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REVIEW

Vaginal Microbiota and HPV Infection: Novel Mechanistic Insights and Therapeutic Strategies

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Abstract: Cervical cancer is a global public health concern. The complex interaction of genetic and environmental factors is critical for the progress of cervical cancer. Growing evidence suggests that microbes, human papillomavirus (HPV), and the immune system interact closely with each other to govern homeostasis of the vaginal environment and the health of the lower genital tract of females. Certain vaginal microbial strains may play either a protective or a pathogenic role in carcinogenesis of the cervix after HPV persistent infection. Probiotics can therefore present a putative therapeutic approach for cervical cancer. However, work in this field remains limited. Recent technological developments have allowed us to identify microbes and their products using culture-independent molecular detection techniques. In this review, we discuss the composition of the vaginal bacterial community, its commensal flora and the protective impact this has on the health of the female genital tract. This review will also describe critical immune factors in lower genital tract health and summarize the role of the vaginal microbiota in cervical carcinogenesis. Knowledge in this field has provided researchers with the clues and tools to propose the use of probiotics as a potential line of treatment for cervical cancer and has provided valuable insights into host–pathogen interaction dynamics within the female genital tract.

Keywords: vaginal microbiota, human papillomavirus, cervical cancer, immune system, probiotic therapy

Introduction

Cervical cancer is a global public health issue, having an estimated 530,000 annual cases and 270,0000 deaths according to the World Health Organization.[1] Development of cervical cancer is a stepwise process by which localized cervical intra-epithelial neoplasia develops in the cervix and progresses into invasive and metastatic carcinoma forms. Human papillomavirus (HPV) is the major cause of cervical squamous cell carcinoma, its precursor lesions (cervical intraepithelial neoplasia), and several other benign and malignant clinical manifestations. HPVs are a family of circular double-stranded DNA viruses, 396 different subtypes of which have been reported.[2] Genital HPVs, subdivided into high- and low-risk types, are frequently associated with invasive cervical cancers.[3] High-risk HPVs (HR-HPVs) are the major drivers of cervical carcinogenesis with integrated HPV DNA present in almost all cervical cancer biopsies. Major HPV oncogenic proteins, particularly E6 and E7, are involved in cervical cancer transformation, immortalization, and malignancy.[4,5] The human vagina harbors affluent microorganisms that play essential roles in human health and disease.[6] Work to date supports the notion that the vaginal microbiota could play a role in HPV acquisition and persistence in

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cervical cancers.[7–15] In this review, we discuss the composition, function and adaptive mechanisms of the vaginal microbiome in healthy females. We then discuss the impact of the vaginal microbiota on HPV infection acquisition and persistence, potential immune factors related to cervical carcinogenesis, and explore the potential use of probiotic therapy for the control of HPV infection in the cervix.

Vaginal Microbiota

Over time, humans have coexisted with complicated bacterial communities, which are unique to specific niches.[16] The vaginal tract is colonized by various microorganisms forming the vaginal microbiota. In the past, the bacteria colonizing the vaginal tract were preliminarily isolated and confirmed by traditional bacterial culturing and biochemical identification methods. The composition of the vaginal microbiome remained obscure because the fraction of microbes failed to be cultured *ex vivo*until now. Present-day techniques, such as next-generation sequencing (NGS) and shotgun metagenomics sequencing, have helped us define the complex vaginal microbiota.[17]

The dominant microbial genus group found in healthy vaginal bacterial communities in women of childbearing age is *Lactobacillus*, commonly regarded as the first line of defense against pathogenic agents. This is different to the majority of body sites; to date, a variety of microbial communities is generally considered a signature of health. Ravel et al[18] were the first to define the composition of the vaginal microbiota using bacterial 16S RNA sequencing technology. 16S RNA is the component of the 30S small subunit of a prokaryotic ribosome. 16S rRNA gene sequencing is highly useful in regard to bacterial classification; it has low phylogenetic power at the species level and poor discriminatory power for some genera. The genes coding for it are referred to as 16S rRNA genes and are used in reconstructing phylogenies due to the slow rates of evolution of this region of the gene. They defined the vaginal microbiota in to five community state types (CSTs) (I, II, III, IV, and V) based on the presence or absence of a particular *Lactobacillus*spp. CST I, II, III, and V microbiota belong to *L. crispatus, L. gasseri, L. iners*, and *L. jessenii*, respectively. CSTI V is heterogeneous on account of depletion of *Lactobacillus*spp. and the presence of strictly anaerobic species such as *Prevotella, Sneathia, Megasphera*, and *Gardnerella*. Longitudinal studies found that the diversity and abundance of microbes within subjects vary widely both within and among subjects in the vagina over time, which also presented superior longitudinal stability than other mucosal sites.[6]

The genus *Lactobacillus* is taxonomically complex and is composed of more than 170 species, which cannot be differentiated phenotypically by traditional molecular identification methods.[19] *L. crispatus, L. gasseri, L. jensenii, L. iners*, and *L. vaginalis* are commonly populated in the vagina. *Lactobacillus*spp. are able to produce numerous protective peptides and metabolic products, such as lactic acid and other acidic compounds that are capable of inhibiting pathogenic bacteria adhesion and growth. In addition, bacteriocin-like compounds, such as bio-surfactants and hydrogen peroxide, may be key for *Lactobacillus*spp. persistence in the vagina.[20] These could explain why vaginal epithelial cells from healthy females are lightly colonized by *Lactobacillus*spp., as these metabolites counter the adhesion and growth of other bacteria.[21] It also controls bacteria which ascend the cervix to the uterus and enhance sperm motility.[22] Studies of chronic wounds showed that *Lactobacillus*spp. supernatants contain antimicrobials (mevalonolactone, benzoic acid, and 5-methyl-hydantoin), surfactants (distearin, dipalmitin, and 1,5-monolinolein), anesthetics (barbituric acid derivatives), and autoinducer type 2 precursors (4,5-dihydroxy-2,3-pentanedioneand2methyl-2,3,3,4-tetrahydroxytetrahydrofurane). In addition to lactic acid, *Lactobacillus*spp. supernatant also contains phenolics, hydrogen peroxide, sodium, calcium, potassium, magnesium, and DNAases.[23] In all, *Lactobacillus*spp. play a protective function in maintaining vaginal health. As microbes struggle for nutrients and adherence to the vaginal epithelium, the local immune system may activate the production of host antibacterial substances to further control microbial growth.[24] Furthermore, a high lactobacilli population is crucial because it protects the host vaginal epithelia against sexually transmitted infections (STIs). Therefore, a complex interaction and synergy between host and microorganism to maintain the stability in the vaginal epithelia exists. A high population of *Lactobacillus*spp. has been reported in healthy vagina followed by other microorganisms[25] such as *Gardnerella*, *Candida*, *Prevotella*, *Ureaplasma*, *Peptostreptococcus*, *Mycoplasma*, *Streptococcus*, *Corynebacterium*, *Clostridium*, *Staphylococcus*, *Bacteroides*, *Enterococcus*, *Bifidobacterium*, *Veillonella*, and *Escherichia*. In short, the vaginal microbial population continually changes throughout life, but it may be consistent and stable in a certain period of time in healthy women. However,

when there is a disorder in the microbial flora, such as the loss of *Lactobacillus*spp. resulting in the overgrowth of anaerobic bacteria as in the case of bacterial vaginitis (BV), this is associated with the higher incidence, prevalence, and persistence of HPV infection, which can contribute to the development of cervical intraepithelial neoplasias.[26–28]

Local Immunity in the Vaginal Microbiota

The vaginal mucosal barrier possesses specialized features to protect against infectious and noxious environmental damage, at the same time maintaining symbiotic mutualism with commensal microbes. Microbes, immune regulatory actions, and host genes interact closely to govern the homeostasis of the vaginal environment.[29] The cervicovaginal mucosa is a critical site for protection against urogenital pathogens. The female genital tract consists of two types of mucosal surface. The lower genital tract (vagina and ectocervix) represents the type II mucosal surface, lined by stratified squamous epithelia. Defining features include the presence of mucus-secreting cells and the expression of polymeric immune-globulin R (pIgR) on the basolateral surface of the epithelia. pIgR binds to polymeric IgA (pIgA) secreted by plasma cells and exports pIgA transepithelially.[30] Plasma cells also release secretory IgA (SIgA) into the lumen. In contrast, the upper genital tract (endocervix and endometrium) represents the type I mucosal surface, which is covered by a monolayer of columnar epithelial cells with tight junctions and SIgA present in the microenvironment. The boundary between type I and type II mucosa, also known as the cervical transformation zone, is the most vulnerable region invaded by pathogens and heavily populated with T cells and antigen-presenting cells (APCs).[31]

In the human vaginal mucosa, four unique myeloid-derived APC subsets have been characterized including, cervicovaginal langerhans cells (cvLCs), $CD14^-$ dendritic cells (DCs), $CD14^+$, DCs, and $CD14^+$ macrophages.[32] Langerhans cells are also found in the epidermis (epLCs) and express the C-type lectin molecule. cvLCs are found in the human cervicovaginal epithelium, whereas $CD14^-$ DCs, $CD14^+$, DCs, and $CD14^+$ macrophages are found in the subepithelial lamina propria region.[32] Transcriptional profiling showed that $CD14^-$ cells (cvLCs and $CD14^-$ DCs) displayed Th_2-inducing and regulatory properties, whereas the $CD14^+$ APCs ($CD14^+$ DCs and macrophages) exhibited signatures of innate immune defense and pro-inflammatory responses.[33] Resident memory T cells in the vaginal mucosa mediate rapid protection upon infection. $CD4^+$ T-cell entry into the cervicovaginal region facilitates the entry of circulating memory $CD8^+$ T cells via the production of IFN-γ. Innate immune sensing and surveillance of commensal and pathogenic microbes in the female genital tract is achieved by microbial motif pattern recognition via pattern recognition receptors (PRRs), such as toll-like receptors,[34] dectin-1 receptor, and nucleotide-binding oligomerization domain receptors present in and on both squamous epithelial cells (Ref). PRR stimulation in the vaginal environment initiates various cytokine and chemokine signaling cascades, including secretion of interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α), to activate or recruit macrophages, natural killer cells, $CD4^+$ and $CD8^+$T-cell lymphocytes, and B lymphocytes.[29] Other factors that strength vaginal defense system include IgA, IgG, vaginal antimicrobial peptides (AMPs) and mannose binding lectin (MBL). *N*-acetyl glucosamine and MBL bind mannose, which is present on the cell surface of microbes.[35] IgG and IgA may assist to avert vaginal epithelial cell adherence and uptake, playing a role in neutralizing and clearing infectious microbes from host sites.[36] Similarly, there are various classes of vaginal AMPs which normally engage immune cells via chemotaxis or possess anti-endotoxin activity. Mechanisms for AMP have been reported in detail previously.[37] However, the detailed relationship among AMPs and vaginal microbiota remains understudied. AMPs, such as defensins, are small cysteine-rich cationic proteins that have varied mechanisms of action against common vaginal microbes, including HPV (Ref). Other host–microbiota interactions occur at the epithelial cell interface; many of them are not well understood. For example, bacterial strains produce a range of metabolites, some of which could cross the epithelial barrier and affect cellular function in other sites, which are influenced by host factors such as hormones and mucins.[38]

HPV and the Microbiota

It is well established that cervical cancer development is the final stage of a sequence of cellular and molecular changes instigated with HPV infection. Host immune response and evasion mechanisms involve a termination host response against tumor antigens. In order to know the local and systemic modifications during interactions between immune system and HPV-related cervical lesions progress to cancer it has already been reported that

elevated cervical leukocytes in cervical cancer patients infiltrate and imitate with increase of M2 macrophages, neutrophils and T lymphocytes. Additionally, a sturdy negative correlation among incidence of T cells, neutrophils, and cancer patients' samples has been reported. Similarly, neutrophils stop T-cell activity and show extended viability and CD16 expression in 3D tumor cell cultures compared to normal neutrophil culture. They also found a high rate of immature low-density neutrophils, elevated plasma concentration and tolerogenic monocyte-based dendritic cells in cancer cases compared to controls. These results showed that G-CSF and neutrophils might be a piece of the immune escape mechanism locally and systemically controlled by cervical cancer cells.[39]

Women diagnosed with CST IV-like condition showed substantially increases in FMS-like tyrosine kinase 3 ligand, TNF-α, IL-1α, IFN-γ, IL-1β, IL-4, IL-12p70, IL-10, and IL-8, and then those of CST I. Additionally, compared with CST I, CST III showed significantly elevated IFN-γ. Recently, it has been shown that there is significant TNF-α, IL-1β and IL-1α longitudinally in cases which developed from a CST I, CST III and CST III to a CST IV.[40,41] On the contrary, mock communities normally dominated by *L. jensenii* (CST V) and *L. crispatus* (CST I) on restructured 3D vaginal epithelial models did not sturdily evoke cytokine IL-8 or IL-1β secretion compared with controls.[40] All observations persistently support the concept that the innate immune response is mainly operated with vaginal microbiota.

HPV infection associated with the vaginal microbiota. Cervical carcinogenesis related with the vaginal microbiota immune response in cervix. Hence, overall, microbes, environments, immune regulatory actions, and gene expression interact closely to govern the homeostasis of the vaginal environment.

Probiotic Therapy

According to the World Health Organization and the United Nations' Food and Agriculture Organization, probiotics are defined as "live microorganisms, which when administered in adequate amounts confer a health benefit on the host".[42] Probiotics comprise living bacteria (eg, *Bifidobacteria* species, *Lactobacilli*, and *Streptococci*) that can modulate the composition of microbiota in an attempt to improve the health, immune system, inflammatory state, and bioavailability of micronutrients. They also reduce the risks of diabetes, and ameliorate dyslipidemia, allergic disorders, and certain cancers.[43] The mechanism by which probiotics confer their conducive functions may be elaborated by alterations in pH, inhibition of pathogens (via the generation of antibacterial compounds, competitive elimination of pathogens in receptor binding sites, and contention for available nutrients), mutagenic, carcinogenic production, and maintenance of the mucosal barrier.[44]

There is a potential to maximize a woman's first line of defense by understanding the vaginal microbiota in depth, which will ultimately help to develop practical and low-cost therapeutics to decrease the susceptibility of STIs, particularly HPV. According to the literature, fifty-four HPV-positive women diagnosed with low-grade squamous intraepithelial lesions were offered a probiotic containing *Lactobacillus casei* on a daily basis and interestingly *Lactobacillus casei* appeared to be helpful in HPV clearance.[45] In HIV-infected individuals, due to the impairment of the immune response and the loss of gut intraepithelial lymphocytes, lower rates of HPV clearance are observed and consequently spontaneous resolution of multiple large anal condylomas is rare among this population. For this reason, it has been suggested that direct efficacy of oral and rectal multistrain probiotic administration in the treatment of anal condylomatosis in HIV-infected patients was effective.[46] In addition, it has been reported that appropriate probiotics would be most useful for women's health. It will be exciting and valuable to establish whether probiotics have the potential to reduce productive viral infection and lead to a clinically "latent" HPV stage.

In the last century, different research groups worked on urogenital lactobacilli based upon their global prevalence in the vagina. In 2002, it was found that fastidious *L. iners* is a highly populated microbe in the healthy vagina.[47–49] In a clinical study, they successfully combined different microbial strains such as RC-14 with GR-1 and administered these orally to interfere with a range of urogenital pathogens to restore the host's own lactobacilli.[50] The idea of oral administration of probiotics came from the knowledge that many urogenital infections arise from the entrance of a pathogen from the rectum to the perineal skin and then the vagina. Therefore, if the other pathogens could do it, why could not lactobacilli? Different studies have already verified that orally administered lactobacilli can reach the vagina.[51–54]

Probiotic lactobacilli have been frequently prescribed against urogynecologic infections. Although clinical data results show poor support, probiotic application effectively cures urogenital infections compared with other treatments. Additionally, probiotic application has no serious adverse

side effects. However, well-designed clinical trials of probiotics for curing urogenital infections are needed. Moreover, co-treatments suggested that probiotic and antibiotic application should be used at least 2 to 4 hours apart to prevent the live microorganisms' destruction in the gastrointestinal tract.[55] Thus, probiotics used for curing genitourinary infections contain *Lactobacillus* species, as the objective is the vaginal microflora. The mechanisms of action of probiotics include acidification of the mucosal surface, prevention of the adherence of pathogens, production of substances such as vitamins and immune modulators, and synergistic action with the host immune system.

It has also been suggested that the microbiome and radiation provoked enteritis. Radiation therapy, either alone or in combination with chemotherapy or surgery, is the standard treatment option for a clinician to treat advanced cervical cancer cases. Unluckily, radiation therapy can affect the bowel as a side effect. According to investigation, almost 80% of cases showed bowel symptoms such as urgency, abdominal pain, bloating and diarrhea during radiation treatment.[56] This dysbiosis and local inflammatory condition could contribute to cause sexual dysfunction and pelvic pain in cervical cancer cases. However, limited data have been reported on this topic, so additional research should be conducted in this area.[57]

Together with the primary treatment, they are able to restore depleted microflora. Therefore, vaginal probiotics might be valuable for this purpose, and oral probiotics may be useful to conserve normal vaginal microflora and help preserve normal vaginal flora during antibiotic therapy.[50] Direct use of vaginal probiotics such as *Lactobacillus* species have been effectively used,[58] together with a reduction in BV.[59] Probiotics might have substantially higher effects on carcinogenesis. It has already been recommended that regular intake of oral probiotics can reduce the threat of gastrointestinal cancer.[60] We suggested that vaginal probiotics work simultaneously to decrease HPV infection rate and escalate HPV infection clearance. Moreover, during treatment, the microbiome can modulate the tumor microenvironment.[61] Data suggested that CpG oligonucleotide or platinum chemotherapy regimens had better efficiency against tumors with regular gut micro-biomes as compared to antibiotic-treated mice.[62] Further, the presence of microorganisms (*Bacteroides taiotaomicron* or *B. fragilis*) was required for CTLA-4 targeted immunotherapy.[63] Clinicians are looking to treat gynecological cancer using this immunotherapy, such that vaginal microbiomes could help influence the efficiency.[64,65] Probiotics prevent sarcoma by reducing the level of gut inflammatory (Th17) cells and promoting anti-inflammatory (Treg) cells in mice.[63,64] The promotion of Treg cells leads to a reduction in gynecological cancers and chronic inflammation. The probiotic can change the tumor microenvironment by direct application to the gynecological tumor. Furthermore, in-vitro studies suggested that vaginal microbiota may increase the apoptosis of cancer cells or may provoke the anti-inflammatory cells like dendritic, Treg and cytokines.[66–69]

The lack of efficient treatment to counter antibiotic resistance to bacterial infections and the intravaginal application of lactobacilli and lactoferrin may be the narrative and capable therapeutic approach to reinstate the mucosal immune homeostasis.[25]

Concluding Remarks

There are many imminent approaches that can be used to evaluate intestinal and related mucosal regions, but they require concrete capabilities that traverse mucosal immunology and reproductive biology. Mmodern technology like single-cell RNA sequencing, mass cytometry, deep sequencing of bacterial 16S rRNA, and internal transcribed spacer regions of fungal rRNA genes, metagenomics, and metabolomics play an important role in immunology and reproductive health. These may find unprecedented purposes. Human vaginal hosts possess millions of microorganisms, also collectively known as the bacterial flora. An increasing number of studies are progressively unraveling the fascinating interaction between hosts and microbiota. Probiotics can confer health by modulating the composition of gut microbiota and restoring the physiological bacterial flora. That being said, limited data are available in this area, and the relevant scientific work is still at the early stage. A lot of studies need to be carried out in the future, as below. Initially, a host provides a large and complex environment for gut bacterial flora. It is unclear yet whether the alteration of intestinal microbiota contributes to the development of eczema or its presence reflects the primary cause of changes in gut microbiota. There is need for a profound understanding of the interactive mechanism between vaginal microbiota and host. As such, it is critical to offer an entirely novel approach to cure diseases by monitoring and controlling vaginal microbiota. Compared with developing novel drugs for inflammation, it might cost less to seek novel approaches, such as monitoring and manipulating human vaginal microbiota if required using probiotics. The opportunity exists to develop updated probiotics in accordance with the interaction between specific microbiota and HPV infection. In addition, it is plausible to develop

probiotics from vaginal microbiota in healthy groups. Similarly, it is significant to determine how probiotics change the constitution of the vaginal microbiota (reconstructing bacterial flora) and how relevant it is to HPV infection. Given little relevant research in the area, more studies should be carried out to elucidate the interaction between specific microbiota and cervical carcinogenesis related with HPV infection and a wealth of well-controlled clinical studies on vaginal microbiota are required to ensure safety in patients. Synergistic efforts in vivo and in vitro are also required so as to advance our knowledge in the area, which is promising for its potential in biomarker reorganization and novel therapeutic targets. All in all, further understanding of the vaginal microbiota may uncover more mysteries of HPV persistent infection and related diseases and provide a promising therapeutic target.

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Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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