



New Insides on Vaginal Immunity and Recurrent Infections

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Major advances in gene amplification and DNA sequencing technology over the last decade have fundamentally changed our knowledge about the vaginal milieu [1].

Professor Albert Doderlein first reported culturing bacteria from vaginal secretions that were classified in 1928 as *Lactobacillus acidophilus*, a genus of Gram-positive facultative anaerobic or micro-aerophilic rod-shaped bacteria. Doderlein found that these bacteria produced lactic acid, which in turn inhibited growth of pathogens both *in vitro* and *in vivo* [2].

In the 1980s, it was determined that *L. acidophilus* was not a single species, but rather a group of closely related, obligate homo-fermentative species known as the *Lactobacillus acidophilus complex*. These species are difficult to distinguish phenotypically or biochemically, therefore they were differentiated on the basis of DNA homology. All of the *Lactobacillus* found in the vagina today are members of this complex [3].

The vaginal microbial milieu undergoes significant changes at various stages in a woman's life that are dependent on estrogen level. During childhood the vaginal pH is neutral, and the vaginal milieu is colonized by diverse aerobic, strictly anaerobic, and enteric bacterial species. Pubertal changes in the vulva and vagina occur and are induced by adrenal and gonadal maturation. Most women in the reproductive age have a predominance of one or more species of *Lactobacillus*: *L. crispatus*, *L. inners*, *L.gasseri* and *L. jensenii*. This is surprising given the number of known species of lactobacilli and suggests that vaginal lactobacilli have characteristics allowing them to successfully thrive in the vaginal milieu [2].

However, in other healthy women *Lactobacilli* are scarce or absent and they are substituted by other lactic acid-producing bacteria: *Atobium*, *Megasphaera* and/or *Leptotrichia* species. They all contribute to a low pH vaginal milieu (4.0–4.5) when glycogen present in sloughed vaginal epithelial cells is metabolized to produce organic acids. Some vaginal species of *Lactobacillus* also produce hydrogen peroxide *in vitro* however; recent studies have shown this is unlikely the case *in vivo* since dissolved oxygen levels in the vagina is remarkably low. Maintaining low pH is highly conserved among women despite the difference in their vaginal microbial milieu [2].

The supply of vaginal bacteria varies significantly among women from different ethnic backgrounds. Vaginal bacterial milieu

dominated by *Lactobacillus* species were found most abundant in Caucasian and Asian but were scarcer in African American and Hispanic women. Moreover, vaginal pH was found to differ among ethnic groups as well, with the overall median vaginal pH of African American and Hispanic women being slightly elevated over what is typically considered to be the norm [2].

Likewise the vaginal milieu continually experience changes produced by vaginal hygiene, the use of prescribed or over the counter medications, birth control methods and sexual activities, among others. Additionally, the vaginal milieu dynamic equilibrium is influenced by the phases of menstrual cycle, pregnancy, aging and stress. The innate immune system is present and functions in synchrony with the adaptive immune system in the vagina. It has evolved to meet the unique requirements to protect from the proliferation of bacterial, viral and fungal pathogens, allogeneic spermatozoa and to enhance the chances for survival of an immunologically distinct fetus [1].

Vaginal flora and immunity are important defense mechanisms. The spread of pathogenic bacteria in the vagina is controlled through lactose and other sugars conversion to lactic acid, by byproducts of endogenous bacteria and by activation of local innate and acquired immunity.

One of the characteristics of the innate immune system is recognizing molecular patterns associated with the pathogens (PAMPs) in the invading microbes. Factors acting in the vagina are soluble components (mannose-binding lectin [MBL], components of the complement, defensins, secretory leukocyte protease inhibitor [SLPI] and nitric oxide), components associated to the membrane: Toll-like receptors (TLR) and phagocytic cells. The recognition of a PAMP triggers the release of pro-inflammatory cytokines and the activation of the acquired immune system (lymphocytes T and B). Once activated these lymphocytes initiate the cells and humoral immunity. The activation of the innate immune system occurs immediately; however several days are necessary for the acquired immunity to become functional [1].

The epithelial cells of the vagina possess "Toll-like receptors" (TLR) in their surface. Eleven TLRs have been identified: TLR1 and TLR2 complexes recognize lipoproteins and peptidoglycan in gram-positive bacteria. TLR3 is specific for the double DNA chain in the replication of many viruses. TLR4 recognizes liposaccharide of the gram-negative bacteria. TLR5 reacts with flagellins of the bacterial flagella. TLR9 distinguish DNA sequences containing the CpG dinucleotide exclusively in the non-methylated state (in humans the DNA sequence is highly methylated). Vaginal cells also release molecules with non-specific antimicrobial activity like defensins, positively-charged peptides that rapidly bind negatively-charged bacterial surfaces. This binding disrupts the microorganism membrane and lyses it. Human defensins HBD-1 and HBD-2 are produced by epithelial cells. In women with infections HBD-2 production is stimulated by estrogens and inhibited by progesterone. Oral contraceptives may decrease the release of HBD-2 therefore increasing the susceptibility to infections [1-3].

The SLPI inhibits proteases, destroying gram-positive and gram-negative bacteria and blocking the action of the human

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immunodeficiency virus. Vaginal levels of this protein are decreased in women with bacterial vaginosis [1].

Mannose-binding lectin (MBL) is an antimicrobial protein present in the vagina although synthesized by the liver. The MBL binds mannose residues, N-acetyl-glucosamine and fucosyl present in microorganisms. This binding induces complement system activation causing bacteria lysis or their opsonization. Women with MBL deficiency are more susceptible to recurrent infections by *Candida albicans* [2].

Heat shock proteins are among the more highly conserved proteins in the evolution of living beings. Fundamental for life conservation, they help cell survival in diverse environmental conditions (high temperatures, chemical poisons, inflammation or microbial pathogen aggression). Heat shock protein HSP-70kDa (hsp70) was recently recognized in the vagina, its synthesis is stimulated in response to inflammation and infection. Intracellular hsp70 binds other proteins (under adverse conditions) and avoids their degradation. Extracellular hsp70 binds TLR stimulating the immune response to pathogens and induces the release of nitric acid in the vagina which has an antimicrobial activity [2].

Antibodies are found in the vagina from transudation of the systemic circulation; they bind and provoke microbial death by complement-dependent mechanism or by opsonization. Antibodies-producing B-lymphocytes are present in the vagina, locally producing IgG and IgA. Local antibodies production differing from the systemic peripheral blood ones represents a rapid mechanism for fighting pathogenic microorganisms, without the need of systemic immune response. Vaginal epithelial cells produce several byproducts with antimicrobial activity (bacteriocins) [1,2].

The vaginal immune system is under hormonal control and confers protection when adaptive immunity is down-regulated by sex hormones to meet the constraints of procreation.

The most common complaint of reproductive age women is abnormal vaginal discharge. Likewise bacterial vaginosis (BV) originates millions of health care visits in the United States, annually. BV is associated with gynecological and obstetrical complications and an increased risk of acquiring other sexually transmitted diseases. The prevalence of BV among women varies widely and is population dependent. The exact etiology of BV remains elusive, but involves deep alteration in the physiological vaginal milieu. Recently the use of broad-range PCR and sequencing of 16S rRNA genes have shown high prevalence bacteria from species as *Atopobium*, *Leptotrichia*, *Sneathia*, *Porphyromonas*, *Gardnerella vaginalis*, and novel members of the *Clostridiales* referred to as *BV-associated bacteria*. It is unclear if these bacterial species are causally related to BV or whether the association is contingent on the criteria used to diagnose BV [2,3].

One difficulty faced regarding a broader characterization of the vaginal microbial flora is the presence of biofilms. Biofilms are formed by colonies of microorganisms that adhere among themselves and cover a flat surface. Biofilms have been reported in the surface of vaginal cells in women with bacterial vaginosis, where *Gardnerella vaginalis* and *Atopobium* predominate. Another difficulty is that *Megaesphaera* and *Leptotrichia* bacteria are also capable of producing unpleasantly smelling metabolites. Furthermore, the variable morphology of *Atopobium* makes its visual detection problematic and is easily confused with other bacteria associated to bacterial vaginosis [2].

Another significant number of women during their reproductive years is affected by Vulvovaginal candidiasis (VVC) caused by *Candida albicans*. Adaptive immunity by Th1-type CD4+ T cells and downstream cytokine responses were considered to be the predominant host defense mechanisms against mucosal *Candida* infections. However newly identified chemotactic danger signals (alarmins) mediators of the acute polymorph nucleates response appear to be secreted by vaginal epithelial cells upon interaction and early adherence of *Candida*. Today the pathological inflammation in VVC is considered to be a consequence of a non-productive innate response initiated by non-classical immune mediators [1].

It is essential that we expand our knowledge regarding the vaginal milieu, the ways in which vaginal innate and adaptive immunity work and the hormone regulation that confers continuous protection. Understanding the nature of this protection may be essential in ensuring optimal reproductive health. More advanced laboratory techniques to outshine the limits of the current research laboratories and quicker available results to be incorporated into practice by women's health care providers in clinical practice are urgently needed.

New knowledge will certainly contribute to the preservation of women's vaginal milieu's homeostasis and more advanced diagnostic and therapeutic performances will improve reproductive health practices fundamental to women's quality of life.

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