HIV Inhibition by Lactobacilli: Easier in a Test Tube Than in Real Life

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ABSTRACT A lactobacillus-dominant vaginal microbiota has been shown to decrease heterosexual HIV transmission. Nunn et al. now report that a vaginal microbiota dominated by *Lactobacillus crispatus* is associated with a relative inability of HIV pseudoviral particles to transverse cervicovaginal mucus (CVM) *in vitro* [mBio 6(5):e01084-15, 2015, doi:10.1128/mBio.01084-15]. The purported inhibitory mechanism is the interaction between carboxyl groups present on HIV and in CVM that occurred only under acidic conditions when carboxyl groups were protonated. *L. crispatus* produces high levels of lactic acid and results in the lowest vaginal pH when it is the dominant vaginal bacterium. In addition, high levels of lactic acid inhibit the proliferation of other bacteria that might negatively affect CVM structure. The utility of enhancing *L. crispatus* dominance to inhibit HIV transmission awaits assessment of the influence of ejaculated semen on this property and investigations on the role of *Lactobacillus* products such as D-lactic acid in this property.

In many regions of the world, women have little or no authority or independence and are not in a position to demand safe sexual practices such as the use of barrier contraceptives. There is a great need, therefore, to identify methodologies that can be used discretely by women to prevent HIV transmission. An enhanced understanding of the factors that facilitate or retard male-to-female HIV transmission would enable the design and development of effective anti-HIV strategies. In a recent article in *mBio*, Nunn et al. provide evidence of a previously unsuspected mechanism that may participate in the regulation of HIV passage through the vaginal mucosa (1). In attempting to reconcile two contradictory published studies claiming that cervicovaginal mucus (CVM) did or did not retard HIV virion transmission, the authors astutely reasoned that the observed differences could be due to variations in the composition of the vaginal microbiota. They first observed that the ability of HIV to pass through CVM was inversely related to the vaginal concentration of the D-isomer of lactic acid. This isomer is preferentially produced by *Lactobacillus crispatus*, *L. gasseri*, and *L. jensenii* in the vagina. Utilizing an *in vitro* system composed of HIV pseudovirions and minimally handled CVM, it was next shown that the secretions from women whose vaginal microbiota was dominated by *L. crispatus* retarded HIV passage to a much greater extent than did the secretions from women in whom other bacteria, including *L. iners*, were present at high levels in the vagina. Addition of exogenous D-lactic acid to the secretions had no effect on the extent of HIV transmission through the CVM. Further investigation provided evidence that it was the interaction between carboxyl groups on the HIV surface and in CVM, in the acidic environment normally present in the vagina when lactobacilli predominate, that inhibited HIV passage. The authors concluded that a vaginal microbiota dominated by *L. crispatus* optimizes the capacity of secretions in the vagina to inhibit HIV from reaching the underlying epithelial cells and initiating a productive infection. Thus, the preferential promotion of *L. crispatus* proliferation in the vagina, by the use of prebiotics or probiotics or other means, may be an effective, low-cost maneuver to reduce the rate of male-to-female HIV transmission. Others have previously reported that a *Lactobacillus*-dominant vaginal microbiota reduces susceptibility to HIV (reviewed in reference 2).

Of course, what is observed in a test tube does not necessarily translate to the *in vivo* environment. For many years, it was largely accepted that hydrogen peroxide production by lactobacilli was a major mechanism that inhibited heterosexual HIV transmission. This was based on *in vitro* experiments where the supernatant from aerobically grown lactobacillus cultures killed HIV. However, as shown by coauthors of the Nunn paper, as well as by other investigators, hydrogen peroxide production is greatly reduced under the anaerobic conditions present in the vagina and, furthermore, CVM as well as semen totally inactivated any remaining hydrogen peroxide (3). To cite another example, the detergent Nonoxynol 9 was remarkably efficient in killing HIV in a test tube. Unfortunately, when provided to women at risk for acquiring HIV, Nonoxynol 9 actually increased the rate of transmission (4). This was due to a localized inflammation induced by the detergent that resulted in migration to the vaginal lumen of lymphoid cells that HIV could infect. Thus, the induction of inflammation in the vagina and influx of target cells for HIV were dominant over detergent-mediated HIV lysis. It has also been shown that other potential HIV microbicides alter the vaginal microbiota from one of lactobacillus dominance to a more diverse bacterial repertoire (5). As suggested by the Nunn study, this alteration may facilitate HIV passage through CVM.

Similarly to the situations described above, much remains to be analyzed before the unique *in vitro* observations in the Nunn study are deemed to have clinical relevance. Male-to-female HIV transmission occurs via sexual intercourse, and the virus is associated with both soluble and particulate semen components, possibly including spermatozoa. It remains to be determined whether CVM is altered by exposure to semen components and/or whether virions that are covered in seminal constituents are trapped by

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vaginal mucus. Semen also alters the vaginal pH from acidity to neutrality or even to slight alkalinity and has immunosuppressive properties. As shown by Nunn et al., HIV inhibition by mucus requires an acidic pH. Thus, as mentioned by the authors, additional studies incorporating the male ejaculate are necessary to determine the clinical relevance of their observations. In reality, all potential HIV microbicides must be pretested in the presence of semen before their utility can be accurately assessed. Importantly, there are limitations in evaluating the mechanisms of vaginal HIV transmission, as well as the efficacy or side effects of vaginal microbicides, in animal models, including nonhuman primates. Only human females have a vagina that is dominated by lactobacilli and whose secretions are rich in lactic acid and have a very acidic pH (6). Thus, examining the effects of these characteristics, individually and in tandem, is necessary to predict clinical efficacy.

It has been extensively reported that women with a lactobacillus-dominated vaginal microbiota are at decreased risk for HIV acquisition, while women with bacterial vaginosis (BV), an alteration of the vaginal microbiota leading to the predominance of Gram-negative anaerobic bacteria, are at an increased risk of becoming infected with HIV (2). A recent study established that a predominance of Gram-negative anaerobic bacteria leads to activation of Toll-like receptors on vaginal epithelial cells, resulting in induction of inflammation and an influx of lymphocytes into the vagina (7). The appearance of target cells for HIV in the vaginal lumen of women with BV or with a sexually transmitted infection by organisms such as *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and herpesviruses, or by any other microbe that elicits a genital ulcer, might minimize the role of CVM in preventing HIV transmission. Similarly, HIV in semen is not only present as free virions but is also associated with lymphoid cells present in the ejaculate. Passage of cell-associated HIV through mucus might very well differ from that of free virus.

Since vaginal *Lactobacillus* spp. other than *L. iners* are the principal producers of D-lactic acid in the vagina, the reported association between this lactic acid isomer and the predominance of *L. crispatus* is not surprising. However, in contrast to the conclusions of the authors, D-lactic acid may be more than merely a marker for the presence of lactobacilli. The production by vaginal epithelial cells of extracellular matrix metalloproteinase inducer and matrix metalloproteinase (MMP)-8 increases as the ratio of L-lactic acid to D-lactic acid in the vaginal lumen increases (8). It is likely that MMPs alter properties of the CVM, thereby facilitating HIV passage. MMP-8 weakens the integrity of the cervical mucus plug and reduces its ability to block bacterial migration from the lower to the upper genital tract (9). The force involved in the release of spermatozoa and secretions of the prostate gland and seminal vesicles from the male to the female results in semen deposition against the cervical os. It is the mucus at this site, rather than mucus covering the vaginal epithelial cells, that comes into initial contact with HIV, as well as with other viruses and bacteria in semen. Thus, regulation of MMP production may have a substantial impact on HIV transmission. The reality that HIV in semen is deposited against the cervical os in most episodes of sexual intercourse has been a neglected aspect of microbicide research and needs be taken into consideration. Addition of exogenous D-lactic acid to decrease MMP levels may, therefore, be a viable alternative or a supplement to manipulation of levels of lactobacilli in the vagina. It should also be remarked that the CVM utilized in the Nunn study was not sterile and that the experiments with HIV pseudovirions were conducted in an aerobic environment. The inhibitory effect of mucus from women positive for *L. crispatus* might have also been due, at least in part, to the production of hydrogen peroxide by *L. crispatus* present in the mucus.

In conclusion, Nunn and coworkers deserve credit for their novel experimentation and insightful analysis. Further studies are needed, however, to evaluate the potential of altering the vaginal microbiota and/or the concentration of vaginal components as effective mechanisms to enhance protection against male-to-female HIV transmission.

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