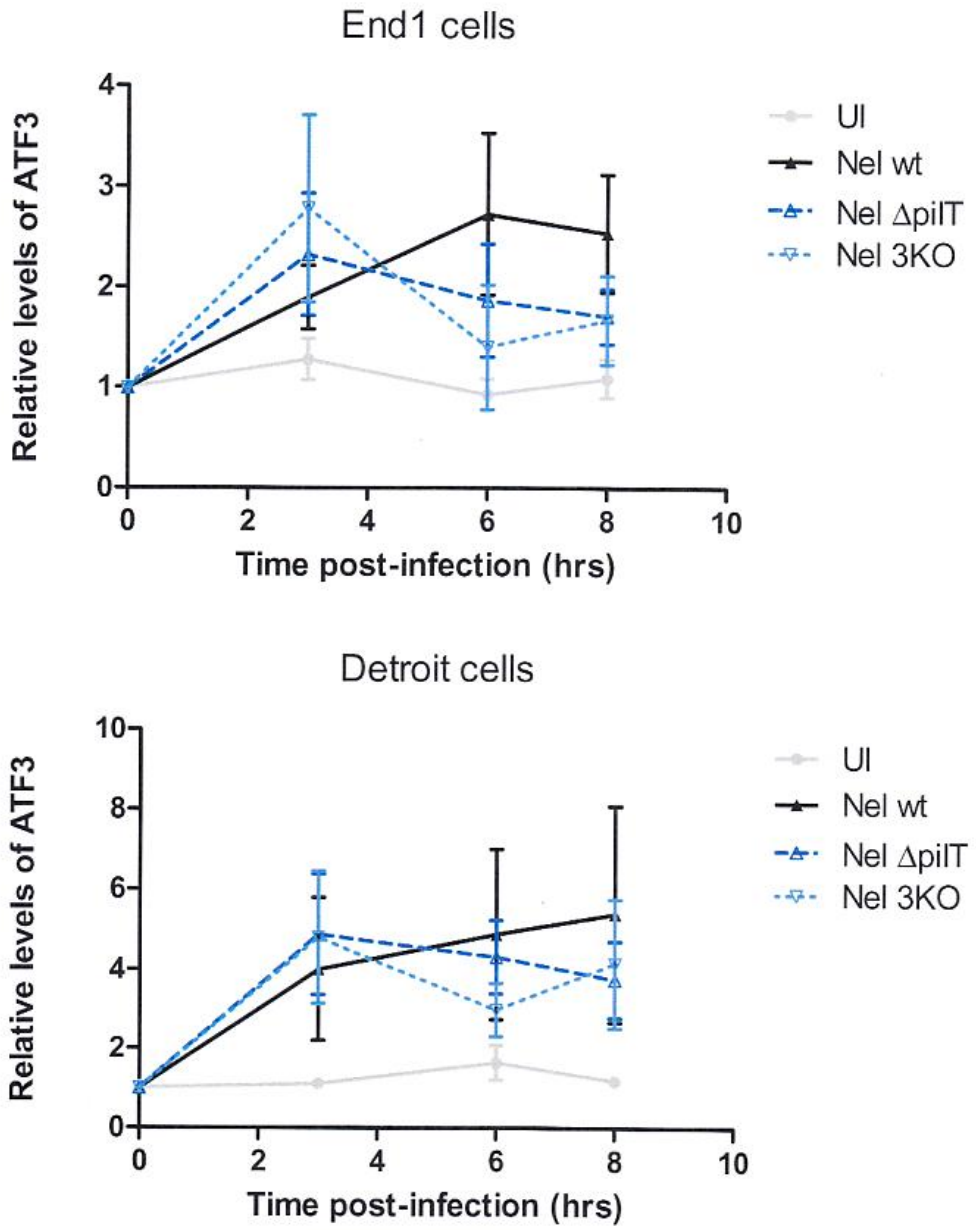


Figure 3a: ATF3 induction by *N.elongata* wild type and *ΔpilT* mutants. End1 or Detroit cells were infected with *N. elongata* wild type type (Nel wt) or *ΔpilT* mutant (Nel ΔpilT) or *ΔpilT1*, *ΔpilT2*, *ΔpilU* mutant (Nel 3KO) at an MOI of 50 for varying lengths of time. Cell lysates separated by 13.5% SDS-PAGE and Western blotted using mAb against ATF3 and β-tubulin. Protein levels were quantified by densitometry and normalized to β-tubulin loading control. Mean values of ATF3 protein relative to zero hour time point, which is set at one (±SEM, n=6).

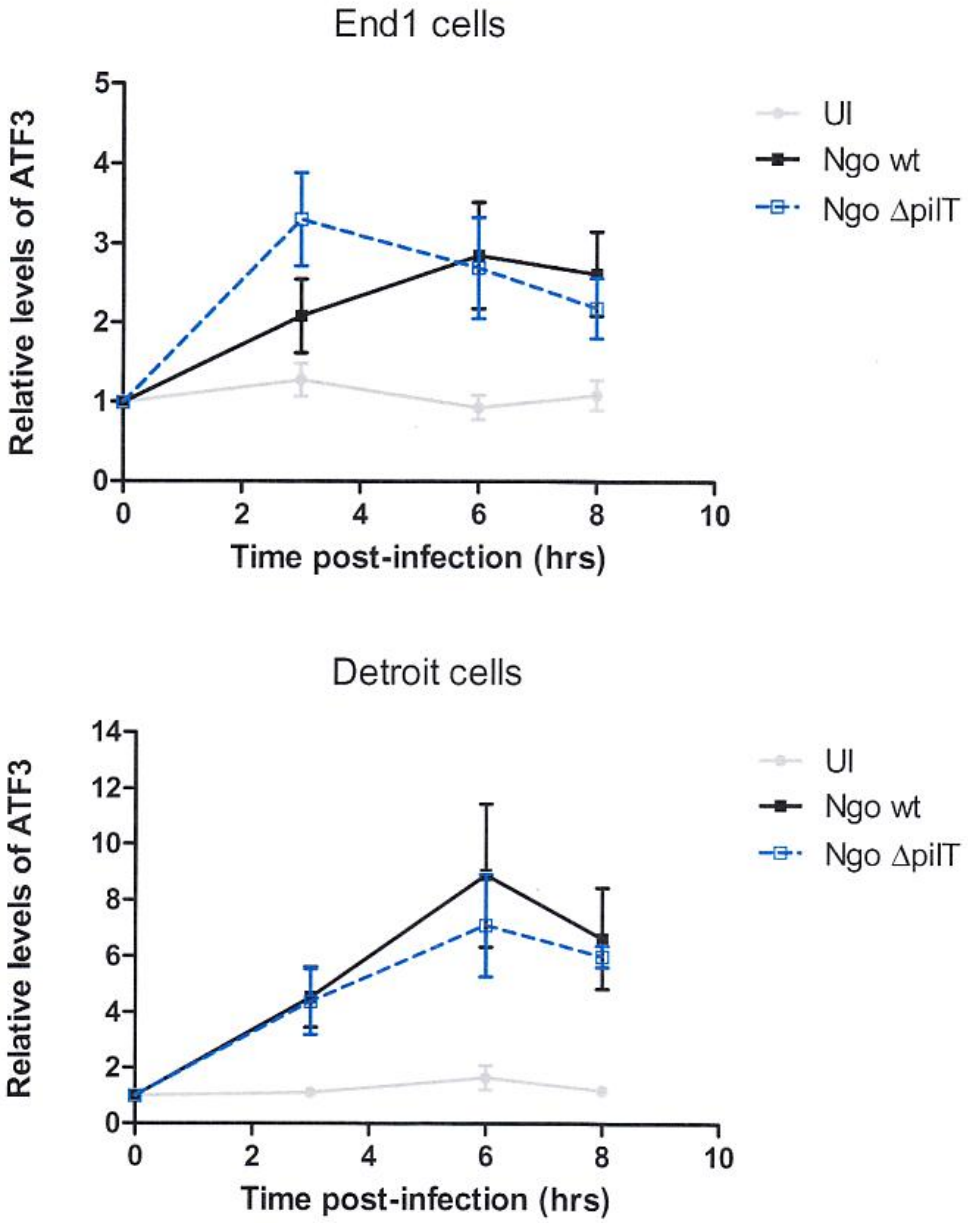


Figure 3b: ATF3 induction by *N. gonorrhoeae* wild type and *ΔpilT* mutant. End1 or Detroit cells were infected with *N. gonorrhoeae* wild type type (Ngo wt) or *ΔpilT* mutant (Ngo ΔpilT). Cell lysates separated by 13.5% SDS-PAGE and Western blotted using mAb against ATF3 and β-tubulin. Protein levels were quantified by densitometry and normalized to β-tubulin loading control. Mean values of ATF3 protein relative to zero hour time point, which is set at one (±SEM, n=6).

Discussion

Multiple *Neisseria* species are capable of colonizing human epithelial cells at various sites of the body. Many times this colonization occurs asymptomatically which suggests that the bacteria are able to dampen the immune response. The results show that both the commensal *N. elongata* and the pathogen *N. gonorrhoeae* activate signaling pathways in End1 and Detroit cells as demonstrated by NFkB activation and the upregulation of ATF3 levels. NFκB is able to activate the transcription of the IL-6 promoter [11], which subsequently leads to the secretion of the pro-inflammatory cytokine IL-6. In contrast, ATF3 negatively regulates expression of IL-6 [13] and acts to dampen the host cell immune response. The opposing nature of these pathways leads to the possibility that even though an NFκB-mediated inflammatory response is triggered at the early stage of infection, this response can be subsequently dampened through the ATF3 pathway.

The competition between signaling pathways can be seen in End1 cells infected with *N. gonorrhoeae* or *N. elongata*. The cells infected with *N. gonorrhoeae* have slightly elevated levels of p-NFκB compared to *N. elongata*-infected cells, particularly at 90 minutes post-infection. This suggests that the pathogen is initially inducing a stronger immune response than the commensal by activating the NFκB pathway to a greater extent. However, *N. gonorrhoeae* and *N. elongata* both activate similar levels of ATF3 in End1 cells indicating that the initial inflammatory response can be subsequently dampened by both the pathogen and the commensal. This pattern of signaling pathway activation is seen in Detroit cells infected with *N. gonorrhoeae* but not in Detroit cells infected with *N. elongata*. The pathogen appears to be using the ATF3 pathway to aid in asymptomatic colonization of Detroit cells, similar to colonization of End1 cells. However, this does not fit the data from N. elongata-infected Detroit cells. The nasopharynx, as represented

by Detroit cells, is a common location for commensal *Neisseria* colonization [5], suggesting that those species do not activate a significant immune response there. It is therefore slightly surprising that *N. elongata*-infected Detroit cells show similar levels of p-NFκB as *N. gonorrhoeae*-infected cells. This would suggest that the commensal and pathogen are both able to elicit a similar immune response. In addition, ATF3 levels in *N. elongata*-infected Detroit cells are lower than in *N. gonorrhoeae*-infected cells. Since ATF3 negatively regulates the immune response, the lower levels induced by *N. elongata* seem to demonstrate a decreased ability to dampen the immune response on the part of the commensal. This is not reflected *in vivo*, so the commensal bacteria may be using additional alternative methods to establish an asymptomatic infection of the nasopharynx.

Tfp retraction has recently been shown to enhance the expression of ATF3 in *N. gonorrhoeae*-infected colorectal cells [13]. Our current results do not demonstrate as strong a connection between Tfp retraction and ATF3 induction in two other cell lines. When End1 cells and Detroit cells are infected with either wild type or Δ*pilT* mutant of *N. gonorrhoeae* only slight differences in ATF3 levels are seen. End1 cells infected with the Δ*pilT* mutant have slightly higher levels of ATF3 early on, but these levels are not sustained throughout the infection. Detroit cells show almost identical levels of ATF3 regardless of Tfp retraction. These differing observations may be reflecting differences in the cell lines response to infection. The results also support the existence of a retraction-independent pathway for bacteria-host cell interactions. It has been suggested that toll-like receptors (TLRs) may be a key factor in this alternative pathway [13]. This pathway is likely utilized by the commensal as well. The data from *N. elongata* wild type and *pilT* mutant infections show that Tfp retraction is minimally important for ATF3 upregulation. There may be a slight dependence at six and eight hours post-infection as indicated

by the decreased levels of ATF3 induced by the mutants at these time points. Overall, Tfp retraction may play a role in enhancing ATF3 expression, but not to the same extent that has been found in other cell lines. This may be due to tissue tropism and the bacteria adapting to colonization of specific mucous membranes.

The results of my experiments lead to more questions of immune regulation. In order to see the downstream effects of NFκB and ATF3, both of which are transcriptional regulators, we will perform cytokine profiling. Levels of cytokines secreted by epithelial cells upon infection by commensal and pathogenic *Neisseria* will give a clearer picture of the immune system response. It will be interesting to see the comparison between commensal infection, which by definition does not cause disease, and pathogenic infection.

So far our approach relies on a tissue culture model of *Neisseria* infection. An improvement of this system would involve using primary cells instead transformed cells. Primary cell lines are more difficult to maintain, but are a better approximation of the *in vivo* situation. An animal model of human *Neisseria* is not currently available but progress is being made toward developing a mouse model which can better represent infections in humans. Studying infection *in vivo* will allow a broader overview of these interactions. An *in vivo* model will allow us to see how secreted cytokines then interact with the host immune system beyond the epithelium. Another avenue of investigation is looking at mice infected with mouse-specific *Neisseria*. Comparisons between how the bacteria has adapted to different hosts may lead to novel forms of interactions and manipulations. In summary, this project has opened up new avenues for investigation that will provide new insights into *Neisseria* and host cell interactions.

Methods and Materials

Reagents

Antibodies against the following proteins were used in this project: ATF3, total NFκB (Santa Cruz Biotechnology), β-tubulin (E7, University of Iowa Developmental Studies Hybridoma Bank), and phospho-NFκB Ser536 (Cell Signaling).

Epithelial Cell Tissue Culture

End1 endocervical cells were grown in EpiLife media (Invitrogen) with human keratinocyte growth supplement (0.2%v/v bovine pituitary extract, 5mg/ml bovine insulin, 0.18 mg/ml hydrocortisone, 5mg/ml bovine transferrin, 0.2ng/ml human epidermal growth factor) and 0.4mM calcium chloride. Detroit 526 nasopharyngeal cells (ATCC #CCL-138) were grown in Modified Eagle medium supplemented with non-essential amino acids and 10% fetal bovine serum. All cells were maintained at 37°C with 5% CO_2 and passed when cells reached approximately 80% confluency. For all experiments, cells were seeded into 6-well plates and used at 85-95% confluency.

Bacterial Strains

The following strains were used: *N. gonorrhoeae* MS11 (wild type) and MS11Δ*pilT1* and *N. elongata* 29315 (wild type), 29315Δ*pilT1* and 29315Δ*pilT1*Δ*pilT2* Δ*pilU*. Bacteria was grown on gonococcal medium base (GCB) agar (Difco) plates with Kellogg's supplements I and II at 37°C with 5%CO_2.

Epithelial Cell Infections and Sample Preparation

Bacteria were harvested from GCB agar plates at 18 hours, and resuspended in 1ml liquid GCB by vortexing. Epithelial cells were infected at a multiplicity of infection (MOI) of 50 with 100µl of bacteria, or mock infected with 100µl of liquid GCB. Cells were incubated at 37°C with 5%

CO_2 until designated time points. For ATF3 samples, cells were washed twice with 1ml ice cold phosphate buffered saline and lysed with 200µl cold RIPA buffer (10 mM sodium phosphate, pH 7.2, 150 mM NaCl, 5 mM EDTA, 50 mM NaF, 0.1% w/v SDS, 1% deoxycholate, 1% v/v Triton X-100) with 1X protease inhibitor cocktail (Roche) and 1X phosSTOP phosphatase inhibitor cocktail (Roche). Wells gently scraped and then incubated on ice for 20 minutes before transferring samples to a centrifuge tube. Lysates were run through a 23 gauge syringe 7-9 times then centrifuged at 16,000 × *g* for 20 minutes at 4°C. Supernatants were then transferred to a clean tube and stored at -80°C for future analysis. For NFκB samples, cells were lysed with 1X SDS-PAGE sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% w/v SDS, 10% v/v glycerol, 50 mM DTT, 0.1% w/v bromophenol blue) with added phosphatase and protease inhibitors. Samples were stored at -80°C for future analysis.

Western Blot Analysis

Samples were separated by 13.5% (ATF3, β-tubulin) or 10% (NFκB) SDS-PAGE and transferred onto 0.45µm nitrocellulose (GE Osmonics). Membranes were blocked for one hour in Tris-buffered saline (TBS) containing 5% powdered milk and 0.1% Tween. Primary antibodies were diluted in 5% milk/TBST (β-tubulin) or 5% BSA/TBST (ATF3, NFκB) and incubated overnight at 4°C. Fluorophore-conjugated secondary antibodies (Pierce) were diluted in 5% milk/TBST and incubated for one hour (phospho-NFκB) or two hours (β-tubulin, ATF3, NFκB) at room temperature. Blots were scanned on the LICOR Odyssey Infrared Imaging system and densitometry was performed using ImageJ. Protein levels were normalized to a loading control (NFκB or β-tubulin) and expressed as a fold change from protein level in time zero samples.

Statistics

Data was analyzed using Prism 5 (GraphPad Software). Two-way ANOVA test was run.

References

1. World Health Organization. *Sexually Transmitted Diseases: Gonorrhoea*. 2012; Available from: http://www.who.int/vaccine_research/diseases/soa_std/en/index2.html.
2. Gerbase A., C. Stein, J. Levison, Y. Htun, *Global burden of sexually transmitted Diseases (excluding HIV) in the Year 2000,* in *Sexually Transmitted Diseases,* 2006, World Health Organization.
3. Janda, W.M. and J.S. Knapp, *Neisseria and Moraxella catarrhalis,* in *Manual of Clinical Microbiology 8th ed.,* P.R. Murray, Editor 2003, ASM Press: Washington DC. P. 585-608.
4. Tonjum, T., *Genus Neisseris.* In Bergey's Manual of Systemic Bacteriology, 2nd ed., G.M. Garrity, Editor, 2005, Springer-Verlag.: New York.
5. Centers for Disease Control. *Identification fo N. gonorrhoeae and related species.* 2011; Available from: http://www.cdc.gov/std/Gonorrhea/lab/ident.htm.
6. Stadnyk, A.W., *Cytokine production by epithelial cells.* The Federation of American Societies for Experimental Biology, 1994. 8(13): p. 1041-7.
7. Ramsey, K.H., et al., *Inflammatory cytokines produced in response to experimental human gonorrhea.* The Journal of Infectious Diseases, 1995. 172(1): p. 186-91.
8. Hedges, S.R., et al., *Cytokine and antibody response in women infected with Neisseria gonorrhoeae: effects of concomitant infections.* The Journal of Infectious Diseases, 1998. 178(3): p. 742-51.
9. Fichorova, R.N., et al., *Distinct proinflammatory host responses to Neisseria gonorrhoeae infection in immortalized human cervical and vaginal epithelial cells.* Infection and Immunity, 2001. 69(9): p. 5840-8.

10. Muller, J.M., H.W. Ziegler-Heitbrock, and P.A. Baeuerle, *Nuclear factor kappa B, a mediator of lipopolysaccharide effects.* Immunobiology, 1993. 187(3-5): p. 233-56.
11. Naumann, M., et al., *Neisseria gonorrhoeae epithelial cell interaction leads to the activation of the transcription factors nuclear factor kappa B and activator protein 1 and the induction of inflammatory cytokines.* The Journal of Experimental Medicine, 1997. 186(2): p. 247-58.
12. Thompson, M.R., D. Xu, and B.R. Williams, *ATF3 transcription factor and its emerging roles in immunity and cancer.* Journal of Molecular Medicine, 2009. 87(11): p. 1053-60.
13. Calton, C.M. *Neisseria infection activates cytoprotective pathways in human epithelial cells*. Thesis (Ph.D.)--Oregon Health and Science University, 2011.
14. Merz, A.J., C.A. Enns, and M. So, *Type IV pili of pathogenic Neisseriae elicit cortical plaque formation in epithelial cells.* Molecular Microbiology, 1999. 32(6): p. 1316-32.
15. Meyer, T.F., et al., *Pilus genes of Neisseria gonorrhoeae: chromosomal organization and DNA sequence.* Proceedings of the Nation Academy of Sciences of the United States of America, 1984. 81(19): p. 6110-4.
16. Freitag, N.E., H.S. Seifert, and M. Koomey, *Characterization of the pilF-pilD pilus assembly locus of Niesseria gonorrhoeae.* Molecular Microbiology, 1995. 16(3): p. 575-86.
17. Drake, S.L. and M. Koomey, *The product of the pilQ gene is essential for the biogenesis of type IV pili in Neisseria gonorrhoeae.* Molecular Microbiology, 1995. 18(5): p. 975-86.
18. Collins, R.F., et al., *Analysis of the PilQ secretin from Neisseria meningitidis by transmission electron microscopy reveals a dodecameric quaternary structure.* Journal of Bacteriology, 2001. 183(13): p. 3825-32.

19. Collins, R.F, et al., *Interaction with type IV pili induced structural changes in the bacterial outer membrane secretin PilQ.* The Journal of Biological Chemistry, 2005. 280(19): p. 18923-30.
20. Merz, A.J., M. So, and M.P. Sheetz, *Pilus retraction powers bacterial twitching motility.* Nature, 2000. 407(6800): p. 98-102.
21. Wolfgang, M., et al., *Components and dynamics of fiber formation define a ubiquitous biogenesis pathway for bacterial pili.* The EMBO Journal, 2000. 19(23): 6408-18.
22. Marri, P.R. et al., *Genome sequencing reveals widespread virulence gene exchange among human Neisseria species.* PLoS One, 2010. 5(7): p. e11835.
23. Higashi, D.L., et al., *N. elongata Produces Type IV Pili That Mediate Interspecies Gene Transfer with N. gonorrhoeae.* PLoS One, 2011. 6(6): p. e21373.
24. Lee, S.W., et al., *PilT is required for PI(3,4,5)P3-mediated crosstalk between Neisseria gonorrhoeae and epithelial cells.* Cellular Microbiology, 2005. 7(9): p. 1271-84
25. Howie, H.L., M. Glogauer, and M. So, *The N. gonorrhoeae type IV pilus stimulates mechanosensitive pathways and cytoprotection through a pilT-dependent mechanism.* PLoS Biology, 2005. 3(4): p. e100.
26. Howie, H.L., S.L., Shiflett, and M. So, *Extracellular signal-regulated kinase activation by Neisseria gonorrhoeae downregulates epithelial cell proapaptotic proteins Bad and Bim.* Infection and Immunity, 2008. 76(6): p. 2715-21.
27. Dietrich, M., et al., *Activation of NF-kappaB by Neisseria gonorrhoeae is associated with microcolony formation and type IV pilus retraction.* Cellular Microbiology, 2011. 13(8): p. 1168-82.