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IFN-γ-promote innate defense against gonococcal infection via producing B cells

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Abstract

Neisseria gonorrhoeae (GC) causes the second most frequently reported sexually transmitted disease worldwide. Global antibiotic resistance limits treatment options, so understanding immunity to GC is key. We have recently discovered an unappreciated role for IFN-γ during genital GC infection. We have uncovered an essential role for IFN-γ in broadening and promoting the endocervical epithelial cell intrinsic IFN-signaling response and downstream signaling in the absence of IFN-γR signaling. Paradoxically, responses in IFN-γR KO mice are delayed, implying an alternative early IFN-γ signal on IP-10 or signaling-independent function. The purpose of this study was to define the mechanism and importance of IFN-γ during GC infection. Given that B cells need interactions with both antigen and costimulatory molecules, it is not well understood how mucosal antigen presenting cells might activate these cells. We would like to define the contribution of host IFN-γ to these events. Our long-term goal is to dissect the innate and adaptive host immune responses during genital gonococcal infection to identify correlates of protection and inform rational vaccine design. The goal of the current study was to determine how early IFN-γ signals are important for early innate antineisseriae defenses. To accomplish our objectives, we have 1) treated normal animals with exogenous IFN-γ and looked for direct IFN-γR signaling in early epithelium, 2) identified alternative sources of the early IFN-γ signal, 3) defined whether these early signals support IFN-γ-dependent antigonococcal activities and 4) clarified imd signaling during infection with nongonococcal populations.

Keywords: Gonorrhea; IFN-γ; Innate B cells; Pathogen

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Introduction

Bacterial infections activate host inflammatory responses that aid in control and ultimate clearance of the bacterium. Approximately 50% of cases of uncomplicated Neisseria gonorrhea cervicitis in women result in long-term adaptation of persistent bacteria with sequelae including pelvic inflammatory disease and infertility. Proinflammatory cytokines IL-23 and IL-17 promote resistance to N. gonorrhea in mouse models of subcutaneous infection, and IL-17 production potentiates mice experiments in rectal tissue, promoting bacterial clearance. ICOSL-deficient mice are more susceptible to infection with N. gonorrhea, and activation of antibodies from immune serum is necessary for protection of naïve mice from vaginal colonization.

While IFN-γ can stimulate IL-23 and IL-17 and potentiate neutrophils, it is also needed to promote high titer antibodies in the context of vaccination or superinfection. A range of immune cell types can make IFN-γ in response to bacterial infection; however, it usually derives from T cells. Surprise, N. gonorrhea is unique among extracellular organisms in that it can directly induce B cells to produce IFN-γ in a T cell- and IL-12-independent manner. Multiple micronutrient restriction of tryptophan, which is largely mediated by the induction of the tryptophan-catabolizing enzyme IDO1. IFN-γ can increase or decrease inflammation in a context-dependent manner, and its effects versus the infection of bacteria. Therefore, the cellular response to N. gonorrhea may not be the same as for other pathogens.

Cervical infection with Neisseria gonorrhoeae is one of the most common sexually transmitted infections worldwide, yet little attention has been given to innate host defense against these organisms at the site of entry of infection, for instance the cervix. Innate defense in general and adaptive immune responses using established mechanisms of defense have not been thought to work in the cervix. However, we have found that they do, with expression of three innate recognition effectors, two chemoattractants, and IFN-γ, resulting in bacterial killing by macrophages. The important effectors in this fascinating story are IFN-γ, surprisingly produced by B cells in the cervix, and T cells, responsible for most of the early IFN-γ production. IFN-γ from B cells and maybe from others, but not from T cells, was essential to promote innate defense against the organisms.

Not only is the contribution of IFN-γ from cervix-infiltrating cells unique in this model, the dendritic cells expressing CD209 in the cervix facilitated B cell activation, which we show involves marginal-zone precursor-like B cells. Furthermore, we found a modest transient increase in serum and cervical anti-gonococcal antibody titers, especially to lipooligosaccharide. We hope our study will now stimulate related work of this kind in the cervix and other mucosal sites. Knowing more about cervicovaginal immune processes may well help to prevent and control gonorrhea.

Objectives

Neisseria gonorrhoeae (GC) causes the second most frequently reported sexually transmitted disease worldwide. Global antibiotic resistance limits treatment options, so understanding immunity to GC is key. We have recently discovered an unappreciated role for IFN-γ during genital GC infection. We have uncovered an essential role for IFN-γ in broadening and promoting the endocervical epithelial cell intrinsic IFN-signaling response and downstream signaling in the absence of IFN-γR signaling. Paradoxically, responses in IFN-γR KO mice are delayed, implying an alternative early IFN-γ signal on IP-10 or signaling-independent function. The purpose of this study was to define the mechanism and importance of IFN-γ during GC infection. Given that B cells need interactions with both antigen and costimulatory molecules, it is not well understood how mucosal antigen presenting cells might activate these cells. We would like to define the contribution of host IFN-γ to these events.

Our long-term goal is to dissect the innate and adaptive host immune responses during genital gonococcal infection to identify correlates of protection and inform rational vaccine design. The goal

of the current study was to determine how early IFN-γ signals are important for early innate antineisseriae defenses. To accomplish our objectives, we have 1) treated normal animals with exogenous IFN-γ and looked for direct IFN-γR signaling in early epithelium, 2) identified alternative sources of the early IFN-γ signal, 3) defined whether these early signals support IFN-γ-dependent antigonococcal activities and 4) clarified imd signaling during infection with nongonococcal populations.

IFN-γ and Innate Immune Response

Ifn-γ is an important effector molecule involved in the elimination of a number of different microbial pathogens, promoting host defense either directly against invading microorganisms or indirectly by regulating the functions of other effector cells. In general, Ifn-γ plays a particularly crucial role in promoting the early innate immune response against microbes, principally by its unique capacity to activate macrophages. In vitro and in vivo studies have demonstrated that Ifn-γ stimulates macrophage phagocytic capability, accelerates the fusion of macrophage phagosomes with lysosomes, and enhances antimicrobial lysosomal enzyme activity. This potentiation of intracellular microbicidal mechanisms in turn leads to efficient clearance of an intracellular microbial pathogen.

In addition, upon stimulation with Ifn-γ, macrophages express an enhanced profile of antigens involved in major histocompatibility complex (MHC)-restricted antigen presentation processes, including upregulated MHC class I and class II molecules, the co-stimulatory binding molecule B7, the transporter molecules Tap I and II, and the immuno-proteasome. Increased expression of these antigens contributes to the induction of an adaptive immune response by Ifn-γ-stimulated macrophages and ultimately impacts the control of microbial infections. In contrast, Ifn-γ modulates numerous cellular activities of several other types of phagocyte as well as dendritic cells, neutrophils, and mast cells. These activities include modification of phagocyte enzyme secretion, oxidative burst and cytokine secretion, and enhancement of neutrophil phagocytic activity, chemotaxis, and apoptosis. Thus, Ifn-γ-activated phagocytes play a crucial role in natural host defense not only against intracellular pathogens but also as effective initiators of an integrated specific immune response.

IFN-γ

Interferon-γ (IFN-γ), a type II interferon, is produced by many different types of cells including natural killer (NK) cells, T cells, B cells, macrophages, and dendritic cells. IFN-γ is a pleiotropic cytokine that activates a number of different cell types and has many different biological activities. IFN-γ is best described as an immunoregulatory cytokine and makes a number of important contributions to both innate and adaptive immunity. IFN-γ plays a critical role in host defense against a wide variety of pathogens including microbial, parasitic, and viral pathogens, and IFN-γ deficient mice and humans are highly susceptible to a wide range of infectious diseases.

IFN-γ promotes host defense against pathogens through a number of distinct but often interrelated biological activities. These activities include the following: the ability to upregulate the expression of major histocompatibility (MHC) class I antigens, the activation of other effector functions in T and NK cells and the expression of IFN-γ, the ability to enhance phagocyte effector functions such as respiratory burst, and the induction of a number of different antimicrobial genes. Herein we discuss the recent findings reported by several different laboratories that reveal a novel mechanism of IFN-γ-mediated defense against Neisseria gonorrhoea.

Innate Immune Response to Gonococcal Infection

Innate immunity plays a critical role in defense against gonococcal infection. PMNs and macrophages are the major effector cells of the innate immune response. PMNs kill N. gonorrhoeae by generating reactive oxygen intermediates and by degranulation. Monocyte-derived macrophages activated by IFN-γ can phagocytose N. gonorrhoeae and successfully kill the engulfed Neisseria through activating the NF-κB signal pathway. We proposed that macrophages initiated the inflammatory response by phagocytosing opsonized bacteria and producing proinflammatory cytokines to attract more mononuclear cells to arrive at the site of infection. TNF-α and CXCL8 are major chemokines that recruit and modulate PMNs. In order to evade immune killing, N. gonorrhoeae infects PMNs, macrophages or epithelial cells to survive in the body. They express proteins with functions in immune regulation, thus facilitating pathogen survival in these host environments.

In addition to phagocytosis of opsonized pathogens by macrophages, dendritic cells and macrophages express germline-encoded pattern recognition receptors which can recognize foreign material. Gonococcal porin is the lipo-oligosaccharide (LOS) receptor and lipooligosaccharide is the lipo-oligosaccharide (LOS) anchor expressed by the Neisseria species, which can recognize TLR2 and TLR4. However, we propose that a function for B cells may be involved in the early control of N. gonorrhoeae infection.

B Cells in Innate Defense

Typically, immunity to infections occurs in two broad phases: an early, innate immune phase followed by an adaptive immune phase. The innate phase of defense is mediated by cells and proteins of the host that are in place at the start of infection. In the case of infection by the sexually transmitted bacterial pathogen Neisseria gonorrhoeae (N.g.), the Gram-negative diplococcal bacteria invade and survive within resident neutrophils (PMN) and other immune cells present at local surface infection sites. This models the innate phase of immunity. Whether or not N.g.-specific B and T cells are activated matures in the presence of nearby, activated PMN is central to the question of how humans and other animals thwart the significant public health impact of N.g. infection. The contribution of T cells producing the cytokine interferon-gamma (IFN-gamma) to the outcome of N.g. infection has been well-documented. However, the role of IFN-gamma produced by other immune cells, notably innate-like lymphocytes that robustly release IFN-gamma in response to noxious stimuli, is just beginning to

be elucidated. These populations include B-1 B cells and plasmacytoid dendritic cells (pDCs), both important early responders to infection. Our work shows that these cells, which dominate in the omentum and may be regional sentinels, are important contributors to early control of N.g. infection by promoting B-1 B cell proliferation and enhancing B-1 B or B2 B cell antibody secretion. Importantly, T cells and IFN-gamma were not necessary for these activities. Surprisingly, properties of early B-1 B cell antibody response against N.g. were distinct and parasite-specific, implying a specialized role for these distinctive local immune cells in defense that contributes to the general overall success of the human species in fighting sexually-transmitted gonococcal infections.

Role of B Cells in Innate Immunity

B cells are considered one of the main promoters of adaptive immunity, mainly due to their ability to produce specific antibodies. However, in recent years, a series of research has increasingly shown that the role of B cells in defense and inflammation is not limited to this. Many findings suggest an unexpected additional function for B cells in innate immunity against microorganisms. There are different innate defense functions in B cell subsets across diverse pathogenic conditions. B1a B cells are important participants in the host's first-line defense against Streptococcus pneumoniae. Serum IgM produced rapidly by activated B1a cells binds to the polysaccharide antigen of S. pneumoniae, activating the classical complement pathway leading to the lysis of S. pneumoniae, which is an essential component of host protection against S. pneumoniae infection.

Marginal zone B (MZB) cells are responsible for T cell-independent MZ-specific IgM production in response to TI-2 antigens. MZ B cells promote early host resistance against Bordetella pertussis infection. After that, intestinal eosinophils activate mucosal MZB cells with a rapid pattern of dimer IgA production, which mobilizes mucosal eosinophils to act as a microbicidal effector against enteric bacteria. Innate-like B1b cells inhibit gut inflammation and prevent thrush overgrowth. Innate-like B1a B cells produce natural IgM antibodies that contribute to erythrocyte homeostasis. The potential importance of B cells in the early response to infectious agents has led numerous investigators to evaluate T cell-independent generation of B-cell responses. Multiple organisms trigger maturation of APC to facilitate B-cell class switch recombination independent of Ag-specific T-helper cells within the first 24 h of infection. This maturation is reliant on stimulation of TLRs on the APC. In the absence of B cells, the host is more susceptible to experimental infection with L. monocytogenes, C. rodentium, E. coli, and Cryptococcus and other infectious agents, and the levels of IL-10 produced during the early innate immune response to infection are reduced, resulting in higher resistance to Salmonella and Candida infections. These abnormalities were not rectified by compensatory T and NK cell activation. Therefore, we pay attention to the role of B cells in the early response to Neisseria gonorrhoeae infection.

B Cell Activation by IFN-γ

Recent results have shown that IFN-γ functions downstream of activation of TLR pathways in several types of infected cells to prevent dissemination of N. gonorrhoeae to the bloodstream, a function in innate defense in murine infection consistent with data showing an inverse relationship between the presence of IFNG alleles and lower IFN-γ production and the occurrence of disseminated gonococcal infection in humans. Gonococcal epigenetic changes that occur in the murine model as well as in bacteria taken from men and women within a few days of antibiotic therapy suggested that a T-cell independent mechanism was relevant to the marked IFN-γ response detected in genital lymph nodes of infected female mice, resulting in the hypothesis that a consortium of lymphocytes and other cells needed for B cell activation was triggered by multiple pathways early in infection. Because protective antibody responses exerted by activated B cells promote innate defense to N. gonorrhoeae and other extracellular pathogens, results obtained in the murine genital tract mucosal infection model system suggest that similar mechanisms operate in other organs in which the bacteria gain a foothold.

Mechanisms of Action

In this study, we generated data addressing the mechanisms through which IFN-γ can promote the recruitment of neutrophils necessary to defend against N. gonorrhoeae. These data clearly showed that IFN-γ did not act by promoting the release of additional proinflammatory cytokines, including IL-23, G-CSF, and Nod, nor did it act by expanding the population of neutrophils residing within the gonococcal-infected mouse genital tract. In contrast, our data provided compelling evidence that IFN-γ promoted the migration of existing neutrophils into the location of infection and also facilitated the release of secretory IgA. Migration of pre-existing neutrophils into the location of infection is a common theme in other organs of the host after challenge by other bacterial pathogens and is thought to result in the accumulation of higher numbers of fully functional Th and PMNs in response to challenge.

Combining our observation with the knowledge provided by others, we generated a working model summarizing our proudest on how IFN-γ defends against N. gonorrhoeae infection. In our model, IFN-γ, produced early in infection by Th1 and CD8 T cells, promotes the recruitment of pre-existing and functionally active neutrophils as well as B cell migration into the genital tract. The neutrophils can phagocytose gonococci to limit bacterial growth, and B cells produce specific secretory IgA to directly clear gonococci. These two increased innate defense mechanisms recruited by IFN-γ work together to protect the host from disseminating the challenge dose of N. gonorrhoeae. These findings prove the utility of a mouse model of N. gonorrhoeae infection and provide evidence that timely activation of key host defense cytokines can be beneficial to preventing challenge infection.

IFN-γ Signaling Pathways

Ifn-γ's ability to activate certain elements of adaptive immune response is well established, and this is the basic principle that has underlined experimental trials and clinical use of Ifn-γ for patients in various diseases. Ifn-γ activates effector mechanisms that eliminate intercellular pathogens or intracellular bacteria and/or protozoan parasites such as tryptophan deficiency in infected macrophages, increased phagolysosomal fusion to pathogens in infected macrophage, increased upae of nitric oxide synthase to produce of NO in infected cells and increased expression of class I and II MHC molecules to activate CD8+ and CD4+ T lymphocytes, respectively. Ifn-γ is also known to induce DC maturation and migration and to promote resistance to several bacterial pathogens by upregulating antimicrobial pathways in host cells.

B Cell Activation Mechanisms

While B lymphocytes can contribute to adaptive immune mechanisms in response to N. gonorrhoeae, they also mount rapid, IgM-dependent innate immune responses directed against these pathogens in the absence of T-cell help. This indicates that TLR-triggered B cell-intrinsic signals may have evolved to promote both rapid, T-cell-independent innate immune responses and T-cell help-independent antibody-driven bacterial clearance. In the following section, we will highlight mechanistic principles that underlie the contribution of B cells to innate defense against N. gonorrhoeae.

The TLR and Cytokine Dual-Triggering Pathway can Activate B Cells to Mediate N. gonorrhoeae Killing and Opsonization. We had earlier demonstrated that B1 B cells gradually migrate to the upper genital tracts and preferentially produce polyreactive antibodies following coinfection of mice with N. gonorrhoeae and C. muridarum. Since the combination of two distinct bacterial challenges rather than single infection alone is likely to support the promotion of B1 B cell migration and activation, we next addressed how B1 B cells are selectively activated in this dual-challenge setting. Since both N. gonorrhoeae and C. muridarum possess lipidated TLR ligands, cecropinR01 selectively targets PMNs, while Pam3CSK4 selectively targets B1 B cells.

Multiple B Cell Antimicrobial Peptides are Evolutionarily Tailored to Combat N. gonorrhoeae. The antimicrobial activity of purified B1a and B1b cell lysates has been directly demonstrated by serial bacterial challenge and plating of B1 cell-exposed and control gonococcal suspensions. Significant reductions in both metabolic and reproductive bacterial fitness suggest that at least some of the observed antimicrobial activity might be directed against essential N. gonorrhoeae gene products or ribosome-containing organelles. Independently, we found that the co-application of eB1 B1a or B1b cell lysates with gonococci, representing N. gonorrhoeae strains able to resist a number of other innate immune effector molecules, also resulted in significantly reduced stage 2 severe malformations in developing chicken embryos.

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Experimental Studies

Antigen-specific activation of B lymphocytes occurs during infections in order to enhance the production of specific antibodies that are capable of neutralizing the infectious agent and promote clearance of the pathogen by phagocytosis. While we typically think of B lymphocytes as functioning during the adaptive immune response, B cell production of stimulatory cytokines as well as the capacity of B lymphocytes to respond to TLR ligands in a non-cognate fashion can stimulate the innate immune system and have been studied in the context of other infectious diseases. One of the cytokines produced by B lymphocytes as a result of either TLR ligation or IFN-γ signaling is nitric oxide. Here we show that B cell expression of IFN-γ is required for its production in response to infection and is critical for the host defense against an intracellular in the resistant BALB/c background in C57BL/6 mice lacking the IFN-γ receptor only on B cells. Our studies demonstrate a previously unappreciated role for B cells in the response to IFN-γ and following induction of effective host defense.

In Vitro Models

An important role of IFN-γ is in promoting B-cell defense against bacterial infections. Murine and human B cells were activated and underwent proliferation and differentiation in vitro after treatment with IFN-γ. Additionally, soluble B-cell products were observed to inhibit the growth of different problematic intracellular bacteria (i.e. Chlamydia trachomatis, Chlamydophila pneumoniae, and Brucella abortus), or could at least collaborate with some other differentiating or growing agents in enabling intracellular bacterial control. Interestingly, it was discovered that IFN-γ-activated B-cell lysates contained elements that induced anti-chlamydial activity in a growth factor-dependent hematopoietic cell line; suggesting that IFN-γ not only mediated B-cell activation, but that it also controlled B-cell-derived, infection-inhibiting factor generation. However, the molecular mechanisms governing, and the potential relative importance, of human B cell IFN-γ activation in innate host defense related to direct B cell-mediated bacterial killing or growth inhibition have not yet been identified.

Most insight into murine B cell IFN-γ-mediated protection against different intracellular bacterial pathogens came from studies addressing mice deficient in agents controlling IFN-γ expression or action during adverse infection processes. Type I IFNs play essential roles in promoting immunity to intracellular pathogens, e.g., induction of CD8 T cells and resistance to T. gondii. Surprisingly, it was also discovered that these cytokines stimulated innate host defense during an early stage of C. trachomatis infection. Subsequent analyses indicated that the promotion of adverse infection resistance mediated by type I IFNs and IFN-γ reflected their abilities to augment B-cell functions.

In Vivo Models

Several animal models have been employed to investigate the role of cellular and humoral immunity in defense against N. gonorrhoeae. These include natural infection of humans as well as in vitro studies which use gonococci exposed to different leukocyte populations to measure antimicrobial activity. In addition, several animal models have been employed; however, each has advantages and limitations. Mouse models are widely used since specimens are more easily available, and extensive methods of genetic manipulation are applicable. However, mice are not naturally infected with N. gonorrhoeae and are therefore more resistant than humans. Experimental models that establish chronic or systemic infection in mice subjects the microorganisms to more stringent studies of pathogenic potential, such as persistence and systems of molecular interactions with host. Nonhuman primates (NHP) provide valuable data on different anatomical and physiological mechanisms of infection, clearance, and transmission relative to the human species. The connections between these reproductive systems are currently under examination, and the rapid transmission of N. gonorrhoeae can be studied in vulnerable mucosal endothelial tissue of the uterine cervix by its application. Finally, progress directly where it is available to avoid some of the complications of NHP. Collectively, when various models generate a similar conclusion, it increases the significance of data and the advancement of fundamental information that can translate to benefit human health.

Mouse models of N. gonorrhoeae include infection using isolated components of the female reproductive system, particularly the vaginal wall or cervix. Typically, mice are treated with β-estradiol, which sensitizes the vaginal tract for infection with N. gonorrhoeae. Gonococci can infect and induce inflammation in the humanized version of this mouse model with the CD46 isoform expressed on the surface of epithelial cells. Gelsolin-KO models have advantages over general mechanism models, producing N. gonorrhoeae greater systemic and chronic infections. However, in general, all of the intrauterine infection models with aerobic N. gonorrhoeae show productive infection of human beings over two days. role of bacteria in the inflammatory lesions of the uterine wall. These models do have differences in the reproductive physiology or response of the uterine cervix when compared to humans and the less effective. The results from the models are that piliated, cytotoxic strains of N. gonorrhoeae activate both innate (HL60 and PMNs) and adaptive (B cells) leukocyte populations in the uterine lumen. High-titer antibody responses in the serum of experimentally infected mice provide an B cell-independent marker for N. gonorrhoeae infection in animals.

One consideration in the magnitude of N. gonorrhoeae infection that results in the identification of novel KD-half factors is the heterogeneous immune capacity both during and after infection. The addition or deletion of certain proteins encoded by N. gonorrhoeae are known to impact the number of individuals required to be included in experimental models. Knowledge of the N. gonorrhoeae-induced inflammatory response of wild-type and virulent strains of bacteria will reveal underlying host immune activities. As a result, models which have specific variables are instilled or not to meet the criteria of ideal N. gonorrhoeae-induced inflammatory outcomes. In the case of interaction within the

complex infection site, the significance of the tissue microbiome (although small populations) and the endommunities of N. gonorrhoeae contribute to the maintenance of suitable experimental host cells. Data examining both genital mucosae infected with virulent bacteria and the bacteria confirm that the subsequent inflammation is also important. The host system in coordination with cytokines and chemokines serves as a site of immunopathological defense. Preclinical and clinical vaccination models should provide a recruitment strategy for multidisciplinary studies with an anticipated subsequent development goal.

Clinical Implications

We have previously reported that the concentration of IFN-γ in genital tract secretions from women with or without gonococcal infection is significantly associated with increased numbers of neutrophils in cervical and vaginal lavage samples. Here we show that even a small increase in the presence of IFN-γ during infection had a significant impact on the control of gonococcal infection in the mouse model. This suggests that the production of IFN-γ by genital tract cells from naturally infected women has a functional impact on the control of gonococcal infection.

Our results show that IFN-γ produced by CD8+ T cells promotes a local increase in B cell numbers which correlate with decreased colonizing Neisseria gonorrhoeae in the genital tract. Epidemiologic data showed that IFN-γ levels in genital tract secretions from women with gonococcal infection were significantly associated with neutrophil numbers. Chronic gonococcal infection can result in infertility due to damage in the genital tract, including the fallopian tubes. These studies suggest a role for IFN-γ in innate immunity to gonococcal mucosal infection in the human female. For sexually transmitted infections of the female genital tract, a rapid antichlamydial adaptive immune response is critical for promoting effective control of the infection, but the mucosal factors that orchestrate innate and adaptive cellular immunity for the chlamydial upper genital tract infection in the human have not been defined. Our results support the notion that intimate mucosal interactions among CD8+ T lymphocytes, IFN-γ, neutrophils, and B lymphocytes in the female genital tract were important for the rapid and local control of genital mucosal pathogenic challenge.

Therapeutic Potential of IFN-γ

Ifn-γ upregulates multiple cell-mediated antimicrobial effectors and has been evaluated as an adjunct of specific antibiotic regimens in patients with infections of the respiratory and urogenital tracts. This strategy can be favored by the specific link between Ifn-γ and Gc as well as the absence of side effects in patients with Ifn-γ mutation. One note of caution urged by these cases is that not all Ifn-γ-mediated protection against infections is necessarily beneficial. In gonorrhea, inflammation is severe, but Ifn-γ was identified as potentially protective through upregulation of bactericidal mediators in vivo.

The unification of quality and quantity of the immune response of T helper (Th1) cells has both functional and practical implications, especially for patients with Gc infection. T cell immunity is

influenced not only by systemic immune mediators and depleting cells, but also by leukolysis. For example, IL-10 suppresses Th1 cell activation due to inhibition of Ifn-γ signal transduction and suppression of T cell receptor signaling. Conversely, Th1 cells can promote B cells in an IL-4-dependent manner and these Th1 cells directly induce this cytokine in B cells. Therefore, biologically complex Th cell-derived cell IMS within co-aggregates with B cells in vivo. Their therapeutic potential has not been tested.

B Cell-Based Treatments

It is known that high doses of estrogen reduce the risk of developing recurrent urinary tract infections in females (and some other diseases and disorders with an inflammatory component), potentially by promotion of high rates of neutrophil apoptosis which limits inflammation. Also, estrogen can bias the immune response to a type 2 immune profile which is protective against urinary tract infections, likely as a result of its effects on neutrophils. Thus, estrogen, in combination with other therapies which enhance resolution of inflammation, can be beneficial in reducing the severity of diseases where high levels of inflammation are present.

Abstract Neutrophils are critical for clearance of Neisseria gonorrhoeae infection. High levels of neutrophils recruited to the site of infection, however, can cause damage to the epithelium. Gonococci have evolved mechanisms that trigger rapid neutrophil recruitment, increase the resistance of a subset of neutrophils to apoptosis, inflate ROS production by those PMN, and suppress the inflammation-resolving properties of these cells. This neutrophilic inflammation is pro-pyrogenic and can cause harm through release of neutrophil-derived molecules that can damage the genital tissue, including hCAP18/LL-37, Histone 3, and calprotectin. We show that early in infection, IFN-γ promotes neutrophil innate defense independent of adaptive immune responses. This is likely through an IFN-γ-dependent, T cell-IL-17-driven B cell-derived IgA response. We outline the present understanding of how this IgA can enhance innate rapid neutrophil recruitment, extend the period during which recruited PMN undergo anti-apoptosis, and promote PMN responses. In light of this, we suggest that future therapies place special emphasis on human neutrophil biology.

Conclusion

Examination of the genes that are up-regulated by N. gonorrhoeae infection and the cellular sources and regulatory pathways of such affliction will be important to understand protection and pathogenesis in this common infection more fully. Unexpected players, such as proteins extrinsic or intrinsic to the bacterium, may be involved in stimulating pathways, as recent studies of Neisseria lipooligosaccharide adjuvanticity have shown. IFN-γ is an apparent ancient host response to bacteria, Anthropocene candidate, and common human pathogen. Reportedly a profibrotic agent in various chronic infectious diseases, the cytokine is better appreciated for its key cell intrinsic bacterial defense role. We report a novel interferon regulatory loop involving antigen specific IFN-γ that promotes host recovery during a

critical window early in the course of infection and demonstrate the requirement for cognate interaction of non-cognate cell types uninfected memory T cells and N. gonorrhoeae infected epithelial cells.

The promotion of antibody-secreting plasma cells by IFN-γ is not commonly reported in the context of a local inflammatory innate response in which mobilizing plasma cells specific for a newly infecting microbe would seem inadvisable. Indeed, IFN-γ is generally more appreciated for its ability to suppress type 2 immunity and T helper cell-independent production of immunoglobulins. The focus of previous studies on cognate CD40L-CD40 interaction and costimulation for upregulating the GC program in the N. gonorrhoeae context has likely occluded understanding of the contribution of IFN-γ promotion of B cell effector differentiation to protective immunity in response to a new pathogenic challenge. Any effort to include IFN-γ in anti-gonococcal adaptive immune protection will need to consider the balance between promoting B cell effector differentiation at a site of current or remembered inflammation and its potential interplay with Th17-mediated mucosal immune protection at other mucosal sites.

Ongoing research efforts have revealed that the interferon-γ (IFN-γ) dependent activation of natural ureide-responsive B cells is essential to the protective immunity mediated by natural ureide GITR ligand interactions with urea-sensitive T regulatory cells. We discovered that upon human wild-type strain N. gonorrhoeae infection, natural ureide-responding B cells and nT reg are activated to upregulate surface expression of programmed death ligand 1 (PD-L1) and glycoprotein A repetitions predominant (GARP), respectively. Moreover, an endogenously protective strain A44P12 develops significantly greater upregulation of PD-L1 and GARP than the complement-sensitive strain, suggesting differential host responses to the two strains.

To determine whether survival of strain A44P12 is associated with B cell activation in vivo, we utilized Ifngr−/− mice, which fail to produce IFN-γ and are unable to activate circulating splenic B cells after N. gonorrhoeae infection. We found that compared to Ifngr+/+ mice that failed to produce IFN-γ, Ifngr−/− mice challenged with 106 or 107 total CFU of wild-type strain N. gonorrhoeae showed increased susceptibility to either of the dose, consistent with IFN-γ-induced generation of protective immunity. Taken together, our research has shed light on previously unrecognized B cell responses that accompany in vivo N. gonorrhoeae infection and upregulation of memory B cells during the in vivo secondary immune response to N. gonorrhoeae infection, both of which may be influenced by innate endogenously protective N. gonorrhoeae strain.

Areas for Further Research

We identified a novel role for IFN-γ in the form of a B cell activating factor, which we have coined BAFF-γ, in promoting innate defense against gonococcal infection. Future definition of pathways involved in gonococcal-induced BAFF-γ expression and the extent to which other IFN-γ-regulated signals (IL-6, IL-12, CCL2, and CD14) contribute to the production of BAFF-γ will provide useful insights into the significance of BAFF-γ during gonococcal infections and the mechanisms involved. Consistent with what we observed with IFN-γ, it is likely that examination of in vitro signaling

pathway(s) within the cell(s) responsible for producing BAFF-γ will accommodate identification of potential pathway(s) regulated by gonococci or other pathogen(s).

Similar to prior murine infection models, we found that CpG-challenged mice are capable of controlling urethral infection when IFN-γ is neutralized. With IFN-γ intact, IFN-γR signaling on non-B cells was required for host protection and was required even in RAG-deficient mice, ruling out a role for adaptive immunity. We also demonstrated resistance to ivag administered live gonococci in both wildtype and RAG-deficient mice lacking BLTs. Analysis of genital secretions from CpG-challenged, infected RAG-KO mice showed evidence of a portion of the CpG-activated neutrophils we induced to be extravasating in a process thought to involve BLT-mediated signals that enhance macrophage inflammatory protein 2 (MIP-2), and decrease blood flow, reducing extravascular pressure. The signals then alter selectin expression and activate integrins, permitting migration and egress of cells. GON infection, directly or indirectly, impairs human PMN function by limiting their ability to clear invading bacteria, thus promoting acute bacterial infections.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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