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Bacterial flora of the female genital tract: function and immune regulation

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The use of non-culture gene amplification techniques has improved our understanding of the composition of the vaginal bacterial ecosystem. In most healthy women in the reproductive period the predominant vaginal bacteria are one or more of the following species of *Lactobacillus*: *L. crispatus*, *L. iners* and *L. gasseri*. However, in other apparently healthy women lactobacilli may be deficient or absent, being replaced by other lactic-acid-producing bacteria: *Atopobium*, *Megasphaera* and/or *Leptotrichia* species. Infection and/or proliferation of pathogenic bacteria in the vagina is suppressed by lactic acid production, bacteria-generated antimicrobial products, and the local activities of the innate and cell-mediated immune systems. Vaginal epithelial cells produce a range of compounds with antimicrobial activities. These cells also possess membrane-bound Toll-like receptors that recognize pathogen-associated molecular patterns. Recognition leads to pro-inflammatory cytokine production and antigen-specific immunity. Local production of IgG and IgA antibodies can also be initiated in the endocervix and vagina in response to infection.

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The female genital tract is composed of a sequence of cavities. The external genital tract (vulva) leads into the vagina that connects in succession to the endocervix, the uterus and then to the Fallopian tubes. This passageway allows for the migration of the mature fetus and menstrual flow to the exterior, and for the movement of spermatozoa to the interior. This exposure of the female genital tract to the external environment carries with it the risk of potentially compromising reproductive functions. Among the defense mechanisms that are operational in preventing infections in this area, undoubtedly one of the most important is the composition of the microbial flora that colonizes the vagina.

Historically, studies of the components of the vaginal ecosystem relied first on microscopic ëvaluation and then on identification of specific bacteria by culture techniques (reviewed by Larsen and Monif).[1] However, utilization of culture media for the comprehensive identification of bacterial ecosystem diversity at a particular body site is now recognized as being incomplete and fragmentary. Culture-independent techniques have revolutionized bacterial detection. Application of the technique of amplification, cloning and subsequent sequence analysis of the genes encoding bacterial 16S ribosomal RNA directly to vaginal samples has clarified the identification of the most common *Lactobacillus* species, demonstrated that lactobacilli are not always the dominant microbial species in apparently healthy women, and has identified previously undetected bacterial vaginal inhabitants.[2–4]

LACTIC-ACID-PRODUCING BACTERIA

The dominant species in the vagina of healthy premenopausal women was initially identified as *Lactobacillus acidophilus*. This turns out to be an oversimplification, however. In healthy women with a lactobacillus-dominant vaginal microflora, the major phylotypes detected by gene amplification are *L. crispatus* and *L. iners*[2,4] or *L. crispatus* and *L. gasseri*.[3] Additional species, *L. jensenii*, *L. gallinarum* and *L. vaginalis*, have also been identified in some women. A culture-independent investigation of vaginal lactobacilli in women from three continents reported that the dominant species were the same in each region: *L. crispatus*, *L. gasseri* and *L. jensenii*.[5] Most interestingly, apparently healthy vaginal ecosystems are maintained in some women in the absence of a lactobacillus-dominant vaginal microflora. *Atopobium vaginae* was identified as the dominant vaginal phylotype in one woman, and in two others appreciable numbers of *Megasphaera* species and/or *Leptotrichia* species were also reported.[2] *Atopobium*, *Megasphaera* and *Leptotrichia* are all producers of lactic acid[2,6], similar to the lactobacilli. Therefore, the acidic environment of the vagina, recognized as an important defense mechanism against the proliferation of different microbial pathogens, can be maintained by bacterial species other than the lactobacilli. Possibly, when lactobacilli are unable to predominate in a particular vagina for whatever reason, another lactic-acid producing species fills this niche. *Megasphaera* and *Leptotrichia* are also capable of producing metabolites with an unpleasant smell. This renders detection of a vaginal odor in women lacking a lactobacillus-dominated vaginal flora as inconclusive evidence for the identification of a disease entity such as bacterial vaginosis, especially in asymptomatic women. Similarly, the variable morphology of *Atopobium*, from elliptical cocci to rod-shaped organisms occurring singly, in pairs,

or in chains[3], makes its detection by Gram stain or wet mount problematic, and it is easily mistaken for other bacteria purported to be markers of bacterial vaginosis.

BIOFILMS

A further complication to the comprehensive characterization of the vaginal microbial ecosystem is the presence of biofilms. Biofilms are colonies of microorganisms that adhere to and cover a solid surface; they can be identified on the surface of vaginal epithelial cells. Although most prominent in women with bacterial vaginosis where *Gardnerella* and *Atopobium* species predominate[7], the bacterial composition of vaginal biofilms in asymptomatic women still remains to be characterized.

RACE/ETHNICITY

Possible racial/ethnic differences in the composition of the 'normal' microflora of the vagina have also not received appropriate research attention. The occurrence of hydrogen-peroxide-producing lactobacilli, purportedly active in antimicrobial defense, is lower in Black women.[8] It has been reported that the vaginal pH of Black women is higher than that of White women among subjects who were not diagnosed as having bacterial vaginosis.[9] Additional studies suggested that this difference was only statistically significant among women who had abnormal – i.e. non-lactobacilli-dominated – vaginal microflora.[10,11] The enhanced prevalence of bacterial vaginosis as diagnosed by Gram stain in Black women as opposed to Whites might merely reflect an increased likelihood that bacteria other than lactobacilli typically predominate in the former population, and not that these women have an abnormal flora.

FLUCTUATIONS IN VAGINAL FLORA

The composition of the vaginal ecosystem is not static but changes over time and in response to endogenous and exogenous influences.[12–15] Variables include stage of the menstrual cycle, pregnancy, use of contraceptive agents, frequency of sexual intercourse, specific sexual partners, vaginal douching, use of panty liners or vaginal deodorants, and utilization of antibiotics or other medications with immune or endocrine activities. Exposure to an altered milieu will cause a fluctuation in the local environment and heighten or diminish the selective advantage of specific vaginal microbes. For example, the loss of lactobacilli from the vagina has been associated with sexual intercourse or with the use of antibiotics for non-vaginal illnesses.[14] Another study found that sexual intercourse without a condom had no effect on vaginal lactobacilli but led to elevated levels of *Escherichia coli* and facultative Gram-negative bacilli.[15] Over the course of the menstrual cycle, vaginal levels of hormones and glycogen vary, and menstrual blood alters vaginal pH and provides a substrate for many microorganisms. Nevertheless, levels of vaginal lactobacilli appear to remain constant throughout the cycle; non-*Lactobacillus* species increase during the proliferative phase, while *Candida albicans* concentrations are highest towards menstruation (as determined by culture).[13]

Since antibiotics can greatly alter the vaginal ecology, it is debatable whether women who are seemingly healthy and asymptomatic but deficient or lacking in lactobacilli on the basis of a wet mount microscopic examination should be treated. Inducing a perturbation in the endogenous microflora due solely to microscopic findings

may lead to the selective proliferation of microorganisms that had been suppressed and that are detrimental to the individual woman's vaginal health.

The yeast *C. albicans* is tolerant of the acidic vaginal environment and is present in the vagina of approximately 10–20% of women of reproductive age. The concentration of this microbe is low, and carriage is typically asymptomatic. However, under conditions associated with a local immunosuppressive event – such as frequent sexual intercourse or induction of a local allergic response – *C. albicans* can proliferate and also undergo morphogenesis to a more invasive hyphal form.[16] This results in the development of a symptomatic vaginitis.

Regardless of the predominant bacterial species in the vagina of a healthy premenopausal woman, it appears certain that lactic acid production is crucial to the maintenance of a healthy vaginal ecosystem. The resulting acidic pH prevents the overgrowth of potentially pathogenic microorganisms. Additional benefits for the host of lactobacillus predominance are production of hydrogen peroxide and bacteriocins by strains of these microbes.[5]

GENETIC POLYMORPHISMS

A newly recognized important variable that influences the composition of the vaginal microflora, as well as the efficacy of the response to pathogens, is an individual's genetic capacity for production of high or low levels of anti- or pro-microbial factors. Genetic polymorphisms are small changes in the DNA sequence of a gene occurring between healthy individuals. It usually involves either a single base pair change or a variable length of a repeated DNA sequence. A polymorphism can occur in the promoter region of the gene, the part of the DNA that is not translated into protein but that influences the rate of gene transcription. If the polymorphism occurs in the coding region of the gene it can influence the composition of the final protein by the substitution of one amino acid for another. Either change may result in individual, phenotypic alterations in the overall activity of the protein product. Polymorphisms in genes such as the anti-inflammatory mediator interleukin-1 receptor antagonist, or the cell-surface receptor for innate immune recognition of Gram-negative bacteria Toll-like receptor 4, have also been shown to influence the quantitative bacterial composition of the vagina.[17,18] The frequency of many gene polymorphisms vary in different racial/ethnic groups, and this may also relate to population differences in the composition of the vaginal ecosystem.

VAGINAL IMMUNITY

In addition to the protective effects of the endogenous vaginal microflora, the infectivity of pathogenic microorganisms at this site is prevented by local components of the innate and acquired immune systems. The innate immune system is the most primitive and evolutionary conserved arm of the immune system. It recognizes pathogen-associated molecular patterns (PAMPs) on microbial invaders rather than specific antigens. Components of innate immunity operational in the vagina are soluble factors (such as mannose-binding lectin, complement components, defensins, secretory leukocyte protease inhibitor (SLPI) and nitric oxide), membrane-associated components such as the Toll-like receptors, as well as phagocytic cells. Recognition of a PAMP by an innate immune system component triggers a sequence of events leading to the release of pro-inflammatory cytokines and activation of the acquired immune

system (T and B lymphocytes). Once activated, these lymphocytes generate microbial antigen-specific cell-mediated and antibody-mediated immunity. While activation of innate immunity is immediate upon recognition of a pathogen, several days are required for acquired immunity to become functional.

Epithelial cells lining the vagina constitute the initial point of contact between microorganisms and the host's genital tract. These epithelial cells are positive for Toll-like receptors (TLRs) and as such are important components of vaginal innate immunity.[19] Eleven TLRs have been identified so far, each with a distinct specificity.[20] Complexes of TLR1 and TLR2 recognize lipoprotein and peptidoglycan present on the surface of Gram-positive bacteria. TLR3 is specific for double stranded RNA, an intermediate in the replication cycle of many viruses. TLR4 recognizes the lipopolysaccharide surface component of Gram-negative bacteria. TLR5 reacts with flagellin, a component of bacteria flagella. TLR9 is able to distinguish between DNA sequences containing the dinucleotide CpG. In humans this DNA sequence is heavily methylated, while in bacteria CpG is unmethylated. TLR9 reacts only with unmethylated CpG DNA sequences and, therefore, is specific for bacteria.

Vaginal epithelial cells also release molecules with potent non-specific antimicrobial activity. One class of these molecules, called defensins, includes positively charged peptides that rapidly bind to the negatively charged surface of bacteria. The interaction results in the disruption of the bacterial membrane and cell lysis.[21] SLPI, a potent inhibitor of enzymes that degrade proteins (proteases), kills both Gram-positive and Gram-negative bacteria and has also been shown to block infection by the human immunodeficiency virus.[22]

Mannose-binding lectin is an antimicrobial protein present in the circulation as well as in vaginal secretions. It is synthesized primarily in the liver; local vaginal production remains uncertain. This protein recognizes mannose, N-acetylglucosamine and fucose carbohydrate moieties present on microbial surfaces. Binding induces activation of the complement system and deposition of complement components on the microbial membrane. This leads to the direct lysis of sensitive bacteria or to microbial opsonization by phagocytic cells that are positive for complement or mannose-binding lectin receptors.[23] Women deficient in mannose-binding lectin production due to a functional polymorphism in the coding region of the gene have been shown to be more susceptible to developing recurrent vulvovaginal candidiasis.[24]

Heat shock proteins are among the most highly conserved proteins in evolution. They are essential to life in every known microorganism, plant and animal. Heat shock proteins aid cell survival under adverse environmental conditions such as exposure to elevated temperature, toxic chemicals, inflammation or microbial pathogens. The inducible 70-kDa heat shock protein (hsp70) is another recently recognized antimicrobial protein present in the vagina.[25,26] Hsp70 synthesis is greatly up-regulated in response to infection and inflammation. Intracellular hsp70 binds to other proteins and prevents their degradation and incorrect assembly.[27] Extracellular hsp70 binds to TLRs and promotes an immune response to the pathogens.[28] In addition, recent evidence suggests that extracellular hsp70, produced in response to abnormal vaginal microflora, induces the release of nitric oxide in the vagina.[29] Nitric oxide has potent antimicrobial activity against a wide range of microorganisms.[30]

Antibodies that can recognize and bind to specific antigens on microorganisms, resulting in microbial killing by a complement-dependent mechanism or by opsonization, enter the vagina by transudation from the systemic circulation. In addition, a component of the mucosal immune system is located in the female reproductive tract, and antibody-producing B lymphocytes are present principally in the endocervix but also in the

vagina.[31] Both IgG and IgA class of antibodies can be locally produced. The elaboration of antibodies in the female lower genital tract provides a rapid mechanism for combating pathogenic microorganisms without the need to wait for the generation of a systemic humoral immune response. This capability means that the repertoire of antibodies in the vagina may differ from that present in the circulation. It is possible to identify antibodies in cervico-vaginal secretions that are not detectable in peripheral blood.

SUMMARY

The bacterial composition of the vagina, along with the presence of a range of innate and acquired immune system components, combine to prevent microbial pathogens from invading and/or proliferating at this site that is exposed to the external environment. Due to advances in non-culture gene amplification methods of bacterial detection, a more definitive picture of the composition of the bacterial ecosystem of the vagina is emerging. Rather than lumping all women into two categories: a 'normal' lactobacillus-dominated flora and an 'abnormal' flora dominated by other bacterial species, it is becoming increasingly apparent that in some women a non-lactobacillus vaginal flora is 'normal' and not necessarily pathogenic. Advances in genetic polymorphism studies have also led to the realization that functional individual variations exist in the rates of production or concentration of antimicrobial innate immune system components. Observation studies and clinical trials of women with specific vaginal flora, especially those based on Gram stain or other morphological characteristics, are of limited utility if the genetic characteristics of the women studied are not also taken into account.

The sequence of events leading to a profound change in the vaginal microflora in women with symptomatic bacterial vaginosis still remains to be determined. Whether all women diagnosed by a microbiological profile as being positive for 'asymptomatic bacterial vaginosis' in reality have an abnormal condition, or merely possess a normal vaginal ecosystem that is not lactobacillus-dominated, must also be carefully determined on an individual basis prior to initiation of any antibiotic treatment. Gynecologists and other physicians dealing with female patients need to be aware of vaginal microbiology and immunology research studies, and translate the most relevant observations into their clinical practice. Application of bench work to the bedside will lead to improvements in the quality and specificity of care for all women.

Practice points

- in most women of reproductive age the vaginal flora is dominated by species of *Lactobacillus*
- in some apparently healthy women, the presence of a vaginal flora dominated by other bacterial species is not necessarily abnormal or a sign of disease
- there may be racial or ethnic differences in what constitutes a 'normal' flora
- individual differences (genetic polymorphisms) between women in the composition of genes involved in innate immunity also influence the composition of the vaginal flora
- the consequences of possessing a specific vaginal bacterial flora or utilization of antibiotics to change the flora have to be evaluated on an individual basis

Research agenda

- strict criteria are needed to differentiate between women who truly have a disturbed vaginal flora and those who have a normal non-lactobacillus-dominated flora
- what endogenous host factors influence the return of a healthy vaginal ecosystem after an exogenous insult induces a temporary imbalance
- the use of exogenous bacterial preparations to replenish a disturbed vaginal flora has yet to be optimized

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