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The Laboratorian SM

Vaginal Mucosal Immunity

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The human female vagina is a common site of infections, including sexually-transmitted infections (STIs), vaginitis caused by *Trichomonas vaginalis* or *Candida* spp. and bacterial vaginosis (BV). Many of these infections can be chronic or recurrent, and have serious complications with regards to pregnancy and child-birth. In addition, male-to-female transmission of HIV is largely due to viral infection of the vaginal mucosa. Although the vaginal mucosa must defend against pathogens, it must tolerate colonization with commensal bacterial species, which also play a role in host defense. Therefore, the immune mechanisms that defend this site are of interest to clinicians and investigators working on improving the prevention and treatment of gynecological infectious diseases.

Pathogen Detection

The vagina is lined by a stratified squamous, non-keratinized epithelium. This epithelium expresses Toll-like Receptors (TLRs) which recognize broadly conserved microbial molecules and serve as critical sensors of infection. Specifically, the vaginal epithelium expresses TLR2 and its partners TLR1 and TLR6, which in combination (TLR1/2 and TLR2/6) recognize lipopeptides present on both Gram-positive and Gram–negative bacteria; TLR4, which recognizes lipopolysaccharide of Gram-negative bacteria; and TLR5, which recognizes flagellin, a component of the flagellum responsible for bacterial motility. In addition, *Trichomonas vaginalis* is recognized by TLR4 on vaginal epithelial cells (1).

Table 1: Summary of Toll-like Receptors which serve as critical sensors of infection.

TLR2 TLR1 TLR6	• In combination recognize lipopeptides present on Gram-negative and Gram positive bacteria.
TLR4	• Recognizes lipopolysaccharide of Gram-negative bacteria.Recognizes *Trichomonas vaginalis on vaginal epithelial cells.*
TLR5	• Recognizes flagellin, a component of the flagellum responsible for bacterial motility.

Stimulation of these TLRs triggers secretion of the chemokine IL-8, which in turn recruits neutrophils to the vaginal mucosa to combat infection (2, 3), as well as production of antimicrobial peptides such as human Beta Defensin-2 (hBD-2), which can directly inhibit bacteria (4). In addition to epithelial cells, the role of dendritic cells (called Langerhans Cells when present in the epidermis) in detecting vaginal pathogens has been explored. Dendritic cells function primarily as antigen-presenting cells, and play a key role in linking the innate (e.g. inflammation) and adaptive (e.g. antibodies, cell-mediated immunity) arms of the immune response. Dendritic cells in the vaginal mucosa utilize TLR9 to recognize Herpes Simplex Virus-2 and stimulate interferon expression to combat this pathogen ([5). In contrast, TLR stimulation increases the susceptibility of these cells to HIV infection (6). The role of vaginal dendritic cells in immunity to bacterial vaginosis, *Trichomonas* vaginitis and a number of other vaginal infections is yet to be determined.

Antimicrobial Defense

In response to recognition of bacterial pathogens, the vaginal mucosa mobilizes a variety of defenses. Antimicrobial proteins, including lysozyme, lactoferrin, and small antimicrobial peptides such as human β-defensins (HBDs) (7) are secreted in response to infection. These proteins have broad antimicrobial activity against bacterial, viral, and fungal pathogens. Vaginal epithelial cells constitutively produce HBD-1, and produce HBD-2 after TLR stimulation with microbial products (4). Lactoferrin expression is increased during BV and infection by *Chlamydia trachomatis*, *Neisseria gonorrhoeae,* or *T. vaginalis* (8). Whereas defensins are directly antimicrobial, lactoferrin likely limits infections by sequestering iron, an essential nutrient, away from pathogens. Another immune factor produced in the vagina is mannose-binding lectin, and a particular variant of the gene encoding this factor is associated with susceptibility to vulvaginal candidiasis (9). Another antimicrobial defense is the recruitment of neutrophils to the vaginal mucosa, where they eliminate pathogens through phagocytosis and their oxidative burst. For example, neutrophils are required for defense against *Trichomonas* infection (10). In contrast, during vulvovaginal candidiasis the symptoms of infection are mediated by vaginal neutrophil influx that is unable to clear the pathogen (11). Antibodies are also present in vaginal secretions, with IgG being the dominant isotype and IgA a minor constituent. These antibodies are derived in approximately equal proportions from serum and local production. The absence of local lymphoepithelial sites, such as the Peyer's Patches in the gastrointestinal tract, is thought to be the cause of the poor antibody response of the vagina (12). In general, protective antibody responses are not observed for this site, underlying the recurrent and chronic nature of many vaginal infections.

Normal flora

The vagina, similar to the upper respiratory and lower digestive tracts, is a non-sterile mucosal surface that is colonized by normal flora. The principal constituents of the vaginal flora are *Lactobacillus* species that produce lactic acid and hydrogen peroxide, which in turn maintains a low vaginal pH and prevents infections such as

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WHAT'S INSIDE ➤➤

UPCOMING EVENTS ➤➤

08/12-14	**FOGS:** Florida Obstetric & Gynecologic Society's Annual Meeting Orlando, FL
09/14-19	**PCOGS:** Pacific Coast OBGYN Society Annual Meeting SunrIver, OR
09/22-24	**ACOG-DV:** Annual District Meeting (District V) Detroit, MI
09/23-25	**ACOG-DVII:** Annual District Meeting (District VII) Kansas City, MO
10/13-16	**ACOG-DIII & VI:** Annual District Meeting (Districts III, & VI) Philadelphia, PA
10/14-16	**TACOG:** Texas Section of ACOG, Plano, TX
10/21-23	**ACOG-DIV:** Annual District Meeting (District IV) Naples, FL
10/23-26	**ACOG-AFD:** Armed Forces District, San Diego, CA

Vaginal Mucosal Immunity

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vulvovaginal candidiasis and bacterial vaginosis. A recent study found women can be grouped into five categories based on their vaginal flora. Four separate groups were dominated each by one *Lactobacillus* species, (*L. crispatus*, *L. gasseri*, *L. iners* or *L. jensenii)* and a fifth group that was characterized by a diverse group of anaerobic species associated with bacterial vaginosis (13). How the vaginal mucosa tolerates colonization with *Lactobacillus* spp. without inflammation but maintains the ability to perceive and respond to pathogens is an important area of research. In addition to naturally-occurring *Lactobacillus* spp. providing protection from infection, research has also focused on the use of *Lactobacillus* probiotics as therapies. In the United States, a recent Phase 2a study found that introduction of *L. crispatus* was safe and well-tolerated, paving the way for a Phase 3 trial to examine efficacy (14). Encouragingly, several small (<100 patients) international randomized double-blind placebo-controlled trials found that probiotic *Lactobacillus* administration improved cure rates for women suffering from bacterial vaginosis, either alone or as an adjunct to antibiotic therapy (15).

Summary

The vaginal environment is a common site of infections, including *Trichomonas* and *Candida* vaginitis, bacterial vaginosis and many others. This site is defended by a number of innate immune mechanisms. Epithelial cells, as well as dendritic cells, recognize pathogens via TLRs, and respond to this stimulation by producing antimicrobial peptides as well as chemokines to recruit neutrophils to the site of infection. The generally poor adaptive immune responses observed during vaginal infections likely underlie the chronic and recurrent nature of many of these infections. Finding ways to stimulate an adaptive immune response and generate immunity to vaginal pathogens is an important area of research. Importantly, the vaginal mucosa must retain the capacity to respond to pathogens while tolerating the normal flora at this site, largely consisting of *Lactobacillus* spp. The tolerance to normal flora established at this site may also contribute to poor immunoresponsiveness. Lastly, the normal flora itself can be viewed as an innate immune defense, by competing with pathogens and generation of an acidic vaginal pH and hydrogen peroxide. Accordingly, replenishment of vaginal flora with probiotic *Lactobacillus* is being examined as a therapeutic and preventative strategy. Additional research into the complex interplay between vaginal immunity, the normal flora and pathogenic microbes will be necessary to devise novel methods to prevent and treat vaginal infections that reduce patient quality of life, lead to serious complications and consume precious health care funding.

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Recent Publications

MDL: Research & Development Peer-Reviewed Papers:

1. **Villasmil ML, Ansbach A, Nickels JT Jr**. 2011. The putative lipid transporter, Arv1, is required for activating pheremone-induced MAP kinase signaling in *Saccharomyces cerevisiae*. *Genetics.* **187**(2): 466-65.
2. **Nolt J, Rice LM, Gallo-Ebert C, Bisher ME, Nickels JT Jr.** 2011. PP2AC5c55 is required for multiple events during meiosis 1. *Cell Cycle.* **10**(9): 1420-34.
3. **Villasmil ML, Nickels JT Jr.** 2011. Determination of the membrane topology of Arv1 and the requirement of the ER luminal region for Arv1 function in *Saccharomyces cerevisiae*. *FEMS Yeast Res. [Epub ahead of print]*

Abstracts:

1. **Huang L, Libby E, Trama J**. Identify and Evaluate Novel Biomarker CIP2A for Cervical Cancer Diagnostics. 102nd Annual Meeting of the American Association for Cancer Research (AACR), April 2-6, 2011, Orlando, FL.
2. **Hilbert DW, Smith WL, Kaunitz AM, Mordechai E, Adelson ME, Trama JP, Gygax SE.** Analysis of host antimicrobial protein production during bacterial vaginosis. 111th General Meeting of the American Society for Microbiology (ASM). May 21-24, 2011, New Orleans, LA.

Quality Assurance Q&A

Q: My office keeps receiving specimen discrepancy reports stating that there was "No swab in the ***OneSwab***® vial". Why do we keep receiving these?

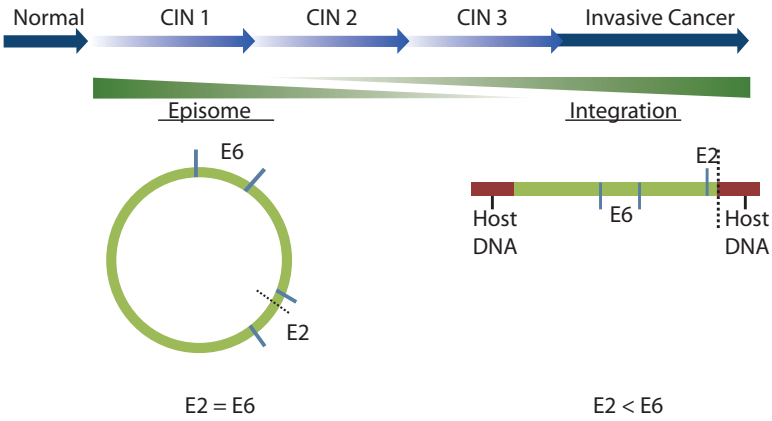
A: Traditionally, when we receive ***OneSwab***® specimens the Dacron swab is inside of the vial resting in the transport media. We understand that some clinicians may choose to collect the specimen, swirl it in the transport media vigorously, and then discard the actual swab. However, we are also aware that some offices will pre-label the actual vial and leave it on the counter top in the exam room area for the clinician to collect the specimen. There have been instances when these empty vials were submitted to our laboratory for testing even though the clinician did not actually collect the swab. Therefore, we do not make automatic assumptions. If we receive a ***OneSwab***® with no Dacon swab inside, we will contact your office as a courtesy so that you can confirm whether or not you would like us to proceed with testing.

If you have a question you would like addressed in future issues, please email your question(s) to QAQ&A@mdlab.com

Medical Diagnostic Laboratories, L.L.C.

New Tests Announcement

Test152 **HPV Type-Detect® 2.0 by Bio-Plex Analysis (Reflex to HPV-16 Risk Assessment Status) (Pos HPV-16 Only Req.)**



HPV-16 Risk Assessment Status Test by Real-Time PCR is unique molecular testing that supplements the information provided by HPV Type-Detect® assay providing an integrated view of three factors reported to be associated with the development of HPV-16-related cervical dysplasia and cervical cancer. The Real-Time PCR method is sensitive and specific and is capable of accurately quantifying the copies of HPV-16 E2, HPV-16 E6, and human GAPDH genes present in ***OneSwab***® and ThinPrep® samples.

Test 166 **Bacterial Vaginosis (BV) Panel PCR [*A. vaginae*, BVAB2, *G. vaginalis, Megasphaera* species (Type 1&2)] (with Lactobacillus Profiling)**

This molecular assay targets a set of bacteria commonly found in normal healthy vaginal microflora and microflora associated with Bacterial Vaginosis (BV). This panel includes tests for four major vaginal *Lactobacillus* species: *L. crispatus, L. gasseri, L. jensenii*, and *L. iners*, and five BV-associated pathogens: *Gardnerella vaginalis, Atopobium vaginae, Megasphaera* Type1 and Type 2, and BVAB2. Even though all of the qPCR tests in the panel can be ordered and performed separately, only the combination of the assays in a single panel allows for the accurate relative quantitative evaluation of the bacterial species composition in a given clinical sample. Generally, molecular diagnostic tests for BV are focused on the detection of recognized pathogenic markers of the disease. Incorporation of the *Lactobacillus* qPCR assays makes our test considerably more comprehensive and greatly extends the diagnostics available for the assessment of vaginal health. The new MDL Bacterial Vaginosis (with *Lactobacillus* Profiling) by qPCR Panel is a significant progress beyond the qualitative identification of BV-associated microorganisms since it now covers microbial markers of the normal vaginal environment. It can be used successfully for the determination of relative vaginal microflora composition and bacterial loads which might facilitate monitoring of the response to antibiotic therapy.

Karlsson CL, Molin G, Cilio CM, Ahrné S. **2011. The pioneer gut microbiota in human neonates vaginally born at term - a pilot study.** *Pediatr Res*. May 27. [Epub ahead of print] PMID: 21629156

It is widely appreciated that the healthy vagina is rich in bacteria and other organisms, and that this population may change, resulting in disorders such as bacterial vaginosis or vaginitis. However, it is less well understood how these populations come to be established. In this report, Karlsson et al describe the composition of intestinal organisms in the immediate neonatal period, on the assumption that these are most likely to have access for vaginal colonization in female children. In addition, they compare average birth-weight children with large-for-gestational-age (LGA) neonates. A total of seventy-nine, full-term, female children delivered vaginally of healthy mothers, were entered into the study. Children of low-birth weight were not included. Parents collected the earliest possible meconium stool, froze it and it was subsequently analyzed by PCR. The PCR was directed towards the 16S ribosomal RNA and results were used to define the presence and relative quantities of a range of bacterial species. *Lactobacillus* was detected in all fecal samples, *Bifidobacterium* in 18%, *Enterococcus* in 27%, *Enterobacteriacae* in 30% and *Bacteroides* in 14%. When present, *Bacteroides* species were present to the greatest amounts (10^9 - 10^{11} 16S copies per gram of feces), while *Lactobacillus* was the lowest (10^6 - 10^7 16S copies per gram of feces). Clear differences were observed between the average birth weight neonates and the LGA neonates: in the LGA group, *Lactobacillus* group GII species were absent, but present in 20% of the other neonates; *Bifidobacterium* were also much reduced in LGA (10% v 19%) and in contrast *Enterobacteriaece* were elevated (50% v 28%). The authors conclude that far from being sterile, the intestine of neonates is populated with organisms that may subsequently populate the vaginal tract of female babies and imply (but do not state) that this is the case. While this study is of interest, it does not take account of the vaginal and intestinal populations of the mothers, nor the ensuing vaginal populations of the children themselves, which are two points the authors suggest as the topic of future work.

Iversen MB, Ank N, Melchjorsen J, Paludan SR. **2010. Expression of type III interferon (IFN) in the vaginal mucosa is mediated primarily by dendritic cells and displays stronger dependence on NF-kappaB than type I IFNs.** *J Virol*. **84**(9):4579-86.

The recent discovery of the type-III family of interferons, known variously as IFN-λ1, λ2 and λ3, or IL-29, IL-28A and IL-28B, respectively, has caused a great deal of research and a certain re-thinking of the role of the type-I IFNS (ie, IFN-α and IFN-β). While type-I IFNs are well established as the key anti-viral mediators in innate immunity, an emerging and unique role for the members of the type-III family at epithelial surfaces (ie where the outside world interfaces with the inside of the body), is becoming clear. In this study, the authors extend these observations to the vaginal tract, describe the main producers of the type-III IFNs as being dendritic cells and illustrates a heavy dependence on one particular transcriptional inducer, NF-κB. In so doing, they position type-III IFNs as key vaginal anti-viral proteins. The authors use HSV2 as the triggering virus, and first show that this agent solicits expression of both type-I and type-III IFNs in the vagina, and that depletion of dendritic cells reduces the presence of type-III IFNs much more than type-I. This observation supports their previous contention that, while many cells (such as stromal cells) can make type-I IFNs, dendritic cells are the main source of type-III IFNs. They next address the role of these IFNs in anti-viral immunity, by using mice whose receptors for type-I or type-III IFNs have been genetically deleted, rendering them incapable of responding to these cytokines. Here, they showed that pathogens that stimulated the pattern-recognition molecule TLR9 (usually associated with the recognition of CpG components of double-stranded DNA viruses and bacteria) required type-III IFNs for complete resistance. Next, they examined the levels of transcription factors commonly associated with IFN activation, showing that NF-κB p65 was upregulated in concordance with type-III expression and that inhibition of this activator abrogated type-III IFN expression, but not that of type-I IFN. Thus, the authors of this paper provide us with a novel insight to the anti-viral responses in the vaginal tract; our natural immunity to many viruses is revealed to be the production of type-III IFNs by vaginal dendritic cells. Since the major clinically-relevant vaginal viral pathogen, HPV, is a double stranded DNA virus, this information may be important in designing future vaginally-directed treatments.

Miura S, Kawana K, Schust DJ, Fujii T, Yokoyama T, Iwasawa Y, Nagamatsu T, Adachi K, Tomio A, Tomio K, Kojima S, Yasugi T, Kozuma S, Taketani Y. 2010. **CD1d, a sentinel molecule bridging innate and adaptive immunity, is downregulated by the human papillomavirus (HPV) E5 protein: a possible mechanism for immune evasion by HPV.** *J Virol.* **84**(22):11614-23.

Human papilloma virus (HPV) is the causative agent of cervical cancer in humans, as well as a number of less serious infectious diseases, such as genital and oral warts and lesions. While only a small number of the 200 or so HPV subtypes are pathogenic, the serious effects of these few have led to vaccine development and research designed to identify pathogenic mechanisms. Much effort has focused on the role of the viral E6 and E7 proteins in tumorigenesis and the loss of the E2 gene that accompanies viral integration. The human immune system is well-equipped to detect and destroy virus-infected cells, using dendritic cells, T-cells and NKT cells amongst others to do this. Pathogens on the other hand have evolves a diverse range of tools with which to thwart these efforts. In the present report, Miura et al focus on a relatively under-researched viral gene, E5, and describe a novel role in evasion of the host immune system. The work focuses on the host detection (or “sentinel”) molecule, CD1d. This molecule is normally expressed on epithelial cells and used to attract and activate NKT cells when those epithelial cells are infected. The work begins with an illustration that the expression of this molecule is severely down regulated in HPV related lesions and cancers. The authors next demonstrated that artificially expressing the HPV E5 protein in epithelial cells caused a loss of CD1d expression from the cell-surface; this was true for E5 from two different HPV sub-types, HPV6 and HPV16. Interestingly however, the CD1d protein was still expressed within the cell, but was held on the endoplasmic reticulum (ER) and not exported to the surface. Focusing now on the ER, the authors showed that the E5 protein was also there, interacting with ER components and likely preventing the final critical folding of CD1d prior to export. Finally, the authors showed that cells expressing E5 were unable to conduct functions normally associated with CD1d expression, particularly the recruitment and activation of NKT cells. The authors concluded that a major role for E5 in the pathology of HPV is to prevent recognition of infected cells by NKT cells. In so doing, the authors illustrate a novel viral escape mechanism that is used by “low cancer risk” and “high cancer risk” HPV strains alike (illustrated by the use of E5 from the HPV6 and HPV16 sub-types, respectively). Thus, E5 does not contribute specifically to tumor development. Rather, it provides HPV with a means to avoid the host immune system generally. However, in the context of the high-risk sub-types, this is critical and so this research provides a potential new therapeutic target for HPV-induced tumors, as well as lesser infectious manifestations such as warts and lesions.

Liu Y, Russell MW. 2011**. Diversion of the Immune Response to Neisseria gonorrhoeae from Th17 to Th1/Th2 by Treatment with Anti-Transforming Growth Factor β Antibody Generates Immunological Memory and Protective Immunity.** *MBio*. **2**(3). pii: e00095-11.

Neisseria gonorrhoeae is a pathogen that is extremely well-adapted to humans as a host. One important feature of all successful pathogens is an ability to evade, negate or modify the host immune response to avoid destruction and therefore survive and flourish. *Neisseria gonorrhoeae* is known to be highly variable in how it presents itself to the immune system and resistant to serum bacteriocidal factors such as complement. In the present report, Lui and Russell describe research suggesting that *Neisseria gonorrhoeae* also effectively evades T-cell immunity, and present a strategy to overcome this. They use a mouse model in which *Neisseria gonorrhoeae* is directly introduced to the genital tract. Characteristically, this model encourages the generation of Th17 T-cells (which produce IL-17) with almost no Th1 or Th2 cells being produced. Most interestingly, the cytokine transforming growth factor -beta (TGF-β) is critical in this process. The work begins by reiterating the presence of IL-17 -bearing T-cells in infected tissues, highlighting the role of mucosally-associated γ/δ T-cells as the chief IL-17 producers. The authors then show that these cells, along with B-cells and myeloid cells are the main

sources of the TGF-β previously shown to be important. This vaginal elevation of TGF-β was evident at 5 days into infection. Next, the authors determined that withdrawing TGF-β by antibody treatment markedly changed the overall phenotype of the immune response, and critically, halved the time it took mice to resolve the infection. When TGF-β was removed, the proportion of Th17 cells was halved and that of Th1 and Th2 cells correspondingly doubled. This was accompanied by a change in disease status; untreated mice took twelve days to resolve their infections and become *Neisseria gonorrhoeae* negative by culture, while anti-TGF-β-treated animals took only six days for this to occur. Finally, the authors investigated the extent to which this treatment resulted in immunological memory and resistance to subsequent infection. Fifty percent of infected anti-TGF-β-treated animals resolved new infections by four days, while untreated (but previously infected) mice took seven days to reach this point. The previously-treated animals maintained their protective Th1/Th2 response. These studies reveal the importance of TGF-β in immune responses that fail to protect against *Neisseria gonorrhoeae* infection, suggesting that vaccination and/or treatment strategies that target this cytokine may be efficacious against this sexually-transmitted pathogen.

Hervouet C, Luci C, Rol N, Rousseau D, Kissenpfennig A, Malissen B, Czerkinsky C, Anjuère F.
2010. Langerhans cells prime IL-17-producing T cells and dampen genital cytotoxic responses following mucosal immunization. *J Immunol*. **184**(9):4842-51.

Mucosal layers constitute the site of the majority of infections of the vaginal tract. As such, its immunological function is key to protection from pathogens that attempt to enter by this route. A critical component of this activity is the recognition of antigens, and correct presentation to effector immune cells, particularly T-cells. In the vagina as elsewhere, a specialized type of antigen-presenting cell, the Langerhans cell, interfaces with the epithelia, and captures and processes antigen in a way that directs the nature and extent of the developing T-cell response. In this report, Hervouet et al use a mouse model to investigate mucosal immunization in the vagina, and define a role for Langerhans cells in directing the development of a particular T-cell subset (the Th17 pro-inflammatory cell) at the expense of T-cell cytotoxicity. Finally, they show that the activities of two Langerhans cell populations, in which the protein "langerin" is present or absent, is critical to the final T-cell balance. In this model, the investigators use a conjugate of the cholera-toxin "B" component to the antigen ovalbumin (CTB-OVA) as the vaginally instilled immunogen. By selective cell depletion experiments, they first show that Langerhans cells are essential to the development of T-cell responses and then, that both types (with or without langerin) capture antigen and migrate to the local lymph-nodes to interact with the T-cells there. Most interestingly however, they show that those without langerin were very good at inducing T-cell cytotoxicity and killing, while those with langerin were more efficient Th17 inducers. With the caveat that this is a murine model and that the question of types of langerhans cells in the human vagina now needs to be investigated, their research opens the way to specifically manipulating the nature of the T-cell response accruing from vaginal immunization in order to specifically target particular classes of pathogens (viruses, bacteria or fungi, for example). It will also be interesting to see the extent to which the hormonal environment influences the efficiency and nature of responses occurring in humans, especially in the face of an established vaginal ecosystem. Nonetheless, this is an exciting advance and one which has great potential utility in both the developed and underdeveloped world.

e-Quiz

1. The following are innate immune mechanisms in the vaginal environment:
   A. Epithelial cells C. TLR's
   B. Dendritic cells D. All of the above

2. **True of False**. The normal flora itself can be viewed as an innate immune defense, by competing with pathogens and generation of an acidic vaginal pH and hydrogen peroxide

3. **True or False**. *Trichomonas vaginalis* is recognized by TLR1 on vaginal epithelial cells.

4. _____ expression is increased during BV and infection by *Chlamydia trachomatis*, *Neisseria gonorrhoeae,* or *T. vaginalis.*

5. *Lactobacillus* species that produce _____ and _____, which in turn maintains a low vaginal pH and prevents infections such as vulvovaginal candidiasis and bacterial vaginosis.

For results to the electronic Epidemiology Quiz,
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