Macrophage–Neisseria gonorrhoeae Interactions: A Better Understanding of Pathogen Mechanisms of Immunomodulation

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Neisseria gonorrhoeae is a significant health problem worldwide due to multi-drug resistance issues and absence of an effective vaccine. Patients infected with N. gonorrhoeae have not shown a better immune response in successive infections. This might be explained by the fact that N. gonorrhoeae possesses several mechanisms to evade the innate and adaptive immune responses at different levels. Macrophages are a key cellular component in the innate immune response against microorganisms. The current information suggests that gonococcus can hijack the host response by mechanisms that involve the control of macrophages activity. In this mini review, we intend to condense the recent knowledge on the macrophage–N. gonorrhoeae interactions with a focus on strategies developed by gonococcus to evade or to exploit immune response to establish a successful infection. Finally, we discuss the opportunities and challenges of therapeutics for controlling immune manipulation by N. gonorrhoeae.

Keywords: innate immunity, macrophages, Neisseria gonorrhoeae, host response, immunomodulation

INTRODUCTION

Neisseria gonorrhoeae or gonococcus, a gram-negative diplococcus, belongs to the genus Neisseria and is the etiological agent of the sexually transmitted bacterial infection (STI) gonorrhea. Nowadays, gonorrhea is the second most common bacterial STI and results in substantial morbidity and economic cost worldwide. The majority of infections are benign mucosal infections of the urogenital tract, pharynx, and rectum. Ascended infections such as endometritis, salpingitis, epididymitis, and pelvic inflammatory disease (PID) are more difficult to treat. Gonococcal PID and its related complications (infertility, ectopic pregnancy, and chronic pelvic pain) constitute the major morbidity and mortality associated with gonorrhea (1). The most recent annual incidence estimates, based on data collected in 2012, indicate 78 million new cases worldwide with a global incidence rate of 19 per 1,000 females and 24 per 1,000 males (2, 3). Another serious concern is the current identification of antimicrobial drug resistance of N. gonorrhoeae, which includes the most recent fluoroquinolones and extended-spectrum cephalosporin (4–6). Indeed, U.S. Centers for Disease Control (CDC) identified multidrug resistance as among the 3 most “urgent” hazard-level threats to the U.S. population (7).
Recent information shows that \textit{N. gonorrhoeae} can prevent the development of a successful protective immune response; the causes of the weak immune response triggered by \textit{N. gonorrhoeae} are varied and correspond to multiple mechanisms that include the immune privilege of the reproductive tissue colonized by bacteria (8, 9), as well as the own strategies developed by the bacteria, such as epitope mimicry, antigenic variations and phase variation. Moreover, gonococci seem to directly interfere with the cells involved in adaptive immune response, such as dendritic cells and B and T lymphocytes (10–13). Regarding innate immunity, phagocytic cells such as macrophages and neutrophils (PMN) represent the earliest line of defense against invading bacteria. However, PMN cannot clear infections by \textit{N. gonorrhoeae} (14). Since gonococcus can survive into PMN and suppress the oxidative burst (14, 15), it has been hypothesized that bacteria actively recruit PMN to permit the spread to profound tissues of the host and even to other hosts.

Moreover, \textit{N. gonorrhoeae} also affects macrophages and their functionality, posing additional difficulties to its detection and elimination by the innate immune system (16).

Considering that macrophages are critical cells in the innate immune response to \textit{N. gonorrhoeae}, and macrophage-driven innate immune response can be subverted by \textit{N. gonorrhoeae}, promoting the persistence of gonorrhea, we will highlight our current knowledge about macrophage–\textit{N. gonorrhoeae} interactions in this mini review with a focus on strategies developed by gonococcus to evade or to exploit immune response to establish a successful infection.

**MACROPHAGES AND ANTIMICROBIAL RESPONSE**

Macrophages are present in almost all tissues and have diverse functions ranging from clearance of microbes, dead, and senescent cells until reparative and regulatory functions. Tissue-resident macrophages can derive from yolk sac macrophages, fetal liver monocytes, or adult bone-marrow monocytes capable of entering in tissues during inflammation (17, 18). When bacteria cross the layer of epithelial cells, accessing submucosa, they have the first encounter with macrophages (19). Macrophages recognize microbial pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs) by genome-encoded pattern recognition receptors (PRRs). It leads the activation of host defense pathways, which include internalization of the pathogen into phagosomes and fusion with lysosomes to form phagolysosomes, where the microbes are killed by reactive oxygen and nitrogen species and proteolytic enzymes, resulting in the clearance of the infection (20). Also, activation of macrophages includes secretion of pro-inflammatory cytokines and antigen-presentation (21). Currently, it has been demonstrated that many signals recognized by macrophages drive to classical activation (M1 macrophages) or alternative activation (M2 macrophages) (22). While M1 macrophages stimulate a robust anti-tumor and microbicide response, M2 macrophages are involved in tumor progression, tissue remodeling, response against parasites and they have regulatory functions (23).

**CELLULAR MODEL FOR EXAMINING MACROPHAGES DURING INFECTION**

Diverse cell models have been used to study the interaction between \textit{N. gonorrhoeae} and macrophages; these include cell lines and primary macrophages. The most common murine line used is RAW 264.7. This is a tumor line induced by Abelson murine leukemia virus (24). Human macrophage cell lines mostly used include U937 and THP-1. THP-1 cells are derived from acute monocytic leukemia (25). U-937 cells were obtained from a patient suffering from histiocytic lymphoma (26). We should consider the differences between these lines based on their diverse origin and maturation stage (27).

Moreover, cell lines generally vary from primary macrophages, since repeated subculture typically results in some abnormalities such as the loss of genes expression (28); although this is not important for proliferation, it is critical for the immune response developed by macrophages. Because of these deficiencies of macrophage cell lines, it may be essential to complement such a model with culture of primary macrophages cells. Cell sources for primary macrophage models include mouse peritoneal macrophages (PM), bone marrow-derived macrophages (BMDM) and human monocyte-derived macrophage (MDM). Murine source of macrophages can be obtained from different strains of wild-type, transgenic and gene-targeted mice. Although PM can be easily harvested, the output is small and we should consider that sanitary and welfare conditions of the animal could affect the physiology of macrophages (29).

Conversely, BMDM do not present problems associated with the health status of the donor mice, because BMDM are obtained from bone marrow stem cells and are completely differentiated \textit{in vitro} (29). In this line, human peripheral blood monocytes are the most commonly used precursors for generating macrophages \textit{in vitro}. The MDM culture allows the monocytes differentiation and polarization toward different macrophage phenotypes using cytokines and/or bacterial products. Thus, M1 and M2 macrophages require GM-CSF or M-CSF in the presence of IFN-\textgamma and/or LPS and IL-4 or IL-13, respectively (30, 31).

**MECHANISMS OF SURVIVAL AND REPLICATION OF \textit{N. gonorrhoeae} DURING MACROPHAGE INFECTION**

\textit{Neisseria gonorrhoeae} is able to prevent some defense mechanisms elicited by macrophages. Our laboratory and others have showed different mechanisms used by gonococcus to evade antimicrobial macrophage response. Below, we discuss some of the most relevant mechanisms observed in Macrophage–\textit{N. gonorrhoeae} interactions.
MODULATION OF PHAGOCYTOSIS BY MACROPHAGE

*Neisseria gonorrhoeae* can avoid phagocytosis mediated by opsonic antibodies. The membrane molecules such as lipoooligosaccharide (LOS), Pili, Opa, and Porin (Por) are the main targets to generate antibodies against gonococci (32–34). By constantly varying these antigens, the bacteria elude antibody opsonization and successive IgG Fc-receptor-mediated phagocytosis (14, 35, 36). Additionally, LOS carbohydrates moiety mimic human surface antigens (37, 38) impairing the recognition by macrophages. Other membrane components such as pili reduce the association *in vitro* between *N. gonorrhoeae* and non-activated mouse PM macrophages, similar to streptococcal M protein which reduce the phagocytosis (39). However, the rate of phagocytosis of pilated gonococcus can be enhanced using immune anti-serum, indicating that this enhancement reflects primarily immunoglobulin G-mediated phagocytosis (opsonization through Fc receptor) rather than surface attachment (40, 41). In spite of this, later studies showed that pili (particularly pilin subunit PilE) acts as a factor that promotes non-opsonic ingestion of gonococci by human monocytes in absence of serum factors rather than as a protective factor (42).

MODULATION OF INTRACELLULAR KILLING BY MACROPHAGE

It has been demonstrated that human monocytes and macrophages can kill intracellular gonococci (39) with diverse kinetics, ranged from a complete killing after 30 min of incubation in mouse peritoneal macrophages (43) to a more prolonged period of survival in RAW 264.7 cells and human MDM (44). When using membrane-impermeable antibiotics, such as gentamicin, our group and others have shown that the number of intracellular gonococcus inside macrophages increases over time (45). Studies in monocytes suggest that the persistence of *N. gonorrhoeae* inside macrophages is indicative of resistance mechanisms to innate host defenses. Thus, studies in monocytes showed that Opa-positive and pilated gonococci caused a differential oxidative response (42). Château et al. showed in differentiated U937 cells that internalized bacteria could escape from phagosome or endosome and retain viability (27).

Moreover, studies using MDM showed that purified PorB modifies phagosomal processing and reduces the delivery of the lysosomal enzyme cathepsin D, suggesting a delay in phagosome maturation and oxidative killing mechanisms (47), which is consistent with the absence of colocalization of *N. gonorrhoeae* with Lysosomal-associated membrane protein 1 (LAMP-1) present in the lysosomal acidic compartment (27). In this way, two other proteins have been described, Ng-MIP (with homology to Macrophage infectivity potentiator), and Ng-OmpA (with homology to the outer membrane protein A). Ng-OmpA, unlike Ng-MIP, has been associated with the adhesion and internalization of *N. gonorrhoeae* in RAW 264.7 cells (44, 48). However, both proteins have shown that they protect bacteria from being killed by macrophages, probably through mechanisms related to the activity of peptidylprolyl cis/trans isomerase (PPIase) (44) and the inhibition of apoptosis of infected macrophages (48), as observed in other pathogens (49–51). Intracellular survival is also favored by the modulation of cellular iron metabolism by gonococcus. Infection of human monocytes, THP-1, MM6, and murine RAW 264.7 cell lines showed an upregulation of hepcidin, neutrophil gelatinase-associated lipocalin (NGAL) and Natural resistance-associated macrophage protein 1 (NRAMP1), suggesting an increase in cellular iron bioavailability (52). Similarly, infection of BMDM with *N. gonorrhoeae* was associated with increased availability of intracellular iron, associated with INF-β and cGAS/STING signaling pathway (53).

MODULATION OF MACROPHAGE CELL DEATH

Apoptosis and autophagy constitute cell death modes for the elimination of infected cells. Failure in these mechanisms can favor the advance of disease (54, 55). *Neisseria gonorrhoeae* infection and its effects on apoptosis have been studied in PMN, and epithelial cells showed different results (56–59). A study using various sources of macrophages showed that gonococcus inhibit apoptosis in U937 and MDM cells in intrinsic and extrinsic pathways, but in THP-1 cells only extrinsic apoptotic pathway was affected (27). Concerning autophagy, Zühraier et al. showed that a phosphoethanolamine (PEA) modification of lipid A increases *N. gonorrhoeae* survival through evasion of autophagy affecting the TLR4-mediated induction of autophagic flux in RAW264 and THP-1 macrophages (60). Additionally, cell death can arise by pyroptosis. Studies using *N. gonorrhoeae* or isolated *N. gonorrhoeae* LOS showed cell death through NLRP3-mediated pyro necrosis in THP-1 cells (61) and cell death in human macrophages by activation of both canonical and non-canonical pyroptosis pathways (62).

MODULATION OF MACROPHAGE ACTIVATION

Several membrane components from *N. gonorrhoeae* including LOS play a role during infection and modulation of antimicrobial response (63, 64). The immunomodulatory potential of LOS is based on lipid A backbone rather than carbohydrates structure. In this context, Patrone et al. showed that the variation in carbohydrates structure from LOS has no influence on production of IL-8, IL-12, and TNF-α cytokines (65). Indeed, THP-1 cells showed differences in their inflammatory response, TLR4/MD2 signaling or TNF-α production, toward LOS derived from a variety of *Neisseria* strains independent of the oligosaccharide truncation (66). Contrarily, the presence of single lauric acid residue in a determinate position of Lipid A and a hexa-acylated lipid A seems to be critical for the initiation of pro-inflammatory responses, since *lpxLIII* and *msbB* mutants...
that lost these features showed a reduced ability to induce pro-inflammatory cytokines and stimulation of TLR-4-inflammatory signaling in U937, THP-1 and mouse primary macrophages (67, 68).

Similarly, modification of gonococcal lipid A with PEA also influences the production of CXCL3 and CXCL10 chemokines in infected macrophages (60). Besides, Knilans et al. showed that other molecules like peptidoglycan monomers released during N. gonorrhoeae cell wall remodeling by LtgA and LtgD lytic transglycosylases, also suppress TNF-α and IL-1β production in THP-1 cells by modulation TLR-2 and NOD2 signaling pathways (69). In this way, it is possible to hypothesize that Opa binding to CEACAM1 present on monocytes may suppress the activation of this cell, similarly to CD4 T cells (70, 71).

**OPPORTUNITIES AND CHALLENGES OF THERAPEUTICS TO CONTROL IMMUNE MANIPULATION**

Due to multi-drug resistance problems related to gonorrhea, researchers and clinicians are dedicated to making preventive vaccines. However, the capacity to create an effective vaccine has not been possible yet due to the characteristics of the immune response against N. gonorrhoeae (76). Today, the development of gonorrhea vaccines has not been effective. Only one study has shown reduced rates of gonorrhea infection in patients immunized with outer membrane vesicle meningococcal B vaccine (MeNZB) with an effectiveness of 31% (77). However, these findings are the first evidence in humans lately. Thus, other types of treatments become necessary. Liu et al. assessed an intravaginal treatment using IL-12–loaded microspheres in a murine model of infection. Microencapsulated IL-12 transforms the infection in a "live vaccine" that triggers the

**MODULATION OF MACROPHAGE PHENOTYPE**

Macrophages are part of reproductive tissues together with epithelial and stromal cells both in humans and mice (72). Considering that reproductive organs, which are targets of N. gonorrhoeae infection, have a privileged immune status (73–75), one can speculate that macrophages could contribute to regulatory response. Findings of our laboratory have demonstrated that gonococcus affects murine RAW264 and its functionality. We showed a shift toward the production of IL-10 and TGF-β and inefficient up-regulation in molecules involved in antigen presentation such as MHC class II and CD86, and therefore a weak allogeneic T-cell stimulatory activity (45).

Similarly, differentiated macrophages from THP-1 and U937 cells challenged with N. gonorrhoeae showed induction of IL-10 and low levels of IL-12 (27). In this line, we have also demonstrated that MDM challenged with N. gonorrhoeae were differentiated toward an M2 profile, expressing the CD163 marker, inducing anti-inflammatory cytokines and inhibitory surface molecules with a low capacity to stimulate CD4 T cells proliferation (46). Regarding this, it is crucial to implement studies that characterize the phenotype of macrophages in a murine model or the genital tract of the patient.
production of local and systemic gonococcus-specific antibodies (78). This method was also evaluated together with a non-living gonococcal vaccine based on gonococcal outer membrane vesicles (OMV) showing an elicitation of long-term humoral protective immunity driven by a Th1 response, which was effective against antigenically diverse strains of N. gonorrhoeae (79). Besides, the blockade of TGF-β1 and IL-10 has been useful to reverse N. gonorrhoeae-mediated suppression of Th1 and Th2 responses and it facilitates the development of specific protective immunity (11, 80). Moreover, Youssef et al. hypothesized the use of curcumin and vitamin D to “rescue” the immunity, by counteracting the different ways used by N. gonorrhoeae to evade the immune response (76). Considering that gonococcus affects macrophages polarization toward M2 phenotype (42), a putative treatment which would reverse M2 to M1 polarization is likely to be beneficial, since M1 polarization is associated with control of acute infections by many intracellular bacteria (81). In this regard, Na et al. blocked M-CSF-induced M2 macrophages differentiation using a COX-2 inhibitor Etdolac driving pro-inflammatory activities in human and murine macrophages and proposing it as a therapy for induction of enhanced antitumor immunity (82). In this line, we think that the M2 phenotype promoted by gonococcus could be reverted using COX-2 inhibitor. We also showed no significant differences in IL-1β levels in MDM infected cells compared to non-infected MDM cells, suggesting that N. gonorrhoeae could trigger insufficient IL-1β levels to activate innate immune response (83). Then, we were able to promote the IL-1β processing and release using exogenous ATP. Interestingly, production of IL-1β did not correlate with the activation of inflammasome-mediated caspase-1, suggesting that ATP could be acting at the level of mechanisms related to vesicle trafficking or pore formation.

We also showed a significant up-regulation of co-inhibitory molecule Programmed Death Ligand 1 (PD-L1) and CD86 down-regulation in macrophages upon gonococcus infection (46). Considering that in the immunological synapse the outcome of the immune response relies at least on balance between positive (CD86) and negative (PD-L1) co-stimulation signals, we hypothesize that an increase in PD-L1 expression, particularly in macrophages, represents a strategy used by N. gonorrhoeae to inhibit previously activated T cells at the later stages of the immune response in peripheral tissues. It could be reversed by PD-L1 blockade, as it has been shown for the treatment of severe chronic infectious diseases such as LCMV, HIV, HCV, HBV, C. trachomatis, and T. cruzi infection (84–87).

CONCLUSION

Until now, much of the research associated with modulation of innate immune cell response has been focused on gonococcus-PMN interaction, and many advances in this field have been achieved. Nevertheless, the interaction of N. gonorrhoeae with the resident or recruited macrophages is less well understood. Our current knowledge of the N. gonorrhoeae interactions with macrophages shows the capacity of gonococcus to avoid the response to an infectious challenge (phagocytosis and effective killing) and the elicitation of acquired immune response (Figure 1). Although we are still investigating which are the molecules from N. gonorrhoeae that confer resistance to macrophage, we have advanced in many questions about modulation of macrophage response and its role in gonococcal pathogenesis. There are still questions associated with the signaling pathways and cellular mechanisms related to inflammatory response, as well as the effects in the T cell activation and differentiation. Accessibility to mouse and human macrophages and the capacity of genetic or pharmacological manipulation will facilitate the studies addressed to find potential targets to be exploited with new therapeutic approaches beyond antimicrobial drugs.

AUTHOR CONTRIBUTIONS

AE conceived and designed the mini review and wrote the mini review. PR contributed to the writing and critically revised the paper. CA-C critically revised the paper. All authors read and approved the submitted version.

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REFERENCES

1. Jerse AE, Deal CD. Vaccine research for gonococcal infections: where are we? Sex Transm Infect. (2013) 89(Suppl. 4):iv63–8. doi: 10.1136/sextrans-2013-051225
2. World Health Organization. Global Incidence and Prevalence of Selected Curable Sexually Transmitted Infections - 2008. (2012).
3. World Health Organization. Global Antimicrobial Resistance Surveillance System (GLASS) Report: Early Implementation 2016-2017. Geneva (2017). Available online at: http://www.who.int/glass/en/
4. Bolan GA, Sparling PF, Wasserheit JN. The emerging threat of untreatable gonococcal infection. N Engl J Med. (2012) 366:485–7. doi: 10.1056/NEJM1112456
5. Fifer H, Natarajan U, Jones L, Alexander S, Hughes G, Golparian D, et al. Failure of dual antimicrobial therapy in treatment of Gonorrhea. N Engl J Med. (2016) 374:2504–6. doi: 10.1056/NEJMtc1512757
6. Unemo M, Shafer WM. Antimicrobial resistance in Neisseria gonorrhoeae in the 21st century: past, evolution, and future. Clin Microbiol Rev. (2014) 27:587–613. doi: 10.1128/CMR.00010-14/27/587
7. Wetzler LM, Feavers IM, Gray-Owen SD, Jerse AE, Rice PA, Deal CD. Summary and recommendations from the National Institute of Allergy
and Infectious Diseases (NIAID) workshop “Gonorrhea Vaccines: the Way Forward.” Clin Vaccine Immunol. (2016) 23:656–63. doi: 10.1128/CVI.00230-16

8. Imarai M, Candia E, Rodriguez-Tirado C, Tognarelli J, Parido M, Perez T, et al. Regulatory T cells are locally induced during intravaginal infection of mice with Neisseria gonorrhoeae. Infect Immun. (2008) 76:5436–5. doi: 10.1128/IJAI.00552-08

9. Niederkorn JY. See no evil, hear no evil, do no evil: the lessons of immune privilege. Nat Immunnol. (2006) 7:354–9. doi: 10.1038/nii1328

10. Arora S, Olszewski MA, Tsang TM, McDonald RA, Toews GB, Huffnagle GB. Effect of cytokine interplay on macrophage polarization during chronic pulmonary infection with Cryptococcus neoforans. Infect Immun. (2011) 79:1915–26. doi: 10.1128/IJAI.01270-10

11. Liu Y, Isam EA, Jarvis GA, Gray-Owen SD, Russell MW. Neisseria gonorrhoeae selectively suppresses the development of TH1 and TH2 cells, and enhances TH17 cell responses, through TGF-beta-dependent mechanisms. Mucosal Immunol. (2012) 5:320–31. doi: 10.1038/mi.2012.12

12. Plant LJ, Jonsson AB. Type IV pili of Neisseria gonorrhoeae influence the activation of human CD4+ T cells. Infect Immun. (2006) 74:442–8. doi: 10.1128/IJAI.74.1.442-448.2006

13. Zhu W, Ventevogel MS, Knillaj KJ, Anderson JE, Oldham LM, McKinnon KP, et al. Neisseria gonorrhoeae suppresses dendritic cell-induced, antigen-dependent CD4 T cell proliferation. PLoS ONE (2012) 7:e41260. doi: 10.1371/journal.pone.0041260

14. Criss KA, Seifert HS. A bacterial siren song: intimate interactions between Neisseria and neutrophils. Nat Rev Microbiol. (2012) 10:178–90. doi: 10.1038/nrmicro2713

15. Palmer A, Criss AK. Gonococcal defenses against antimicrobial activities of neutrophils. Trends Microbiol. (2018) 26:1022–34. doi: 10.1016/j.tim.2018.07.003

16. Quillin SJ, Seifert HS. Neisseria gonorrhoeae host adaptation and pathogenesis. Nat Rev Microbiol. (2018) 16:226–40. doi: 10.1038/nrmicro.2017.169

17. Cole J, Aberdein J, Jubrail J, Dockrell DH. The role of macrophages in the innate immune response to Streptococcus pneumoniae and Staphylococcus aureus: mechanisms and contrasts. Adv Microb Physiol. (2014) 65:125–202. doi: 10.1016/bs.ampbs.2014.08.004

18. van de Laar L, Saelens W, De Prijck S, Martens L, Scott CL, Van Isterdael G, et al. Antibodies to opacity proteins (Opa) correlate with a reduced risk of gonococcal salpingitis. J Clin Invest. (1994) 93:1748–55. doi: 10.1172/JCI117159

19. Zheng H. Analysis of the antigen-antibody specificity in the semen of patients with Neisseria gonorrhoeae. Clin Med Sci J. (1997) 12:47–9.

20. Shafer WM, Datta A, Kolli VS, Rahman MM, Balthazar JT, Martin LE, et al. Phase variable changes in genes lgtA and lgtC within the lgtABCDE operon of Neisseria gonorrhoeae can modulate gonococcal susceptibility to normal human serum. J Endotoxin Res. (2002) 8:47–58. doi: 10.1177/0969909X02008010501

21. Virji M. Pathogenic neisseriae: surface modulation, pathogenesis and infection control. Nat Rev Microbiol. (2009) 7:274–86. doi: 10.1038/nrmicro2097

22. Mandrell RE. Further antigenic similarities of Neisseria gonorrhoeae lipooligosaccharides and human glycoporphinolipids. Infect Immun. (1992) 60:3017–20.

23. Mandrell RE, Griffiths JM, Macher BA. Lipooligosaccharides (LOS) of Neisseria gonorrhoeae and Neisseria meningitidis have components that are immunologically similar to precursors of human blood group antigens. Carbohydrate sequence specificity of the mouse monoclonal antibodies that recognize crossreacting antigens on LOS and human erythrocytes. J Exp Med. (1988) 168:107–26. doi: 10.1084/jem.168.1.107

24. Blake M, Swanson J. Studies on gonococcal infection. IX. In vitro decreased association of piloted gonococci with mouse peritoneal macrophages. Infect Immun. (1975) 11:1402–4.

25. Jones RB, Buchanan TM. Quantitative measurement of phagocytosis of Neisseria gonorrhoeae by mouse peritoneal macrophages. Infect Immun. (1978) 20:732–8.

26. Jones RF, Newland JC, Olsen DA, Buchanan TM. Immune-enhanced phagocytosis of Neisseria gonorrhoeae by macrophages: characterization of the major antigens to which opsonins are directed. J Gen Microbiol. (1980) 121:365–72. doi: 10.1099/00221287-121-2-365

27. Knepper B, Heuer I, Meyer TF, van Putten JP. Differential response of human monocytes to Neisseria gonorrhoeae variants expressing pili and opacity proteins. Infect Immun. (1997) 65:4122–9.

28. Cooper MD, Floyd SA. In vitro kinetics of phagocytosis and intracellular killing of gonococci by peritoneal macrophages from mice deficient in complement component 5. Infect Immun. (1982) 36:363–70.

29. Leuuzzi R, Serino L, Scarselli M, Savino S, Fontana MR, Monaci E, et al. Ng-MIP, a surface-exposed lipoprotein of Neisseria gonorrhoeae, has a peptidyl-prolyl cis/trans isomerase (PPlase) activity and is involved in persistence in macrophages. Mol Microbiol. (2005) 58:669–81. doi: 10.1111/j.1365-2958.2005.04859.x

30. Escobar A, Candia E, Reyes-Cerpa S, Villagras-Valdes B, Neira T, Lopez M, et al. Neisseria gonorrhoeae induces a tolerogenic phenotype in macrophages to modulate host immunity. Mediators Inflamm. (2013) 2013:217017. doi: 10.1155/2013/217017

31. Ortiz MC, Lefimil C, Rodas PI, Vernal R, Lopez M, Acuna-Castillo C, et al. Neisseria gonorrhoeae modulates immunity by Polarizing human macrophages to a M2 profile. PLoS ONE (2015) 10:e0130713. doi: 10.1371/journal.pone.0130713
47. Mosleh IM, Huber LA, Steinlein P, Pasquali C, Gunther D, Meyer TF. Neisseria gonorrhoeae porin modulates phagosome maturation. J Biol Chem. (1998) 273:53323–8. doi: 10.1074/jbc.273.53.53323

48. Serino L, Nesta B, Leuzzi R, Fontana MR, Monaci E, Mocca BT, et al. Identification of a new OmpA-like protein in Neisseria gonorrhoeae involved in the binding to human epithelial cells and in vivo colonization. Mol Microbiol. (2007) 64:1391–403. doi: 10.1111/j.1365-2958.2007.05745.x

49. Lundemose AG, Kay JE, Pearce JH. Chlamydia trachomatis Mip-like protein has peptide-prolyl cis/trans isomerase activity that is inhibited by FK506 and rapamycin and is implicated in initiation of chlamydial infection. Mol Microbiol. (1993) 7:777–83. doi: 10.1111/j.1365-2958.1993.tb01168.x

50. Moro A, Ruiz-Cabello F, Fernandez-Cano A, Stock RP, Gonzalez A. Secretion by Treponema cruzi of a peptide-prolyl cis-trans isomerase involved in cell infection. EMBO J. (1995) 14:4283–90. doi: 10.1002/j.1460-2075.1995.tb07245.x

51. Sukumaran SK, Selvaraj SK, Prasadarao NV. Inhibition of apoptosis by Escherichia coli K1 is accompanied by increased expression of BclXL and blockade of mitochondrial cytochrome c release in macrophages. Infect Immun. (2004) 72:6012–22. doi: 10.1128/IAI.72.10.6012-00422.2004

52. Zughiaer SM, Kandler JL, Shafer WM. Neisseria gonorrhoeae modulates iron-limiting innate immune defenses in macrophages. PLoS ONE (2014) 9:e87688. doi: 10.1371/journal.pone.0087688

53. Andrade WA, Agarwal S, Mo S, Shafer SA, Dillard JP, Schmidt T, et al. Type I interferon induction by Neisseria gonorrhoeae: dual requirement of cyclic GMP-AMP synthase and toll-like receptor 4. Cell Rep. (2016) 15:2438–48. doi: 10.1016/j.celrep.2016.05.030

54. Lamkanfi M, Dixit VM. Manipulation of host cell death pathways by Neisseria gonorrhoeae. J Cell Death. (2008) 2010-145. doi: 10.1155/2010/328724

55. Chen A, Seifert HS. Neisseria gonorrhoeae-mediated inhibition of apoptotic signalling in polymorphonuclear leukocytes. Infect Immun. (2011) 79:4447–58. doi: 10.1128/IAI.01267-10

56. Patrone JB, Stein DC. Effect of gonococcal lipooligosaccharide variation on human monocytic cytokine profile. BMC Microbiol. (2007) 7:7. doi: 10.1186/1471-2180-7-7

57. Pridmore AC, Jarvis GA, John CM, Jack DL, Dower SK, Read RC. Activation of toll-like receptor 2 (TLR2) and TLR4/MD2 by Neisseria is independent of capsule and lipooligosaccharide (LOS) sialylation but varies widely among LOS from different strains. Infect Immun. (2003) 71:3901–8. doi: 10.1128/IAI.71.7.3901-3908.2003

58. Ellis CD, Lindner B, Anjam Khan CM, Zahringer U, Demarco de Hormaeche R. The Neisseria gonorrhoeae lpxLII gene encodes for a late-functioning lauroyl acyl transferase, and a null mutation within the gene has a significant effect on the induction of acute inflammatory responses. Mol Microbiol. (2001) 42:167–81. doi: 10.1046/j.1365-2958.2001.02619.x

59. Zhou X, Gao X, Broglie PM, Kebaier C, Anderson JE, Thom N, et al. Hexa-acylated lipid A is required for host inflammatory response to Neisseria gonorrhoeae in experimental gonorrhoea. Infect Immun. (2014) 82:184–92. doi: 10.1128/IAI.00890-13

60. Knilans KJ, Hackett KT, Anderson JE, Weng C, Dillard JP, Duncan JA. Neisseria gonorrhoeae lytic transglycosylates LTA and LdG reduce host innate immune signaling through TLR2 and NOD2. ACS Infect Dis. (2017) 3:624–33. doi: 10.1021/acssciencemag0000888

61. Lee HS, Boulton IC, Reddin K, Wong H, Halliwell D, Mandelboim O, et al. Neisserial outer membrane vesicles bind the co-inhibitory receptor carcinoembryonic antigen-related cellular adhesion molecule 1 and suppress CD4+ T lymphocyte function. Infect Immun. (2007) 75:4449–55. doi: 10.1128/IAI.00222-07

62. Andrade WA, Agarwal S, Mo S, Shafer SA, Dillard JP, Schmidt T, et al. Type I interferon induction by Neisseria gonorrhoeae: dual requirement of cyclic GMP-AMP synthase and toll-like receptor 4. Cell Rep. (2016) 15:2438–48. doi: 10.1016/j.celrep.2016.05.030

63. Lamkanfi M, Dixit VM. Manipulation of host cell death pathways during microbial infections. Cell Host Microbe. (2010) 8:44–54. doi: 10.1016/j.chom.2010.05.004

64. Chugani N, Gold LI, Williams RS. Localization of transforming growth factor beta isoforms TGF-beta 1, TGF-beta 2, and TGF-beta 3 in surgically induced endometriosis in the rat. Obstet Gynecol. (1994) 83:455–61.

65. Grant KS, Wira CR. Effect of mouse uterine stromal cells on epithelial cell transepithelial resistance (TER) and TNFalpha and TGFbeta release in culture. Biol Reprod. (2003) 69:1091–8. doi: 10.1095/biolreprod.103.015495

66. Wada K, Nomura S, Morii E, Kitamura Y, Nishizawa Y, Miyake A, et al. Changes in levels of mRNAs of transforming growth factor (TGF)-beta1, -beta2, -beta3, TGF-beta type II receptor and sulfated glycoprotein-2 during apoptosis of mouse uterine epithelium. J Steroid Biochem Mol Biol. (1996) 59:367–75.

67. Youssaf DA, Peiris AN, Kelley JL, Grant WB. The possible roles of vitamin D and curcumin in treating gonorrhea. Med Hypotheses. (2013) 81:131–5. doi: 10.1016/j.mehy.2013.04.013

68. Petousis-Harris H, Paynter J, Morgan J, Saxton P, Mc Ardle B, Goodyear-Smith F, et al. Effectiveness of a group B outer membrane vesicle meningococcal vaccine against gonorrhoea in New Zealand: a retrospective case-control study. Lancet. (2017) 390:1603–10. doi: 10.1016/S0140-6736(17)31449-6

69. Liu Y, Egilmez NK, Russell MW. Enhancement of adaptive immunity to Neisseria gonorrhoeae by local intravaginal administration of microencapsulated interleukin 12. J Infect Dis. (2013) 208:1821–9. doi: 10.1093/infdis/jit354

70. Liu Y, Hammer LA, Liu W, Hobbs MM, Zielke RA, Sikora AE, et al. Experimental vaccine induces Th1-driven immune responses and resistance to Neisseria gonorrhoeae infection in a murine model. Mucosal Immunol. (2017) 10:1594–608. doi: 10.1038/mi.2017.171

71. Liu Y, Liu W, Russell MW. Suppression of host adaptive immune responses by Neisseria gonorrhoeae role of interleukin 10 and type 1 regulatory T cells. Mucosal Immunol. (2014) 7:165–76. doi: 10.1038/mi.2013.36

72. Megie JL, Mehrav Y, Capo C. Macrophage polarization and bacterial infections. Curr Opin Infect Dis. (2011) 24:230–4. doi: 10.1097/QCO.0b01328384b37e8

73. Na YR, Yoon YN, Son DI, Seok SH. Cyclooxygenase-2 inhibition blocks macrophage differentiation and suppresses metastasis in murine breast cancer model. PLoS ONE (2013) 8:e68341. doi: 10.1371/journal.pone.0068341

74. Garcia K, Escobar G, Mendoza P, Beltran C, Perez A, Arancibia S, et al. ATP Induces IL-1beta secretion in Neisseria gonorrhoeae-infected human macrophages by a mechanism not related to the NLRP3/ASC/Caspase-1 axis. Mediators Inflamm. (2016) 2016:2158504. doi: 10.1155/2016/2158504
84. Fankhauser SC, Starnbach MN. PD-L1 limits the mucosal CD8+ T cell response to Chlamydia trachomatis. J Immunol. (2014) 192:1079–90. doi: 10.4049/jimmunol.1301657
85. Liu J, Zhang E, Ma Z, Wu W, Kosinska A, Zhang X, et al. Enhancing virus-specific immunity in vivo by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. PLoS Pathog. (2014) 10:e1003856. doi: 10.1371/journal.ppat.1003856
86. Peligero C, Argilaguet J, Guerrero-Fernandez R, Torres B, Ligero C, Colomer P, et al. PD-L1 blockade differentially impacts regulatory T cells from HIV-infected individuals depending on plasma viremia. PLoS Pathog. (2015) 11:e1005270. doi: 10.1371/journal.ppat.1005270
87. Terrazas LI, Montero D, Terrazas CA, Reyes JL, Rodriguez-Sosa M. Role of the programmed Death-1 pathway in the suppressive activity of alternatively activated macrophages in experimental cysticercosis. Int J Parasitol. (2005) 35:1349–58. doi: 10.1016/j.ijpara.2005.06.003

Conflict of Interest Statement: 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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