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Role of the human vaginal microbiota in the regulation of inflammation and sexually transmitted infection acquisition: Contribution of the non-human primate model to a better understanding?

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The human vaginal microbiota has a central role in the regulation of the female reproductive tract (FRT) inflammation. Indeed, on one hand an optimal environment leading to a protection against sexually transmitted infections (STI) is associated with a high proportion of *Lactobacillus* spp. (eubiosis). On the other hand, a more diverse microbiota with a high amount of non-*Lactobacillus* spp. (dysbiosis) is linked to a higher local inflammation and an increased STI susceptibility. The composition of the vaginal microbiota is influenced by numerous factors that may lead to a dysbiotic environment. In this review, we first discuss how the vaginal microbiota composition affects the local inflammation with a focus on the cytokine profiles, the immune cell recruitment/phenotype and a large part devoted on the interactions between the vaginal microbiota and the neutrophils. Secondly, we analyze the interplay between STI and the vaginal microbiota and describe several mechanisms of action of the vaginal microbiota. Finally, the input of the NHP model in research focusing on the FRT health including vaginal microbiota or STI acquisition/control and treatment is discussed.

KEYWORDS

vaginal microbiota, inflammation, cytokines, neutrophils, sexually transmitted infections, non-human primates

1. Introduction

The female reproductive tract (FRT) is one of the entry points of sexually transmitted pathogens and includes a wide array of immune parameters necessary to abrogate pathogen invasion. The FRT is divided into an upper tract, composed of the Fallopian tubes, ovaries, uterus and endocervix and a lower tract, with the ectocervix

and the vagina. The two compartments differ in their architecture, with two distinct types of mucosae (type I and II) (1, 2). The critical zone for pathogen invasion is the transformation zone, located between the upper and lower tract, where the multilayer squamous epithelium changes to a single layer of columnar epithelial cells (3). The transformation zone is immunologically active, the change of epithelium architecture allows the passage of small pathogens to the mucosa. The lower tract epithelium permits the protection of underlying tissues from abrasion during intercourse. However, the lack of tight junction allows intra-epithelial passage of molecules, small pathogens and interaction of these pathogens with potential target cells. The epithelial barrier in the upper and lower tracts acts as a physical barrier but can also recognize Pathogen Associated Molecular Patterns (PAMPs) through Pattern recognition receptors (PRR) like Toll-like receptors (TLR) and NOD-like receptors (NLR) and induces the production of cytokines, chemokines and antimicrobial peptides (AMP) (4, 5). A layer of mucus, covering the epithelium of the FRT, is in direct contact with pathogens. The mucus acts as a physical trap for pathogens thanks to gel-like properties (6).

Macrophages, NK cells, dendritic cells (DC) and neutrophils have a variable distribution according to FRT compartments and hormonal levels (4, 7). Other innate-like lymphocyte cells such as $\gamma\delta$ and mucosal-associated invariant T (MAIT) cells are also present within the FRT (8, 9). T lymphocytes are the most abundant leukocytes in the FRT (10, 11). The transformation zone contains the highest concentration of macrophages, CD4+ and CD8+ T cells compared to all FRT compartments (12). IgA and IgG are found in the FRT, and IgG concentration is higher than IgA in cervicovaginal fluids (13). B cells are mainly found in the cervix and vagina and are more abundant in the ectocervix compared to the endocervix in premenopausal women (11, 14).

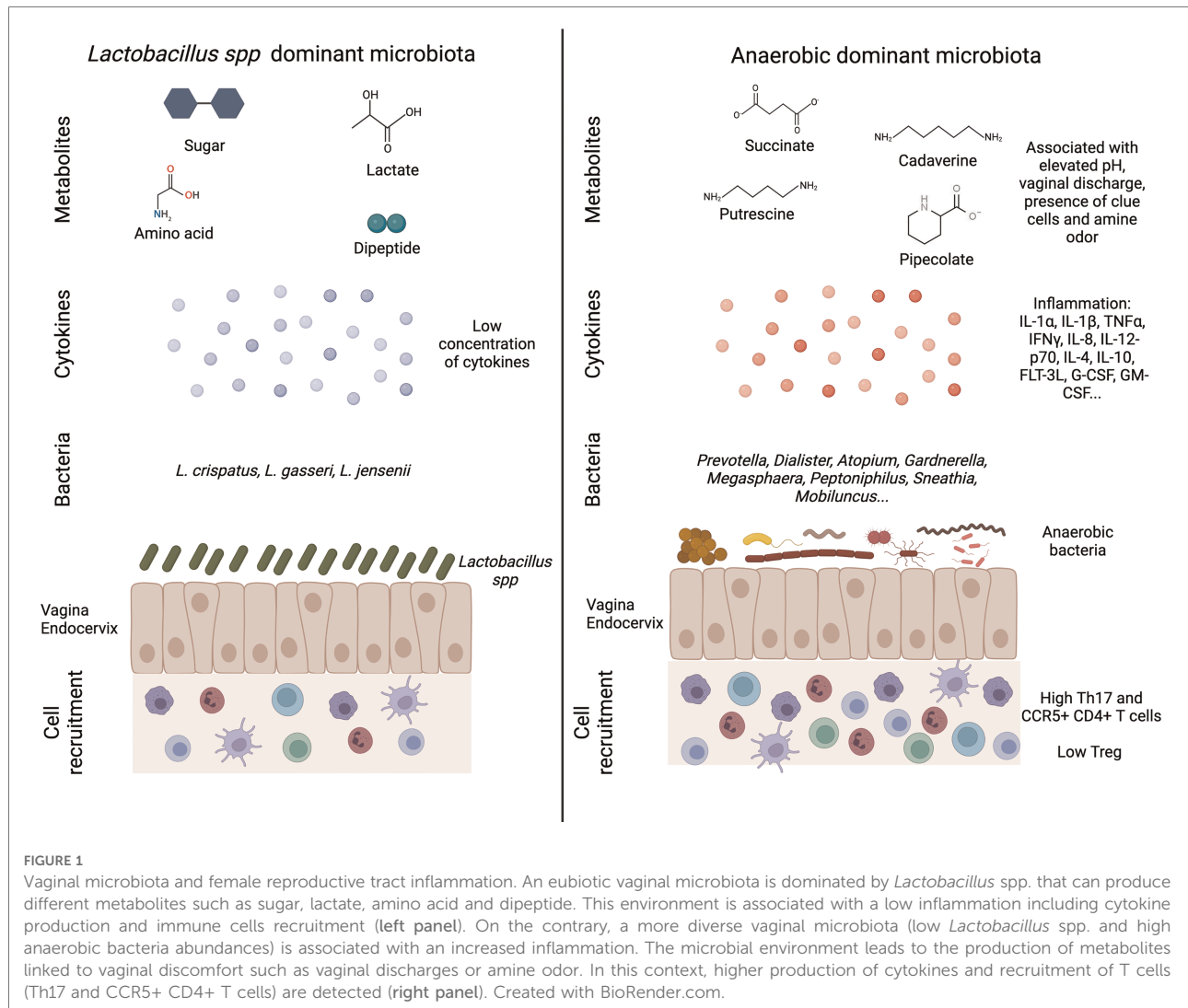
Hormonal changes in the FRT induce several modifications of the immune system involving secreted molecules (AMPs, cytokines and chemokines), epithelial cells, fibroblasts, immune cells and vaginal microbiota (14).

In humans, eubiotic vaginal microbiota is characterized by the dominance of one genus, *Lactobacillus* spp., which maintains an acidic pH in the vagina (pH = 4–5) thanks to lactic acid production. It also maintains a low inflammatory environment through the production of bactericide and bacteriostatic components (Figure 1). Analysis of the vaginal microbiota by 16S rRNA gene sequencing and vaginal pH measurement allowed a community clustering into five groups or community state type (CST) (15). Group I, II, III and V are dominated by one species of *Lactobacillus* spp.: *L. crispatus* (group I), *L. gasseri* (group II), *L. iners* (group III) and *L. jensenii* (group V). Women from the group IV had a vaginal microbiota characterized by higher proportions of anaerobic bacteria (facultative or strict other than

Lactobacillus spp.) including *Prevotella*, *Dialister*, *Atopobium*, *Gardnerella*, *Megasphaera*, *Peptoniphilus*, *Sneathia*, *Mobiluncus*, with a vaginal pH starting at 5 and rising up to more than 5.5. The group IV can be divided into two subgroups: characterized by a microbiota with a modest proportion of *Lactobacillus* spp. and a low number of various species of anaerobic bacteria (such as *Anaerococcus*, *Corynebacterium*, *Fingoldia* or *Streptococcus*) (group IVa), or dominated by diverse anaerobic bacteria belonging to the genus *Atopobium*, *Prevotella*, *Parvimonas*, *Sneathia*, *Gardnerella* or *Mobiluncus* (group IVb) (16). This diverse vaginal microbiota composition has been associated with local inflammation, characterized by an increase in pro-inflammatory cytokines and the presence of activated cells, which in turn increase the susceptibility to sexually transmitted infections (STIs) and poor obstetrics events (17, 18) (Figure 1). This increased bacterial diversity associated with high anaerobic bacteria abundance, low *Lactobacillus* spp. abundance and high local inflammation, leads to a clinical condition called bacterial vaginosis (BV). BV is also associated with a modification of metabolomic profiles. In BV+ women, several metabolites are associated with BV clinical criteria: elevated pH (increase of tyramine, cadaverine, N-acetylputrescine, sphingosine and decrease in tyrosine and glutamate), vaginal discharge (increase of cadaverine and agmatine), presence of clue cells (increase of deoxycamitine, pipecolate and decrease of glutathione, glycylproline) and amine odor (increase of N-acetylputrescine and decrease in lactate, phenylalanine). On the contrary, *L. crispatus* and *L. jensenii* abundance have been described to correlate positively with other metabolites such as sugars, lactate, amino acids and dipeptides, but negatively correlated with tyramine, pipecolate, cadaverine putrescine and agmatine, which are increased in BV+ women (Figure 1). *L. iners* exhibited intermediate correlation patterns between *L. crispatus*/*L. jensenii* and BV associated bacteria (19). *Gardnerella vaginalis* is often associated with BV and the predominant agent inducing biofilm. This bacterium was present in all the patients suffering from BV. However, it was also observed in 59% of BV– patients, suggesting that *G. vaginalis* is also present in healthy women (20). *G. vaginalis* alone does not systematically induce BV (21), but has a central role in BV acquisition by being a part of a group of organisms including *Atopobium*, *Megashaera*, *Sneathia* and BVAB1/2/3 involved in the induction of a dysbiotic environment (22).

Various factors are known to influence the vaginal microbiota composition, such as hormones, contraceptive use, hygiene, sexual behaviors, semen, ethnic status and STIs (23–32). Other factors including nutrition, stress or tobacco use have also been shown to influence the vaginal microbiota composition (33–36) (Figure 2).

Here, we address the interplay between vaginal microbiota composition and vaginal inflammation at steady state and in



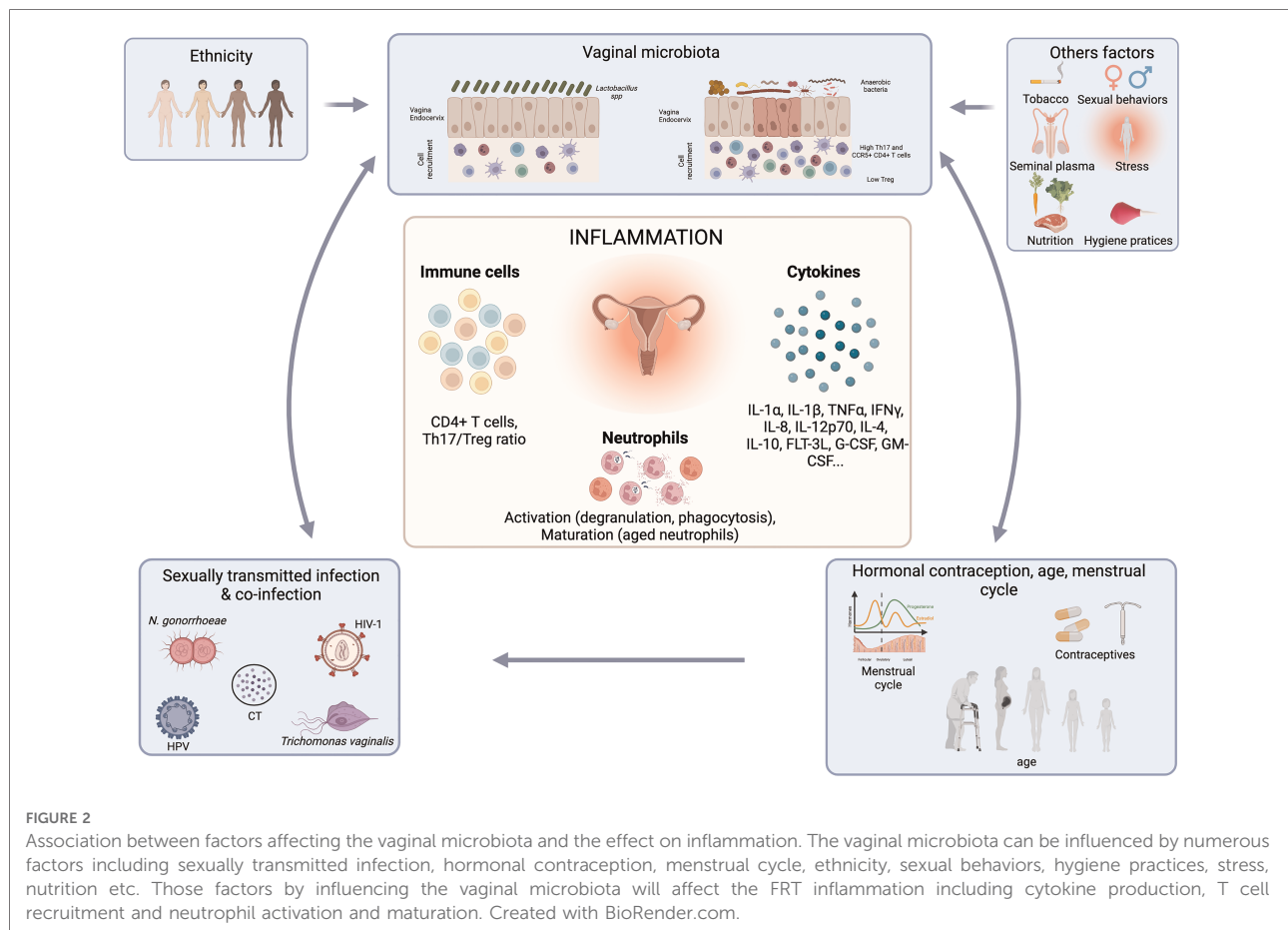
the context of a STI. Finally, we discuss animal models and more precisely non-human primate (NHP) use to study vaginal microbiota composition and its link to local inflammation.

2. The vaginal microbiota and inflammation

2.1. Cytokine production

The vaginal microbiota has been described to be closely linked to FRT inflammation (Figure 1). Women with CST-IV microbiota were observed to be four times more likely to have elevated genital pro-inflammatory cytokines, compared to those with CST-I-III. More precisely, IL-1 α , IL-1 β , TNF α , IFN γ , IL-8, IL-12-p70, IL-4, IL-10, and FMS-like tyrosine 3 ligand (FLT-3L) were significantly increased in women with

CST-IV compared to women with CST-I (17). Moreover, several bacteria were associated with inflammation: *Fusobacterium*, *Aerococcus*, *Sneathia*, *Gemella*, *Mobiluncus* and *Prevotella* (17). BVAB1/2/3, *Prevotella amnii* and *pallens*, *Parvimonas micra*, *Megasphaera*, *G. vaginalis* and *Atopobium vaginae* (37). Elevated concentration of IL-1 α , IL-1 β and decrease of CXCL10 were proposed to be used as biomarker for asymptomatic STI or BV (38). Jespers et al. associated a better vaginal health with a low concentration of IL-1 β , IL-8 and IL-12(p70) but they observed an increase of CXCL10 concentration (39). In another study conducted on cervicovaginal lavages (CVL), obtained from an African cohort, the authors observed an increase of IL-1 α , IL-1 β , G-CSF, GM-CSF and a decrease of CXCL10 in women suffering from dysbiosis (40). In *in vitro* experiments, an increased inflammation (IL-1 β , IL-6, IL-8, G-CSF, PDGF, CXCL10, CCL4 and Gro- α) was detected when cell lines from the FRT were exposed to *G. vaginalis* or *A. vaginae* compared



to *L. johnsonii*. However, a difference in CXCL10 expression has to be noted between *in vitro* and *in vivo* analysis (41). Similar results were observed for *A. vaginae* or *G. vaginalis* in a 3D model of human cervix: polymicrobial exposition led to an increase production of IL-1 α , IL-6, IL-8, CCL2, CCL20 and IL-1 β compared to no bacterial exposition or *L. crispatus* exposition (42).

An analysis of the microbiota and proteome from CVL of Rwandan female sex workers showed that several parameters were increased from groups 1 (*L. crispatus* dominated) to 4 (severe dysbiosis) including histones (HIST3H2A, HIST2H3A/C/D, HIST4H4), AMP (psoriasin, calprotectin), proteasome core complex proteins involved in catabolism and IL-36. MUC5B was also increased from groups 1 (*L. crispatus* dominated) to 4 (severe dysbiosis) and MUC5AC was increased in group 4 compared to other groups, which could be explained by an increased production of mucus, or by the degradation of the mucus by BV associated bacteria. Indeed, *G. vaginalis* was observed to degrade MUC1 compared to *L. crispatus* in a 3D model of human cervix (42). Cytoskeleton alterations (increased actin-organizing proteins, decreased keratins and cornified proteins) were also observed in groups 3 (moderate dysbiosis) and 4 (severe dysbiosis).

Furthermore, there was an increased trend for cell death from group 1–4, reflecting cell damage also described in the 3D model of human cervix exposed to *A. vaginae* (42, 43). Another study evaluated lactic acid treatment of cervicovaginal epithelial cell lines, with physiologically concentrations of short chain fatty acid (SCFA) and described the induction of an anti-inflammatory environment (increase of IL-1RA). Moreover, lactic acid and SCFA mixture significantly decreased the expression of IL-6, IL-8, CXCL10 and CCL20 upon stimulation with TLR1/2 and TLR3 agonists. On the contrary, higher concentration of SCFA, compared to lactic acid, induced an increased expression of TNF α and a decreased expression of CCL5 and CXCL10 upon TLR stimulation, suggesting that elevated SCFA might be responsible for the increased inflammation observed in the vagina of BV+ women (44).

2.2. Cell recruitment and phenotype

The vaginal microbiota has also an impact on immune cell recruitment and phenotype (Figure 1). *L. acidophilus* administration to BV+ mouse model colonized by *G. vaginalis*

efficiently inhibits NF- κ B pathways as well as IL-1 β , IL-17, TNF α and increases IL-10 and Foxp3 expression. Moreover, the *Lactobacillus* species inhibited, *in vitro*, the differentiation of mice splenocytes into Th17 cells but increased their differentiation into Tregs (45). Similarly, *in vitro* experiment described that *G. vaginalis* was able to increase the production of IL-17, whereas *Lactobacillus* spp. induced a higher level of IFN γ . The authors suggested that BV associated bacteria might induce a Th17 response that may impair antiviral response and facilitate disease progression (46). Women with a diverse vaginal microbiota with low *Lactobacillus* spp. abundance (CST-IV) had a 17-fold increase in HIV-1 target cells in the FRT with elevated CCL3 and CCL5, which attracts CCR5+ cells. CVL of women with CST-IV had higher levels of IL-1 α , TNF α , IL-8, IL-12p70, IFN γ , IL10, IL-17 and IL-17 inducing cytokines IL-23 and IL-1 β . Moreover, vaginal inoculation of *P. bivia* in germ free mice promoted the recruitment in the FRT of CCR5+ CD4+ T cells compared to mice inoculated with *L. crispatus* (47). A. Semaganda et al. suggested that Treg might be important in the regulation of inflammation induced by BV associated bacteria but do not affect directly the presence of BV associated bacteria (48). Cervical cytobrushes of HIV exposed seronegative women (HESN) exhibited an increased frequency of NK cells, CXCR5+ CD8+ T cells, follicular T cells compared to HIV unexposed healthy women. In terms of vaginal microbiota, the HESN cohort exhibits a higher bacterial diversity but with an increased abundance of *L. crispatus*, *L. gasseri* and *L. iners* compared to the healthy cohort. These parameters may explain the protection of HESN from HIV-1 infection (49). In contrast, Lennard et al. did not find any differences in the frequency of CD4+ CCR5+ cells in cervical cytobrushes of BV+ women, suggesting the implication of other factors in the increased susceptibility of BV+ women to HIV-1 (37). No differences were detected in CD11+ DC or CD14+ monocytes and macrophages frequency in cervical cytobrushes when comparing BV+ and BV- women. However, in women with highly diverse microbiota, there were gene upregulations for NF- κ B, TLR, NOD like receptor and TNF α signaling pathways in cervical APCs, most likely with LPS acting as the upstream regulator. CD4+ T cells frequency, obtained from cervical cytobrushes for those women, were higher and cells were more activated (HLA-DR+, CD38+, CCR5+) (17). RNA sequencing of isolated cell populations from cervical cytobrushes highlight that antigen presenting cells (APC) is the main cell population involved in FRT inflammation. The authors detected that gene upregulation in APC isolated from dysbiotic patients are involved in cell growth suppression, apoptosis, inflammatory pathways, neoplastic suppression and pro-proliferative pathways (50). Ectocervical tissues of BV- women harbored higher number of CD45+ cells, including CD3+, CD8+ and HLA-DR+ cells. Nevertheless, BV+ women had higher number of CD4+ and CCR5+ cells (51).

TABLE 1 Effects of bacterial vaginosis on the immune responses.

Factors influenced by the vaginal microbiota	Effects of BV	References
Soluble factors	(+) IL-1 α , IL-1 β , TNF α , IFN γ , IL-8, IL-12-p70, IL-4, IL-10, FLT-3L, G-CSF, GM-CSF, PDGF, CCL4, IL-36 (+) Antimicrobial peptides (psoriasin, calprotectin)	(17, 38, 40-42)
Cell recruitment	(+) Th17, CCR5+ CD4+, HLA-DR+ CD38+ CCR5+ (-) Treg	(17, 45, 47)
Other effects (metabolisms, mucus...)	(+) Inflammation pathways (NF- κ B, TLR, NOD, TNF α ...) (+) Epithelial barrier disruption (+) Mucus impairment (MUC5B, MUC5AC) (+) Cell deaths	(17, 42, 50)

(+) Increase; (-) decrease.

Overall, polymicrobial vaginal microbiota composition has been associated with an increased inflammation (higher pro-inflammatory cytokines production, upregulation of signaling pathways involved in inflammation, cell recruitment), epithelial barrier disruption, mucus impairment, cytoskeleton alteration and increased cell deaths (Figure 1; Table 1).

2.3. Neutrophils and microbiota

2.3.1. Neutrophils in the FRT

Neutrophils are key mediators of inflammation and are present in all the compartments of the FRT (Figure 2). The highest presence of neutrophils is in fallopian tubes and this number decreases from the upper to the lower tract (52). In humans, neutrophils in fallopian tubes have been described to be phenotypically and functionally different from the neutrophils in the blood. Indeed, they expressed lower level of CD66b, CD62L, IL-8 receptor (CXCR1, CXCR2) and higher level of bacterial product receptor, CD64, HLA-DR, IFN γ , TNF α , IL-12 and VEGF compared to blood neutrophils (53). In the uterus, a strong increase of IL-8 before the menstruation leads to a significant recruitment of neutrophils, mainly through CXCR2 signaling pathway (54, 55). Their role during menstruation is to allow the disruption of endometrial tissue through the release of elastase, which activates extracellular matrix-metalloproteinases and increases immune defense (4). In a mouse model, neutrophil depletion induces a blockade of the estrous cycle due to a dysregulation of serum steroid hormone levels, suggesting an important role of neutrophils in regulating hormone levels (56). Estradiol treatment of mice infected with *Candida albicans* blocked neutrophil migration to the vagina and accumulated them in the ectocervix and fornix. This phenomenon is mediated by

an altered expression of CD44 and CD47 expression of FRT epithelial cells enabling neutrophils to migrate to the vagina. On the contrary, progesterone treatment facilitates neutrophil migration and killing. These data suggested that neutrophil migration is dependent on sex hormones. Indeed, during the ovulation, neutrophils are not present in the vaginal lumen to facilitate the passage of sperm cells, although it may lead to vaginal infections (57).

Recently, we have described the phenotype of neutrophils in cervicovaginal cytobrushes obtained from female macaques and shown that they are different from neutrophils in the blood. We used several surface markers to characterize the priming (CD11b, CD62L), activation (CD11b, CD32a) or maturation (CD10, CD101) status of the neutrophils. We observed three main cervicovaginal neutrophil populations: CD11b^{high} CD101⁺ CD10⁺ CD32a⁺ (mature/activated), CD11b^{high} CD101⁺ CD10⁻ CD32a⁺ (immature/activated) and CD11b^{low} CD101^{low} CD10⁻ CD32a⁻ (pre-neutrophil like). The proportion of those populations varied during the menstrual cycle. During menstruation, there was an increase of CD11b^{high} CD101⁺ CD10⁺ CD32a⁺ subset of neutrophils, which expressed higher levels of CD62L than other populations of cervicovaginal neutrophils (58).

Very few is known about the relationship between the vaginal microbiota and local neutrophils in the FRT in contrast to what is described in the gut (59). We will now discuss how the relationships between the microbiota and neutrophils are important to modulate the inflammation at the level of the FRT.

2.3.2. Regulation of the microbiota by neutrophils

2.3.2.1. Neutrophil recruitment and activation

Neutrophils are among the most abundant immune cells in the vaginal lumen (58, 60). They are rapidly recruited to the mucosa during the inflammation process and thus are crucial for maintaining the balance between commensals and pathogens at the level of the vaginal microbiota. In BV+ women, elevated vaginal level of IL-8 and IL-1b has been correlated to the presence of pathogenic bacteria, leading to a high neutrophil count (61). On the contrary, many probiotic strains of *Lactobacillus* have been shown to induce lower TLR response compared to pathogenic strains *in vitro* (62, 63). This may lead to an activation of neutrophils that depends on the vaginal microbiota composition as demonstrated in the gut (64, 65).

2.3.2.2. Regulation of neutrophil function

Neutrophils can elicit a vast antimicrobial response and impact the microbiota composition. For example, upon pathogen recognition, neutrophils can produce various AMPs and proteins, through granule secretion, and those AMPs are important for the containment of the microbiota (66, 67).

Reactive oxygen species (ROS) are produced by local neutrophils in the FRT (68), and contribute in the one hand to enhance the oxidative stress induced by *Lactobacillus* H₂O₂ production, leading to killing or restriction of pathogen growth (69), and on the second hand, they mediate neutrophil extracellular trap (NETs) production. NETs are networks composed of DNA and neutrophil proteins that can bind pathogens and facilitate their killing by neutrophils (70). In the FRT, exaggerated NET formation might lead to infertility since NETs can also damage endogenous cells. The vaginal microbiota could also have an indirect effect on NET release by neutrophils. Indeed, cervical mucins have been described to have anti-inflammatory properties, leading to the inhibition of NET release in the FRT (71). The vaginal microbiota composition is able to regulate the cervicovaginal mucus (CVM). Indeed, anaerobic bacteria can degrade the CVM (72), and thus could enhance indirectly NET production through the lifting of NET release inhibition induced by mucins.

In conclusion, neutrophils are important to control the vaginal microbiota and respond to pathogens, but there is also a need to maintain a balance between recruitment/activation of the neutrophils and tolerance of the microbiota to prevent various reproductive health issues including infertility. The microbiota also produces numerous components and metabolites that can have an impact on neutrophil production, maturation and functions.

2.3.3. Regulation of neutrophils by the microbiota

2.3.3.1. Regulation of neutrophil production and maturation

Several studies have shown that the microbiota is essential in the production of neutrophils in the gut but nothing has been reported yet in the FRT (73, 74).

Metronidazole treatment of BV+ women lead to an increased production of GM-CSF which appeared to be associated with the abundance of *L. crispatus* (75). Although the authors did not monitor hematopoietic cell proliferation and differentiation, this result could suggest that the vaginal microbiota could be involved in neutrophil production.

2.3.3.2. Regulation of neutrophil circulation

The circadian circulation of neutrophils is also influenced by the microbiota. In germ free mice, *Klebsiella pneumoniae* lung infection leads to a limited neutrophil mobilization favoring the pathogen growth (76). Microbiota depletion in mice induces a reduced number of aged neutrophils (CD62L⁻, CXCR4⁺) in the blood, and exhibit significant reductions in neutrophil adhesion/Mac-1 activation compared to untreated mice, leading to a retention of neutrophils in the circulation (77). Myd88, TLR2 and TLR4 knockout mice also exhibit less aged neutrophils, suggesting a role of the

microbiota in priming neutrophils (77). Another paper has shown that administration of *Lactobacillus acidophilus* induced a decrease in pro-inflammatory cytokines (IL-8, TNF α , IL-6), immunoregulative cytokines (IL-4, IL-10), neutrophil and macrophage infiltrates as well as a reduced injury severity in rats suffering from colitis (78). The microbiota could have a local effect on epithelial cells leading to the recruitment of neutrophils, and a distal effect through secretion of metabolites that diffuse into the blood. Indeed, it has been shown that low concentrations of TLR ligands such as LPS can be detected in the bone marrow and induced neutrophil trafficking into the blood (79). It has also been shown that bacteria from the gut microbiota can produce short-chain fatty acids (SCFA), and could have an impact on neutrophil recruitment at the site of inflammation (80).

2.3.3.3. Regulation of neutrophil functions

The microbiota can have a direct or indirect effect (for example, by modulating the inflammation) on neutrophil functions. Neutrophils isolated from germ-free or antibiotics treated mice exhibit decreased antimicrobial functions, such as a decreased in myeloperoxidase activity (81), in phagocytic capacity (82) or a reduction in NET formation (77).

Metabolites such as short chain fatty acid, secondary bile acid lithocholic acid or histamine produced by the microbiota, modify ROS production or phagocytosis activity in neutrophils (59, 83). Butyrate and propionate inhibit pro-inflammatory cytokines (TNF α and cytokine induced neutrophil chemoattractant-2) and nitric oxide production by LPS stimulated neutrophils (rat model). Moreover, those SCFA are also able to inhibit HDAC and NF- κ B activation, which might explained the decrease in pro-inflammatory cytokines (84). In a mouse model for endometritis, a dysbiosis was induced in the gut microbiota, and uterine colonization of *S. aureus* led to endometrial inflammation, associated with increased pathogen load in the uteri of the mice with gut microbiota-dysbiosis associated with a low phagocytic capacity and responsiveness of neutrophils. A fecal microbiota transplantation was then performed, and those effects were reversed. The authors demonstrated that protective effect was due to SCFAs present in the feces of mice and that diffuse into the blood but also in uterine tissues.

Most of the mechanisms for neutrophil regulation were observed in the gut, but might also be important in the vagina in terms of neutrophil priming and function for instance. *Lactobacillus* impact on neutrophils might also occurred in the vagina. Interestingly, R. Cheu et al. have observed an activation of cervicovaginal neutrophils, as well as a delay in neutrophils apoptosis leading to neutrophil accumulation within the vagina of women suffering from bacterial vaginosis (Keystone 2018).

3. Vaginal microbiota and STI

3.1. Interaction between STI and the vaginal microbiota

Numerous studies evaluating bacterial vaginosis either by Nugent score, culture or Amsel criteria described association between STI acquisition and BV (85–87).

3.1.1. Bacterial STI including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

The vaginal microbiota of adolescent girls or women dominated by diverse anaerobic bacteria (such as *G. vaginalis*, *Porphyromonas somerae*, *Corynebacterium urealyticum*, *Dialister* spp., *Megasphaera*, *A. vaginae*, *Prevotella disiens*) or *L. iners* are more likely to be infected by bacterial STI agents such as *Chlamydia trachomatis* (CT), compared to women with a vaginal microbiota dominated by *L. crispatus* (88). Similar bacteria are increased in women suffering from chlamydiosis or candidiasis (*Gardnerella*, *Prevotella*, *Faecalibacterium*, *Megasphaera*, *Roseburia* and *Atopobium*) in addition to a *Lactobacillus* spp. depletion (89). BV+ women were at increased risk (3.4 times) to acquire *N. gonorrhoea* and CT, compared to women colonized with hydrogen peroxide producing *Lactobacillus* (29). Interestingly, *N. gonorrhoeae* asymptomatic patients had more frequently a *Lactobacillus* dominant microbiota and a lower bacterial diversity compared to symptomatic patients, suggesting a link between symptoms and the vaginal microbiota (90). Furthermore, women with tubal infertility and CT infection had a vaginal microbiota dominated by *L. iners* and a decrease of other *Lactobacillus* spp., *Bifidobacterium*, *Enterobacter*, *Atopobium* and *Streptococcus* (91). In opposition to CT infected women, *L. iners* was observed to be decreased in *Trichomonas vaginalis* infected women whereas *Streptococcus agalactiae*, *Prevotella bivia*, *Sneathia sanguinegens* and *Gemella asaccharolytica* were increased (92). Presence of *Prevotella amnii* and *S. sanguinegens* have been associated with a 2 fold increased risk for *T. vaginalis* infection (93). Also, the co-occurrence of *Gardnerella*, *Peptoniphilus*, *Dialister*, *Atopobium*, *Parvimonas* and *Metamycoplasma hominis* in CT positive women may lead to biofilm formation (94). *Mycoplasma hominis* and *Ureaplasma urealyticum* are often detected in the vagina/cervix of sexually active women with BV (95–97).

3.1.2. Human papilloma virus (HPV)

Microbial variation is detected in women with high risk HPV infection, characterized by a decrease of *Lactobacillus* spp., *Veillonella*, *Sneathia*, *Sporolactobacillus* and an increase of *Gardnerella*, *Prevotella*, *Dialister*, *Slackia*, *Actinomyces*, *Porphyromonas*, *Peptoniphilus*, *Anaerococcus*,

TABLE 2 Vaginal microbiota composition observed in STI patients or bacteria that are able to enhance pathogen acquisition.

Pathogens	Vaginal microbiota composition in infected individuals	Bacteria that enhance pathogen acquisition	References
<i>Chlamydia trachomatis</i>	(+) <i>Gardnerella</i> , <i>Prevotella</i> , <i>Faecalibacterium</i> , <i>Megasphaera</i> , <i>Roseburia</i> and <i>Atopobium</i> , <i>L. iners</i> (-) <i>Lactobacillus</i> spp., <i>Bifidobacterium</i> , <i>Atopobium</i> , <i>Streptococcus</i>	<i>G. vaginalis</i> , <i>Porphyromonas somerae</i> , <i>Corynebacterium urealyticum</i> , <i>Dialister</i> spp., <i>Megasphaera</i> , <i>A. vaginae</i> , <i>Prevotella disiens</i> and <i>L. iners</i>	(88, 89, 91)
<i>Trichomonas vaginalis</i>	(+) <i>Streptococcus agalactiae</i> , <i>Prevotella bivia</i> , <i>Sneathia sanguinegens</i> , <i>Gemella asaccharolytica</i> (-) <i>Lactobacillus</i> spp.	<i>Prevotella amnii</i> , <i>Sneathia sanguinegens</i>	(92, 93)
HPV	(+) <i>G. vaginalis</i> , <i>Eggerthella</i> , <i>Atopobium</i> spp. (other than <i>A. vaginae</i>), <i>Dialister</i> spp., <i>Veillonellaceae</i> , <i>Aerococcus christensenii</i> , <i>Peptoniphilus asaccharolyticus</i>	<i>G. vaginalis</i> , <i>Prevotella</i> , <i>Dialister</i> , <i>Slackia</i> , <i>Actinomyces</i> , <i>Porphyromonas</i> , <i>Peptoniphilus</i> , <i>Anaerococcus</i> , <i>Peptostreptococcus</i>	(98, 99, 103)
HIV-1	(+) <i>Dialister</i> , <i>Gemella</i> , <i>asaccharolytica</i> , <i>eggerthella</i> , <i>Parvimonas micra</i> , <i>Mycoplasma hominis</i> , <i>Sneathia</i> , <i>Megasphaera</i> , <i>Leptotrichia amnionii</i> , (-) <i>Lactobacillus crispatus</i>	<i>Ruminococcaceae</i> spp., <i>Sneathia sanguinegens</i> , <i>Atopobium vaginae</i> , <i>Aerococcus</i> spp.	(47, 104, 105)

(+) Increase; (-) decrease.

Peptostreptococcus, *Streptococcus*, *Ureaplasma*, *Megasphaera* and *Mycoplasma* (98). In addition, cervicovaginal swabs of HPV⁺ women exhibit higher levels of biogenic amine and phospholipid compared to HPV⁻ women (99). In a meta-analysis performed by Tamarelle et al., a vaginal microbiota dominated by *Lactobacillus* was described to have a protective role against HPV and CT (100). In addition, a higher susceptibility to HPV was determined in BV⁺ women (101). HPV infection was shown to reduce innate peptide expression including defense peptides expressed by the vaginal or uterine epithelium. Moreover, *Lactobacillus* was shown to be able to degrade and use defense peptide as an amino acid source to survive. Therefore, the authors hypothesized that HPV infection, by reducing defense peptide availability, lead to a reduction of *Lactobacillus* and an increase of BV associated bacteria that will promote HPV disease progression (101). In HPV⁺ women that cleared the infection and had a cervical intraepithelial neoplasia removal, an increased abundance of *L. crispatus* was observed compared to women with a persistent infection. Interestingly, *G. vaginalis* was present in all women suffering from persistent infection following surgical treatment (102). Furthermore, during HPV persistence and post-clearance, *G. vaginalis* abundance was increased and associated with a higher cytokine concentration (103).

3.1.3. HIV-1

A higher vaginal bacterial diversity was observed in HIV-1⁺ women and *Dialister*, *Gemella asaccharolytica*, *Eggerthella*, *Parvimonas micra*, *M. hominis*, *Sneathia*, *Megasphaera* and *Leptotrichia amnionii* were associated with higher risk of HIV-1 acquisition (104). In the FRESH cohort, women with high vaginal microbiota diversity and a low abundance of *Lactobacillus* (CST-IV) had a 4-fold increased risk for HIV-1 acquisition compared to women with a microbiota dominated by *L. crispatus* (CST-I). Moreover, the authors observed that none of the women with *L. crispatus* dominated microbiota

acquired HIV-1 (47). In a vaginal mucosal culture model, the cervicovaginal fluids from women with CST-I inhibited HIV-1 infection, compared to women with CST-IV. HIV-1 infection was even enhanced when the *in vitro* model was cultured with vaginal fluids of women with CST-IV containing high abundance of *Ruminococcaceae* spp., *S. sanguinegens*, *A. vaginae* and *Aerococcus* spp. (105).

Overall, a higher bacterial diversity of the vaginal microbiota, associated with an increase of BV associated bacteria and a decrease of *Lactobacillus* spp. (except *L. iners*) abundance induce a higher susceptibility to various viral, bacterial and fungal STI (Figure 2; Table 2).

3.1.4. Co-infections

Co-infections within the FRT are frequent; indeed, a pre-existing infection can facilitate the acquisition of a second STI by modifying the local environment. Numerous epidemiology studies described that women suffering from an STI such as CT, Human Simplex Virus type 2 (HSV-2), *Neisseria gonorrhoeae*, *Treponema pallidum*, or *Trichomonas vaginalis* have an increased risk of HIV-1 acquisition (106–108). Several mechanisms might be involved within the FRT: for instance, CT infection may facilitate HIV-1 acquisition via cytokine production, leading to the recruitment of HIV-1 target cells, higher expression of HIV-1 coreceptors CXCR4 or CCR5, or epithelial cell disruption allowing HIV-1 cell free virus to cross the epithelial barrier... (109–111). Interestingly, vaginal microbiota of CT/HPV or HSV-2/HIV-1 co-infected women have an increased bacterial diversity (112, 113). Furthermore, Borgogna et al. described a distinct metabolic profile between uninfected and CT infected or CT/*Mycoplasma genitalium* coinfecting women (30). The interplay between vaginal microbiota, metabolic signatures and FRT inflammation during an STI needs to be better addressed in order to understand how those modifications may facilitate the acquisition of a second STI.

3.2. Crosstalk between neutrophils and the microbiota during STI exposition

During STI exposition, crosstalk between neutrophils and the microbiota can lead to various outcome in terms of STI acquisition and disease progression (Figure 2). Hensley-McBain et al. have shown that neutrophil accumulation in colorectal biopsies of HIV-1 infected ART treated patients is due to an increase of functional neutrophil survival. The authors observed an altered ratio of *Lactobacillus/Prevotella* in HIV-1 positive individuals, which correlated with neutrophil survival in colorectal tissue from the same patient. *In vitro*, *Prevotella*, *Bacteroides fragilis* and *Ruminococcus bromii* increased neutrophil survival compared to *Lactobacillus plantarum* and *Lactobacillus rhamnosus* (114). Neutrophils are known for being involved in protection against various STI. However, in neutrophils isolated from HIV-1 positive individuals, PRR expression is reduced, and upon TLR stimulation, neutrophils exhibit a reduced cytokine expression (115). During the chronic phase, a dysbiosis occurs (characterized by a *Gammaproteobacteria* enrichment and a *Lachnospiraceae/Luminococcaceae* depletion) (116). The hyporesponsiveness of neutrophils, combined with this dysbiosis could be involved in disease progression, but usually tissue neutrophils are often poorly analyzed, and more studies are necessary to fully understand the role of neutrophils at mucosal surfaces and their association with STI risk disease progression (117).

In the FRT, various STI agents induce neutrophil activation and antimicrobial function, including NET production, which prevents several STI acquisition notably during the early phase of infection (68, 118). However, bacterial STI have also developed mechanisms to counteract neutrophil-mediated immunity. For example, *N. gonorrhoea* and *C. trachomatis* resists neutrophil cytotoxic activity by several mechanisms, including inhibition of NET formation (119–121). However, up to date, there is no study on the impact of the vaginal microbiota composition on neutrophil-mediated immunity during bacterial STI. Probiotics could help maintaining an environment suitable for a balanced neutrophil recruitment and antimicrobial functions. For example, *Lactobacillus* have been used in the gut to reduce infection severity linked to inflammation. Neutrophil accumulation is reduced upon *Lactobacillus* treatment. Oral administration of *L. plantarum* CIRM653 before *K. pneumoniae* infection in mice, induced an immunosuppressive Treg response and decreased the number of immune cells (macrophages and neutrophils) and pro-inflammatory cytokines (IL-6 and TNF α) compared to naïve mice (122).

In conclusion, neutrophils are present at steady state in the FRT, but can also be rapidly recruited upon infection. Thus, they might have various roles in STI acquisition, notably depending on the vaginal microbiota composition, which is an important modulator of the local inflammation.

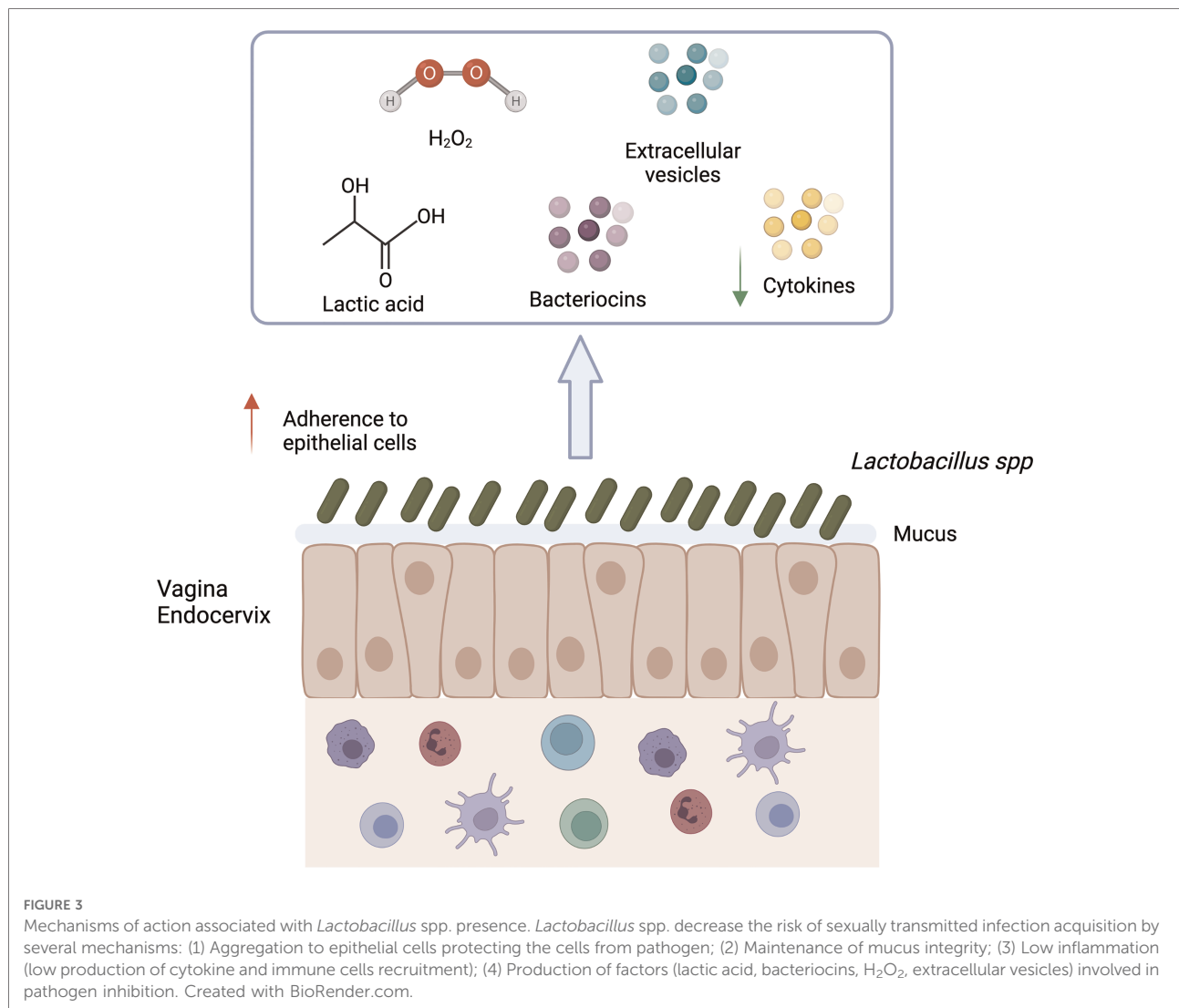
3.3. Mechanisms of action of the vaginal microbiota

Lactobacillus have been proposed as probiotic agents to be used against pathogenic microorganisms in the vagina. They can enter in competition with pathogens for attachment sites on epithelial cells, co-aggregate with pathogens and produce antimicrobial compounds. Indeed, *Lactobacillus* can produce: (1) biofilm allowing to mask epithelial cell receptors, adhesion to mucins and epithelial cells (123, 124) (2) lactic acid that inhibits pathogen growth (3) bacteriocins and bacteriostatic agents (Figure 3).

Lactobacillus are able to decrease bacterial infection such as CT either by aggregating to extracellular elementary body (EB), by competition for epithelial cells attachment or through lactic acid production that inactivate CT (125, 126). *L. crispatus* strains seem to have the best anti-CT effect (127). *In vitro*, *L. crispatus* treatment of a CT infected epithelial cell line (J774) decreased the production of IL-6, IL-8 and TNF α and induced the production of IL-10 (128). In addition, IL-1 α , IL-1 β and TNF α were strongly associated with CT infection in women that had a change in microbial communities (17). Pre-colonisation of epithelial cells with *L. crispatus* or *L. crispatus* enolase/glutamine synthetase decreased the adhesion and invasion of *N. gonorrhoea* probably by entering in competition for epithelial cell attachment. In addition, the expression of TNF α and CCL20 was also decreased in the pre-colonisation model (129). *Lactobacillus* spp. was observed to affect MyD88 and NF- κ B signalling pathways. *L. johnsonii* was able to activate the TLR2/TLR4 mediated NF- κ B pathway (130). In addition, pretreatment of cells with *L. rhamnosus* before *E. coli* infection suppressed Myd88, NF- κ B, TICAM2 expression associated with a decrease of IL-1 β , IL-18, IL-6, IL-8, IL-10, IFN β and TNF α (131). Many *Lactobacillus* spp. were described to be able to inhibit *T. vaginalis* adhesion on epithelial cells (132). Another anti-CT mechanism could result from a decreased availability of nutrients (such as glucose) in the environment, thanks to its consumption by *Lactobacillus* spp. (127). Annelot C. Breedvelt et al. demonstrated that women with a *L. crispatus* dominated microbiota had a higher level of bacterial IgA coating compared to non-*L. crispatus* dominated microbiota. The authors suggested that a higher level of unbound bacteria with IgA in non-*L. crispatus* dominated microbiota might be associated with inflammation, and therefore a higher susceptibility to infection (133).

3.3.1. Effect of H₂O₂ on pathogens

H₂O₂ was described as a potent antimicrobial factor produced by *Lactobacillus*, however, under the hypoxic conditions found in the vagina, *Lactobacillus* production of H₂O₂ is undetectable (134, 135). Moreover, physiological concentrations of H₂O₂ are not sufficient to have a bactericidal



effect on pathogens, suggesting that the main antimicrobial agent produced by *Lactobacillus* is lactic acid (135).

3.3.2. Effect of lactic acid on pathogens

Vaginal lactic acid exists as D and L-isomers. Epithelial cells can only produce L-lactic acid, *L. crispatus* and *L. gasseri* are able to produce both isomers, *L. jensenii* only produces D-lactic acid and *L. iners* only produces L-lactic acid (136). Lactic acid production acidifies the vagina, but *in vitro*, when the pH reaches 3.2–4.8, *Lactobacillus* stop their growth and acid lactic production (137). *L. iners* colonization has been linked to a higher vaginal pH compared to other species of *Lactobacillus* (138). Matrix metalloproteinase-8, which is able to degrade the cervical plug, is down regulated by D-lactic acid through decrease of extracellular metalloproteinase inducer (EMMPRIN), thus inhibiting pathogen migration to the uterus (136).

Lactic acid has an important antimicrobial effect in the vagina. *L. crispatus* pre-colonisation of porcine vaginal mucosa model inhibits *N. gonorrhoeae* growth mainly through lactic acid production (139). A significant association between a low HIV-1 infectivity and a high percentage of D- and L-lactic acid, which also correlated with a high relative abundance of *Lactobacillus* spp. (particularly *L. crispatus*) has been reported (140). Moreover, cervicovaginal mucus with a high concentration in D-lactic acid collected from women demonstrated a better HIV-1 trapping. These mechanisms seem to be mediated through hydrogen bonds between surface carboxyl groups of the virus and host-derived envelope glycolipids or glycoproteins. Furthermore, CVM that trapped HIV-1 were generally associated with a *L. crispatus* dominant microbiota whereas those that failed to trap HIV-1 had either a *L. iners* dominant microbiota or a significant abundance of *G. vaginalis* (141, 142). On the contrary, Nahui

Palomino et al. have shown that L-lactic acid but not D-lactic acid were the main contributors for HIV-1 inhibition on cervicovaginal explants. The authors also demonstrated that *Lactobacillus* were able to bind to HIV-1 virions, thus decreasing HIV-1 infection (143).

3.3.3. Effect of bacteriocins on pathogens

Bacteriocins are antimicrobial peptides or proteins secreted by some but not all vaginal *Lactobacillus* strains (144, 145). Bacteriocins are divided into two classes: class I bacteriocins (lantibiotics) are lanthionine-containing peptides, whereas class II bacteriocins do not contain lanthionine (146). Class I bacteriocins are active through the formation of pores and efflux of small metabolites from sensitive cells or through enzyme inhibition. The majority of class II bacteriocins are active by inducing membrane permeabilization and the subsequent leakage of molecules from target bacteria. Bacteriocins isolated from vaginal *Lactobacilli* were shown to inhibit the growth of various microorganisms including *Klebsiella*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Candida parapsilosis* or *G. vaginalis* (144, 145). Bacteriocins are also active against STI agents such as *N. gonorrhoeae* (147). In a recent study, a *Lactobacillus* strain was even shown to selectively inhibit 100% of *L. iners* strains tested through two different class IIb bacteriocins: gassericin K7A and gassericin K7B (148). Among other strains of the vaginal microbiota including *L. crispatus*, *L. jensenii*, and *L. gasseri*, only few of them (<20%) were also inhibited by these bacteriocins. *In vitro* studies showed that bacteriocin production was not constitutive but only occurred in specific conditions: for example through co-cultivation with several other Gram-positive strains (149) or during the exponential growth phase of *Lactobacilli* (150), when bacteriocin production was probably being enhanced by autoinduction (149). The ability of various *Lactobacillus* strains to produce bacteriocins that efficiently inhibit vaginal pathogens may allow their use as therapeutic agents for the treatment and prevention of urogenital disorders and could be an alternative to conventional antibiotic therapy (150).

3.3.4. Effect of extracellular vesicles on pathogens

A recent study has linked extracellular vesicles (EV) produced by *Lactobacillus* and protection against HIV-1. EV are produced by Gram (+) and Gram (–) bacteria. In Gram (–), they have been described to be produced by the pinching off of the outer membrane of the bacteria. However, the mechanism implied in EV production in Gram (+) bacteria is not yet determined (151). EV produced by *Lactobacillus* spp. isolated from women inhibit HIV-1 replication in CD4⁺ T cells, human tonsillar and cervicovaginal tissues in a dose dependent manner. HIV-1 inhibiting EV were associated with more amino acids, and the expression of several proteins such

as enolase and elongation factor Tu. Those proteins were reported to play a role in adhesion to epithelial cells (152). Interestingly, Paolo E. Costantini et al. described that other Gram (+) bacteria, such as *S. aureus*, *G. vaginalis*, *Enterococcus faecium* and *E. faecalis* were able to produce EV that protect against HIV-1 infection. The mechanism associated with this protection is through a steric hindrance of gp120. Moreover, several proteins present on EV surface are involved in the anti-viral effect (153). This mechanism seems to be not exclusive of *Lactobacillus* spp., therefore evaluation and comparison between species/strains need to be addressed to understand if EV produced by *Lactobacillus* spp. have a better HIV-1 inhibitory effect compared to EV produced by anaerobic bacteria.

3.3.5. Impact of indole production by the vaginal microbiota during the CT infection

The vaginal microbiota can also impact the pathogenesis of STI infection. For example, upon CT infection, IFN γ production induces the activation of the enzyme IDO1 which depletes the tryptophan pool within the epithelial cells into kynurenine. *In vitro*, pretreatment with IFN γ of CT (svD) infected epithelial cell induces the depletion of the tryptophan pool, leading to no infection of the cells. Supernatant of indole producing bacteria such as *Prevotella intermedia* and *Prevotella nigrescens* promotes CT infection in presence of IFN γ (154). Interestingly, *in vitro*, IFN γ induced a persistent CT phenotype which was described to have a distinct proteome profile compared to EB and reticulate body (RB) with a high expression of the tryptophan operon *TrpA* and *TrpB* (155). These proteins are essential for tryptophan synthesis using indole produced by anaerobic bacteria present in the FRT (156). Ratios of kynurenine/tryptophan are higher in vaginal fluids of CT infected women, and correlated with high abundance of *Streptococcus* spp. and *Peptoniphilus* spp. Moreover, 94% of the samples of CT infected women exhibited a high abundance of indole producing bacteria such as *Porphyromonas asacharolytica*, *Propionibacterium acnes*, *Fusobacterium nucleatum*, *E. faecalis*, *Peptoniphilus harei* and *E. coli* (157). CT can retain a subset of genes involved in tryptophan synthesis (*trpA*, *trpB* and *trpR* genes) and use them to synthesize tryptophan from indole, leading to CT growth and infection (158).

3.3.6. Impact of the vaginal microbiota on antiviral drugs

Vaginal microbiota composition has been linked to antiretroviral microbicide gel protection against HIV-1. The efficacy of tenofovir (TFV) to decrease HIV-1 infection was reduced in culture colonized by bacteria from vaginal fluids of women with a diverse microbiota (105). In the CAPRISA study, TFV gel reduced HIV-1 incidence by 61% in women with a vaginal microbiota dominated by *Lactobacillus*, but

only by 18% in women with a non-*Lactobacillus* dominant microbiota. Furthermore, TFV concentration in CVL negatively correlated with BV associated bacteria such as *G. vaginalis* and *Prevotella*. *G. vaginalis* and to a lesser extent *Prevotella* species, are able to rapidly metabolized TFV before drug uptake by target cells. The mechanism involves the production of adenine by cleavage of the side chain component of TFV (oxy-methylphosphonic acid) (159). Moreover, CVL, collected from women suffering from BV, were able to degrade TFV and dapirivine (DPV) but not tenofovir alafenamide (TAF). TAF does not seem to be impacted thanks to a faster internalization in target cells (159, 160). These articles demonstrated the importance of *Lactobacillus* in preventing drug degradation by other species of bacteria, highlighting that microbial communities are critical for TFV and DPV efficacy (160). On the contrary, Taneva et al. suggest a more complex role of the microbiota in the modulation of topical antiretroviral efficacy. The authors observed adenine production in the culture medium, which is able to block endocytosis of TFV into human cells, leading to decrease protection against HIV-1. They also observed that TFV is transported and metabolized by *L. crispatus* but not *L. jensenii* and *L. iners*. The authors hypothesized that *L. crispatus* could be a reservoir that gradually release TFV into the vagina. Finally, they observed that the inhibitory effect of the vaginal microbiota was overcome at higher drug levels, suggesting that a sustained drug delivery could be sufficient to induce protection. However, a decrease in drug levels might lead to a decreased efficacy of antiretroviral due to adenine and high pH (161). Intravaginal rings could be used to induce a sustained delivery of drugs. DPV ring use in adolescents was not associated with cervicovaginal inflammation or microbiota changes, however, a significant increase in *L. crispatus* and a decrease of *L. iners* were observed when both arms were combined (placebo ring and DPV ring), suggesting a beneficial effect of ring use on the microbiota (162). TFV intravaginal ring in adults was also observed to have no impact on vaginal microbiota composition. Moreover, the authors described no effect of BV associated bacteria on TFV availability. The hypothesis was that sustained release of TFV by the ring is sufficient to abrogate the effect of BV associated bacteria (163). The efficacy of daily oral PrEP for HIV-1 prevention among women with highly diverse microbiota were not impacted, compared to women with a *Lactobacillus* dominant microbiota (164).

4. Non-human primate (NHP) models to study vaginal inflammation and microbiota in STI

Animal models including mice, rat or non-human primates (NHP) have been widely used in biomedical research including

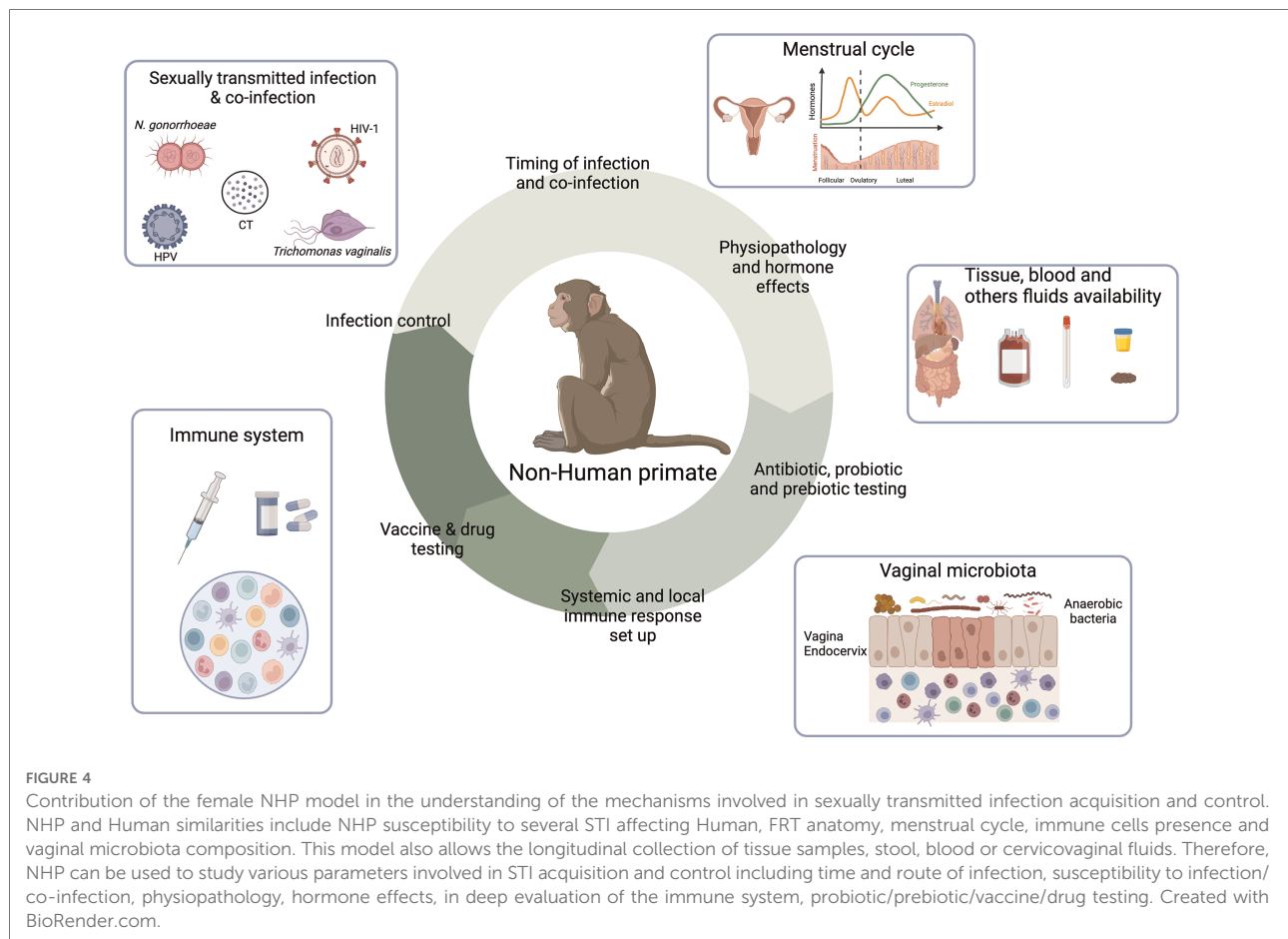
for STI studies. Rodents are particularly used due to their numerous advantages including a low cost in terms of housing, easy breeding and numerous offsprings at each litter. Moreover, genetic engineering or microbiota modification can be easily performed in mice models for instance (165, 166). However, substantial differences can be highlighted between the mice models and humans. Divergence between humans and mice occurs between 65 and 75 million years ago (167, 168). Differences in terms of innate and adaptive immune responses can also be highlighted: the balance of leukocyte subsets (T and B cells), neutrophils and soluble factor production (cytokines and chemokines) are different for instance (168, 169). Moreover, FRT anatomy as well as hormonal secretion differ between humans and mice. For instance, the cervical epithelium is different between the two species (170). Furthermore, several infectious pathogens do not infect or recapitulate human pathology in mice (171). On the contrary, NHP have been described to be susceptible to wide range of human pathogens including viral (Simian Immunodeficiency virus, the simian analogue of HIV-1, ZIKA, Ebola, Chikungunya or Influenza) or bacterial infections (Periodontal disease, Tuberculosis, *Chlamydia trachomatis*) (171–174). They are essential to determine vaccine or drug safety/efficacy, immune responses against infectious pathogens that induce high mortality and tissue specific immune responses for instance. The NHP model can be used to study human STI by controlling various parameters such as the infectious dose, or the time of infection that are more difficult to assess in cohort studies (Figure 4). It is also possible to study the mechanisms involved during co-infections establishment, which is much more difficult in cohort studies.

4.1. Similarities between human and NHP

Humans and NHP are closely similar since their divergence occurs only between 6 and 7 million years ago (167). Therefore, it is not surprising to observe similarities in terms of immune system, female reproductive tract and to a lower extent vaginal microbiota between the two species. Three species of macaques are commonly used: rhesus macaque (*Macaca mulatta*), cynomolgus macaque (*Macaca fascicularis*) and pigtail macaque (*Macaca nemestrina*) (175).

4.1.1. Immune system

Immune cells including neutrophils, basophils, CD8⁺ and CD4⁺ T cells, B cells, NK cells, plasmacytoid DC, classical/intermediate or non-classical monocytes in the blood are observed in rhesus, cynomolgus macaques and humans and have similar surface markers (169, 176). For example, DC subsets represent less than 1% of cells in PBMC and express similar TLR in humans and rhesus macaques (RM). Both



species have similar T cell subsets (naïve, central memory or effector memory) (177). However, some differences can be noticed between NHP and humans. Bjornson-Hooper et al., observed more CD4⁺ CD8⁺ double positive T cells, a lower ratio of classical/non classical DC, low expression of CD11c and CD16 on neutrophils and the CD8 expression on NK cells in the blood of cynomolgus or rhesus macaques compared to humans (169). The lower presence of B cells is also noticed in peripheral blood, mesenteric lymph node and spleen in cynomolgus macaques compared to humans (176).

4.1.2. Female reproductive tract

The female reproductive tract of cynomolgus macaques is closely similar to the one of women in terms of morphology, endocrine system and menstrual cycle (178). The menstrual cycle in rhesus and cynomolgus macaques (28–32 days) is similar to the one observed in humans (28–30 days). The follicular phase lasts 12–14 days and the luteal phase around 14–16 days (179). Menstruation lasts between 1 and 8 days, but 85% of the females exhibit a menstruation cycle of only 3–5 days (180). RM exhibit seasonal variations in their sexual and gonadal activity (only active from October to March), contrary to marmoset and cynomolgus macaques (179).

Marlin et al. have characterized the immune cell in the vagina, cervix, uterus and fallopian tubes of cynomolgus macaques. The localization and phenotype of immune cells are similar in the human and cynomolgus macaque FRT (181).

These similarities between Humans and macaques allow a better characterization of the genital inflammation, the environmental factors impacting the inflammation and thus the development of strategies to modulate this inflammation. Indeed, in cynomolgus macaques, it is possible to test intervention strategies targeting immune cells or using microbicides to modulate the inflammation. Moreover, during those studies, it is possible to access various samples in longitudinal studies, including tissue sample for instance, that can be difficult to obtain in Humans (Figure 4).

4.1.3. Vaginal microbiota

The vaginal microbiota of macaques exhibits low abundance of *Lactobacillus*, contrary to what is observed in eubiosis women. Many species of macaques harbor a diverse vaginal microbiota, composed of a wide range of bacteria including *Saccharofermentans*, *Campylobacter*, *Atopobacter*, *Thioreductor*, *Streptococcus* and interestingly, several BV associated bacteria such as *Gardnerella*, *Prevotella*, *Fusobacterium*, *Sneathia*,

Atopobium, *Peptoniphilus*, *Dialister*, *Mobiluncus* and *Porphyromonas*. The vaginal pH is also higher than in humans, with a median value of 7 (182–186). *Lactobacillus* can be found in all species of macaques, but at a low abundance. In RM, most of the *Lactobacillus* detected are *L. johnsonii*, and few sequences correspond to *L. gasseri* and *L. salivarius*. A comparative study between humans and RM showed that oral, perianal and vaginal microbiota of RM were distinct from the human ones. Indeed, the vaginal microbiota was more diverse than the human vaginal microbiota. The vaginal microbiota of cynomolgus macaques is similar in terms of taxonomic composition and relative abundance to the one of women harboring a high vaginal microbiota diversity (CST-IV). It is also variable according to animals and correlates with hormonal variation during the menstrual cycle (186). The vaginal microbiota is more diverse during lactation and menstruation (187). More precisely, during the menstruation there is an increased concentration of pro-inflammatory cytokines, a higher abundance of mature/activated neutrophils that originate from the blood and vaginal microbiota fluctuation (58)

One hypothesis explaining the low abundance of *Lactobacillus* spp. in macaques concerns the levels of glycogen and lactic acid poorly present in the vaginal fluids of macaques compared to humans (188). Indeed, Miller et al. hypothesized that high level of starch in human diets have induced an increased amount of glycogen within the vagina, which in turn promotes the proliferation of *Lactobacillus* (189). Several research teams have developed macaque's model vaginally enriched with *Lactobacillus*, but were only partially successful (190, 191). The authors observed either a transient colonization with *L. crispatus* and/or no modification of cervicovaginal inflammation and pH. The diverse vaginal microbiota in cynomolgus macaques makes it a good model to study dysbiosis: the NHP model can be used to test various strategies aiming at improving the genital health by modifying the vaginal microbiota for instance using combinations of antibiotics, prebiotic and/or probiotics (Figure 4).

Overall, the NHP model is suitable to study human physiological and pathological situations. It allows the monitoring of the systemic and local immune responses in the FRT. Various factors impacting the local inflammation such as cytokine profiles, immune cell distribution, the vaginal microbiota, ongoing STI ... can also be evaluated individually in this model. It is a relevant model to study the susceptibility and the response to infectious diseases such as STI. Moreover, the NHP model can be manipulated to evaluate safety/efficacy of vaccines and therapies against STI or genital inflammation.

5. Conclusion

Vaginal microbiota composition is increasingly described to have a central role in the regulation of FRT inflammation and

protection against STI acquisition. Indeed, *Lactobacillus* spp. allow the maintenance of a low inflammatory environment and are able to control STI through different mechanisms (lactic acid/bacteriocin production, adhesion to cells/pathogens etc.). Neutrophils are key modulators of the inflammation. In the FRT, their interaction with the microbiota impacts the immune response to STI. However, the mechanisms of action are still poorly understood. New models, including cervicovaginal 3D model or human vagina on chip, will be interesting to study how the vaginal microbiota composition, as well as metabolites, regulate the FRT inflammation (42, 192). However, the use of a suitable animal model that reproduce the FRT environment, including immune cells, soluble factors, vaginal microbiota composition with an environment subject to hormonal fluctuation for instance, is mandatory. Since strain specificity was observed in terms of inflammation and metabolites production (193), deep analysis at the strain level will also be essential to better understand the role of key bacteria in inflammation.

In conclusion, this review highlights the interplay between the vaginal microbiota composition and the local inflammation, including cytokine/chemokine production, cell recruitment and especially neutrophil presence in association with susceptibility to STI. Moreover, several mechanisms of action of how an optimal vaginal microbiota is able to decrease the susceptibility and favor the treatment of STI, are discussed. Finally, the NHP model is described demonstrating its usefulness for the study of vaginal microbiota composition and FRT inflammation, including innate and adaptive immune responses in a context or not of STIs.

Author contributions

CA wrote the first draft of the manuscript, designed the figures and did subsequent edits. LR completed the first draft and subsequent edits in the text and the figures. EM provided critical feedback and edits on the work throughout. The authors approved the final edit of the manuscript as submitted. All authors attest they meet the ICMJE criteria for authorship. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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