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The effects of commensal bacteria on innate immune responses in the female genital tract

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Abstract

The innate and adaptive immune systems are important mechanisms for resistance to pathogens in the female lower genital tract. Lactobacilli at this site help maintain a healthy vagina by producing several factors including lactic acid. Indeed, bacterial vaginosis, a condition in which the genital microbiota is altered, is strongly associated with increased rates of a number of infections including HIV. However, the precise factors that contribute to increased rates of microbial and viral infections in bacterial vaginosis remain to be elucidated.

We have studied the effects of bacterial microbiota in the lower genital tract on innate immunity and have found that Toll-like receptor ligands and short chain fatty acids, produced by bacterial microbiota, have dramatic effects on immune function. In this review, we will discuss these results, in addition to some recent articles that we believe will enhance our understanding of how microbes might interact with the immune system.

Keywords

innate immunity; short chain fatty acids; vaginal microbiota

Introduction

The female genital tract is equipped to deal with a variety of foreign substances including spermatozoa, a fetus that is immunologically distinct from its mother, and a wide array of pathogens. These pathogens include viruses, bacteria, fungi and parasites. To add to this complexity, the various parts of the female genital tract are influenced by sex hormones during menstruation.[1] All of these components act in concert to optimize the conditions for reproduction and a successful pregnancy. The female genital tract is protected against these various invaders by two inter-related mechanisms: the innate and the adaptive immune systems. The intricate interplay between the two arms of the immune system, plus their interaction with the genital tract bacterial microflora and the host epithelial cells, is ultimately responsible for the health of the lower female genital tract.[2, 3]

There are several layers of innate protection in the lower female genital tract. The epithelial cells covering the length of the female genital tract act as the first line of defense and a physical barrier by inhibiting the passage of pathogens and their associated particles. The epithelial cells also produce mucus, which covers the internal surface of the vagina and

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cervix and serves to trap infectious agents.[4, 5] Moreover, the presence of several antimicrobial proteins (such as defensins) in the mucus helps combat many pathogens before they gain entry into the epithelial layer of the genital tract.[6]

The epithelial cells further help protect against pathogens by expressing many receptors known to be important in immune responses, including a number of Toll-like receptors (TLRs), MD-2, as well as major histocompatibility complex (MHC) molecules. These molecules help recognize, process and initiate cellular immune responses to obliterate pathogens. When activated, epithelial cells can also produce a variety of cytokines and chemokines such as TNFα, G-CSF, GM-CSF, IL-6 and IL-8, to help recruit immune cells, induce their differentiation/activation and develop successful immune responses.[1, 2]

If the protection afforded by the epithelial barrier is compromised, however, pathogens encounter a second layer of innate defense consisting of specialized immune cells and their products. These cells, which include macrophages, dendritic cells, neutrophils, and natural killer cells, are dispersed throughout the female genital tract, surveying that environment. Should these cells come across pathogens, they can take up, process, and/or destroy them.[1, 3]

The innate immune system recognizes pathogen-associated molecular patterns (PAMPS) through various pattern recognition receptors such as the TLR and NOD-like receptors (NLRs). The interaction between these receptors and their respective microbial ligands quickly results in the production of cytokines, chemokines, and antimicrobial products in order to slow down or stop pathogen replication and begin to eradicate the pathogen. While such actions occur quickly, they lack in specificity and memory. An antigen-specific attack is launched by the adaptive arm of the immune system, consisting of T and B cells, after the microbial antigens have been recognized, processed and presented by antigen presenting cells. The cross-talk between the innate and the adaptive immune systems, as well as the important role that T and B cells play in conferring long-term immunity in the genital tract, have been discussed elsewhere. Here, we shall focus on another crucial component of this multifaceted system: the commensal bacteria and their effect on mucosal immune responses.

The Lower Genital Tract Bacterial Microbiome

The lower genital tract of women is colonized by commensal bacteria that can affect immune responses. Identifying the types of bacteria present at this mucosal site is therefore a critical step in understanding how immunity is affected by the microbiota. Clinical diagnostic criteria have classically divided genital bacterial microbiota into at least two large groups; microbiota that is considered to be healthy; and bacteria associated with bacterial vaginosis (BV). While healthy microbiota is dominated by *Lactobacillus* species, BV can be variable between women but, in most cases, consists largely of colonization by several types of anaerobic bacteria and *Gardnerella vaginalis*.[7–9] Recent studies have largely confirmed the existence of these two groupings using molecular techniques such as direct sequencing or PCR of the 16S rRNA genes to identify and quantify the types of bacteria present since culture may skew the proportions of bacteria identified at this mucosal site.[10–12] More recently, Ravel et al.[13] sequenced the genital microbiota of 396 asymptomatic North American women and found there were roughly five types of bacterial communities, dominated by either *Lactobacillus iners, L. crispatus, L. gasseri, L. jensenii*, and a fifth group that was not dominated by lactobacilli but instead consisted of mostly anaerobes and *G. vaginalis.* Also recently, Hummelen et al. showed that, in a group of HIV+ African women, *L. iners* and *G. vaginalis* were the main constituents of the vaginal microbiota.[14] *Prevotella bivia*, a pathogen known to invade epithelial cells and to cause inflammatory responses[15], dominated in this cohort of African women who were also BV+. Interestingly, *L. crispatus* was more strongly associated with a healthy vaginal pH than *L. iners* in the HIV

+ African cohort, although at higher frequencies *L. iners* also correlated with a vaginal pH of less than 4.5.[14]

A key feature of colonization by predominantly Lactobacillus is a relatively low pH (pH < 4.5), due to production of large amounts of lactic acid by these bacteria.[16] This low pH is believed to help protect against colonization by pathogens. Lactobacilli are also thought to contribute to the health of the vagina by producing H2O2 and by competing against more pathogenic microorganisms.[17–23] In contrast, bacteria in BV produce relatively little lactic acid, but make a number of immunomodulatory substances including succinate, sialydases and proteases[24], as well as proinflammatory substances such as lipopolysaccharides (LPS), lipoteichoic acids (LTA) and peptidoglycans (PGN).[25, 26] These microbial products can stimulate cells through TLRs or NLRs that are present on immune cells and other cells in the female lower genital tract.

Studies by us show that substances in the mucosal fluid of women with BV can stimulate myeloid cells to secrete several cytokines including IL-8 while fluids from women with healthy microbiota are much less stimulatory ([27, 28] and Fig. 1). Previous studies using cell lines expressing TLR2 showed that some of the stimulatory products in BV mucosal fluids activated cells through this receptor.[28] To determine whether stimulation through either TLR2 or TLR4 was predominant, antibodies to TLR2 or TLR4 were added to cultures. Antibody to TLR2 only slightly reduced stimulation of the myeloid cell line while antibody to TLR4 reduced IL-8 production by 30–60 percent (Fig. 1). These results indicate that while there are some TLR2 ligands present in BV, stimulation through TLR4 is stronger. However, these results also suggest that TLR2 and TLR4 ligands may not account for all the stimulatory substances in BV mucosal fluids.

In addition to TLR ligands, bacteria, especially anaerobes, produce large quantities of short chain fatty acids (SCFAs) in mucosal sites such as the gut[29] and the female genital tract in BV ([30, 31], also Fig. 2). These microbial products, which include acetic, butyric, and propionic acids, have been shown to play an important role in a wide array of immune responses, based largely on studies investigating the gut microflora. Those studies show that SCFAs modulate immune responses by inhibiting the production of proinflammatory cytokines, and by affecting immune cell migration and phagocytosis. Furthermore, these compounds are known to induce apoptosis in various cell types including neutrophils.[32–38] In fact, due to its mostly anti-inflammatory effects on the immune system, butyric acid has long been used to treat inflammatory bowel disease (IBD).[29]

SCFAs are readily taken up by both nonionic diffusion and active transport by cells.[29] More recently, GPR43, a member of the G-protein-coupled receptor family, was shown to bind SCFAs.[39, 40] Interestingly, GPR43 is expressed on the surface of innate immune cells including neutrophils and monocytes,[37, 39, 41, 42] highlighting the important role that the SCFA and GPR43 interactions may play in innate immune responses.

Our own research, among others, confirm the presence of many SCFAs in the lower genital mucosa. The SCFAs at this site include acetic, propionic, isobutyric, *n*-butyric, and isovaleric acids (Fig. 2 and[30, 31, 43]). In fact, we find that the concentration of acetic acid in cervical-vaginal lavage (CVL) samples can be as high as 3 mM (Fig. 2). Given that during the lavage the mucosal fluid is diluted an average of 40 fold, the concentration of acetic acid may reach as high as 120 mM in the lower genital tract. Our data also show that various SCFAs are found at significantly higher concentrations in BV+ women when compared to non-BV women (Fig. 2), which is not surprising given that BV is characterized by the outgrowth of mostly anaerobic bacteria. Despite their abundance in the genital tract,

however, the SCFAs' role in modulating immune responses in the genital mucosa is not well understood.

We and others have found that SCFAs can induce neutrophils to undergo an oxidative burst (Fig. 3, also[37]). Our data further suggest that, whereas SCFAs alone are unable to induce IL-8 production by peripheral blood mononuclear cells (PBMCs), the production of IL-8 and TNFα (Fig. 4 and data not shown) appears to be enhanced when both SCFAs and Pam2CSK4 (a synthetic TLR2 ligand) are used to stimulate these cells. These data suggest that SCFAs, especially in combination with other microbial products, may be involved in recruitment and activation of the innate immune cells in the female genital tract. As the levels of some SCFAs have been reported to fluctuate during menstruation[43], It will be interesting to investigate how various SCFAs, acting alone or in concert, might affect immune responses at different stages of menstrual cycle and what the implications of these changes are in health and disease states.

Concluding Remarks

The female genital tract is a complex and unique environment. It is equipped to promote a successful pregnancy in an ever-changing milieu affected by sex hormones and various foreign invaders. Its protection against infections hangs in balance between commensal versus pathogenic microorganisms. Given the scope of sexually transmitted diseases, and the extent to which they cause morbidity and mortality worldwide, it is crucial to elucidate factors that contribute to the health of the female genital tract. Clearly, more work needs to be done to determine what the important immune mediators are in genital mucosa, what the respective roles of epithelial and immune cells are in production of these mediators, and how the chronologic and reproductive aging impacts immune responses. Furthermore, because of the heterogeneity of microbial flora in different individuals both in health and disease states, it will be important to identify and fully investigate how these bacteria shape mucosal immune responses in the female genital tract.

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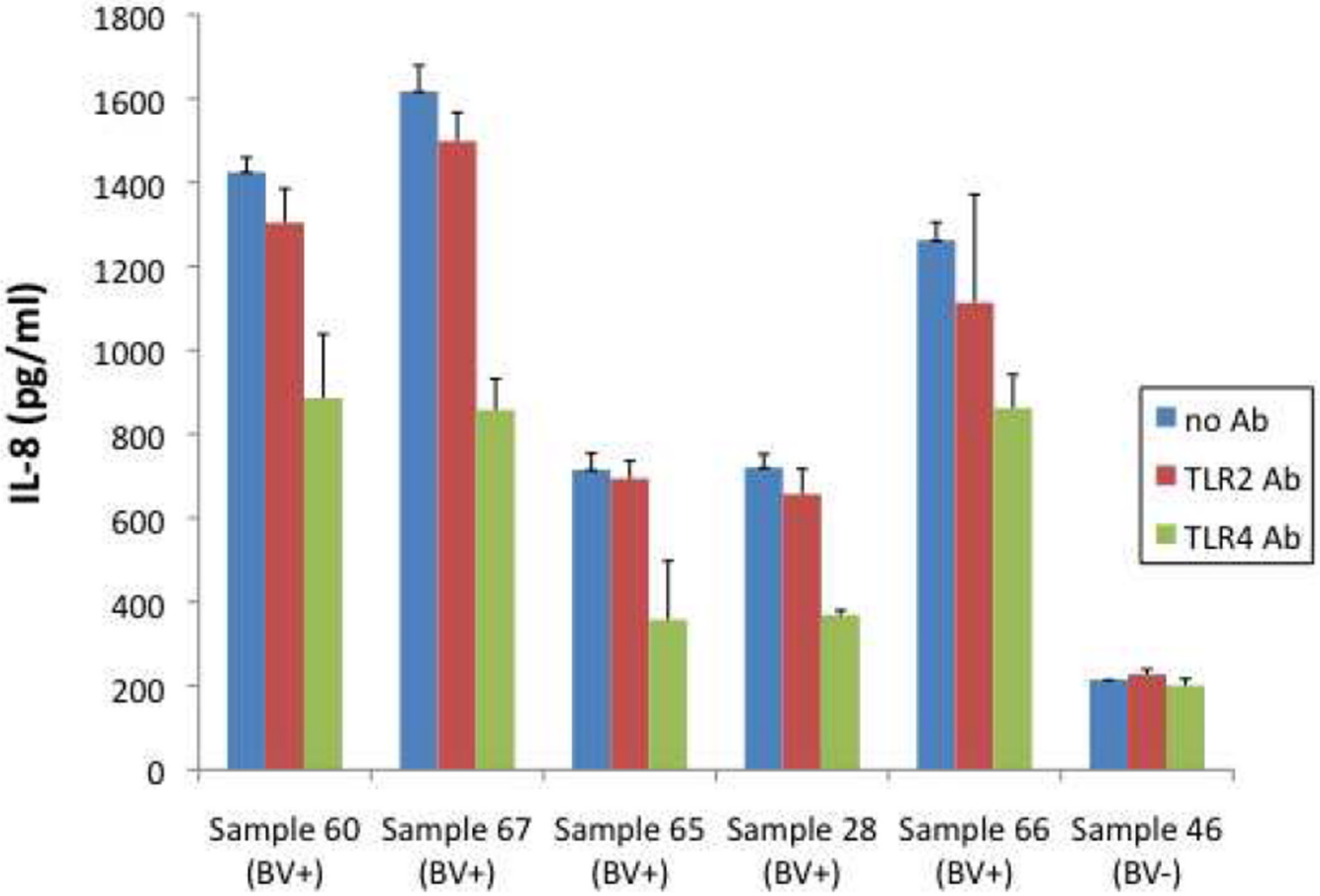

Figure 1. Mucosal fluids from women with BV stimulate cells to secrete IL-8

Mucosal fluids were collected from women by cervical-vaginal lavage. Lavage fluid was cleared by centrifugation, diluted and added to cultures of U937 monocytic cells along with antibodies to TLR2 and TLR4. The final dilution of fluid in cultures was 1:40. After overnight incubation, supernatants were collected and IL-8 levels measured (mean±SD of triplicate cultures).

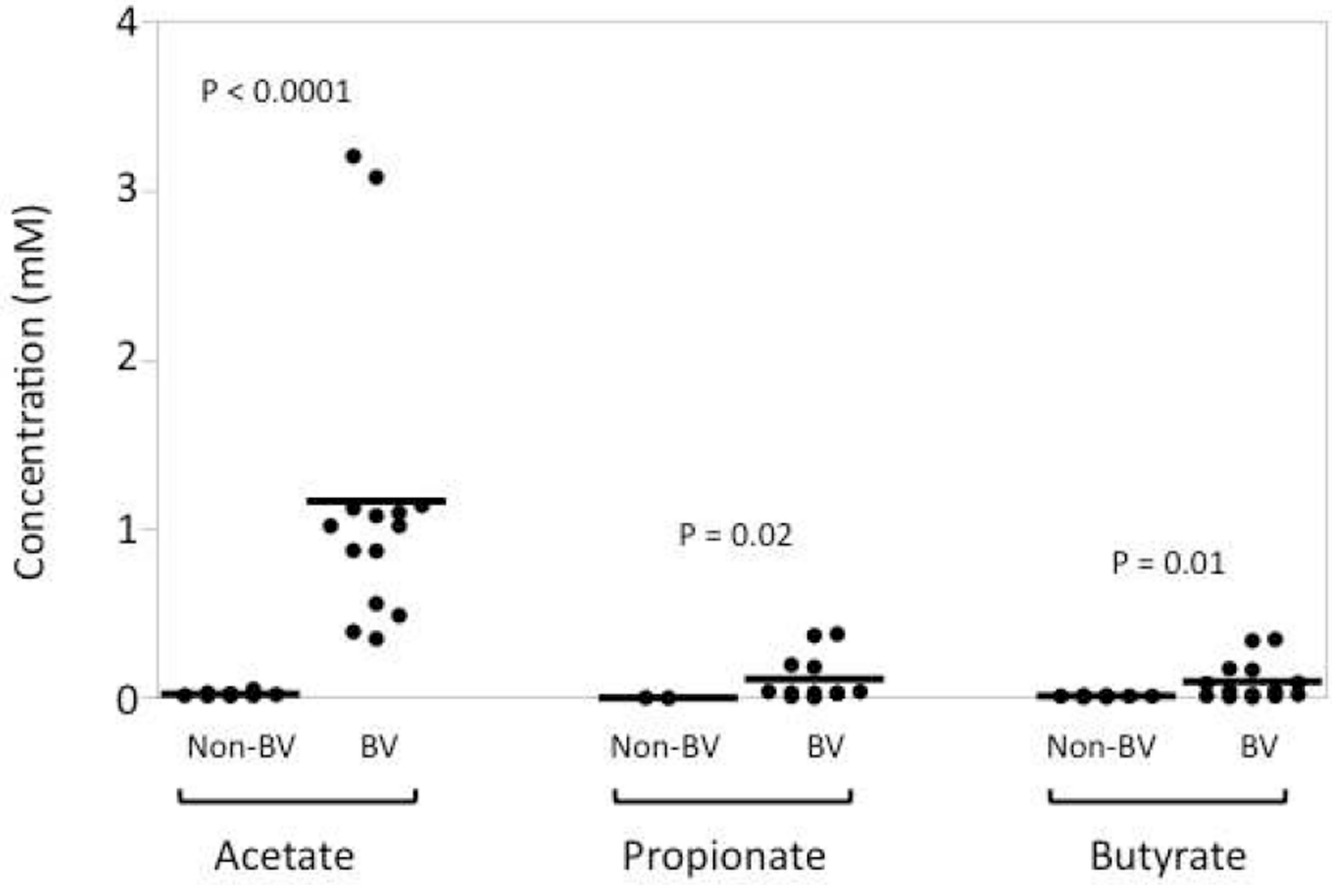

Figure 2. SCFAs are present in vaginal fluid

Vaginal fluids were collected from women with or without BV by cervical-vaginal lavage. The levels of acetate, propionate, and butyrate were measured by gas chromatography. P-values are given comparing non-BV and BV for each SCFA.

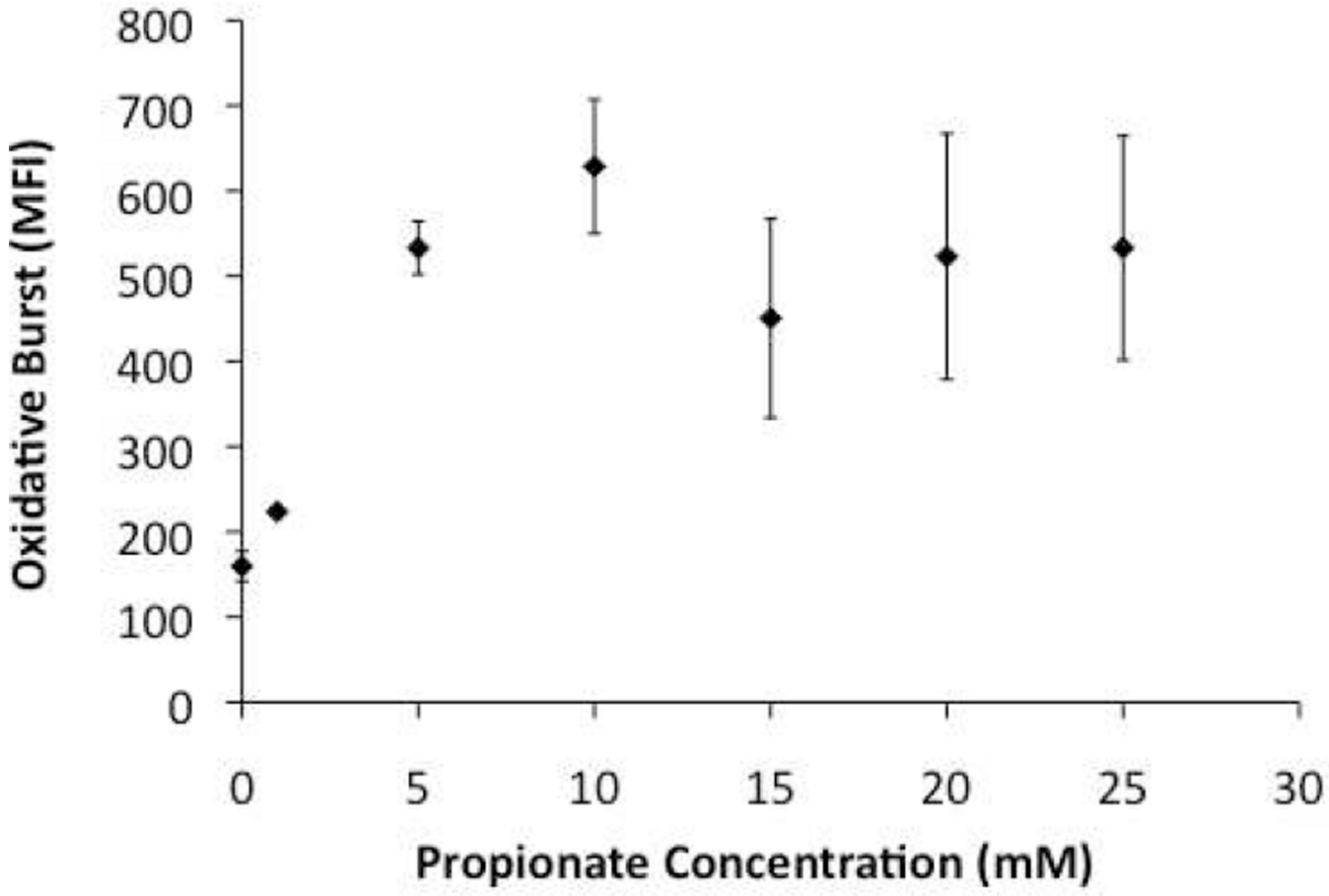

Figure 3. Propionate is a potent activator of neutrophils

Neutrophils were isolated from the blood of healthy donors. They were subsequently loaded with 0.1 μM of dichlorofluorescin diacetate (DCFH-DA) for 15 minutes at 37°C. The oxidative burst was measured as a function of the Mean Fluorescence Intensity (MFI) by flow cytometry.

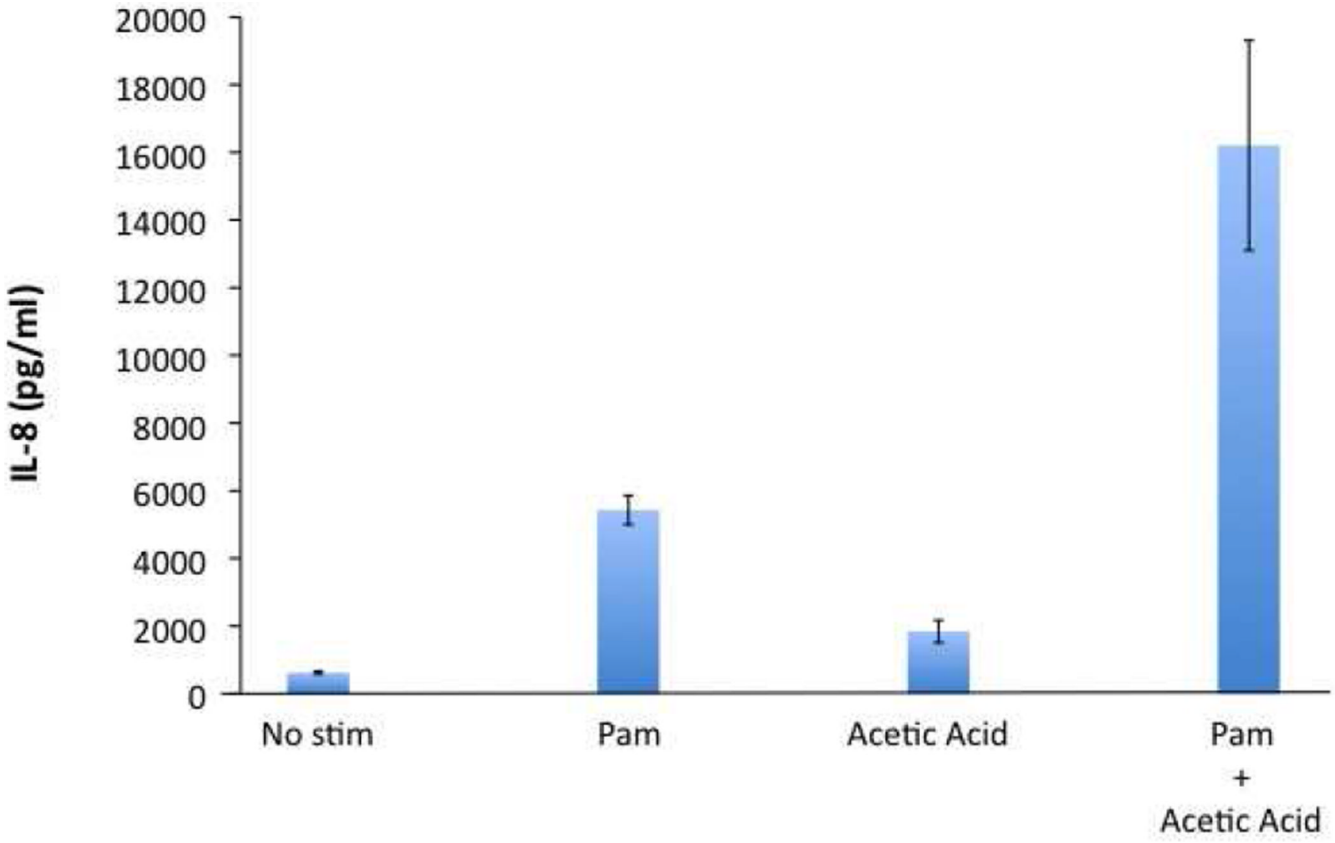

Figure 4. SCFAs enhance the ability of Pam2CSK4 to stimulate IL-8 production by PBMCs

PBMCs were collected from healthy donors. 250,000 cells were cultured with Pam2CSK4 (Pam @ 0.1 μg/ml) and/or Acetic Acid (2 mM), as indicated. IL-8 production was measured by ELISA.