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Innate Immunity in the Lower Female Mucosal Tract

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Abstract

The moist, nonkeratinized surfaces of mucosal tissues face a significant challenge with regard to protection of internal tissues from pathogenic invaders, a situation augmented by the fact that these sites are colonized by commensal microorganisms. The mucosa of the human reproductive tract is unique in that it must also balance the need for immunologic vigilance against pathogenic microorganisms and neoplastic cells with its critical role in reproduction, successfully creating an immune environment that tolerates allogeneic spermatozoa as well as the semi-allogeneic developing fetus. This article reviews the components of innate immunity that are functional in the cervicovaginal environment.

Keywords: Humoral immunity; Innate immunity; Bacteriocins; Cell mediated immunity; Defensins; Mucosal tissue; Commensal microorganisms; Cervicovaginal environment

Introduction

The female reproductive tract consists of a series of cavities lined by a highly vascular and glandular tissue called the mucosa. Mucosal tissues, nonkeratinized epithelia derived mainly of endoderm lined in epithelium, cover the surfaces of numerous body cavities, providing a moist interior lining which provides a hospitable habitat for a rich and dynamic microflora.

The mucosa of the female reproductive tract is unique in that even though it must, like all mucosal surfaces, maintain a vigilant and vigorous immunity against pathogenic intruders [1], it must also maintain an environment favorable to the reproductive process, tolerating the presence of allogeneic spermatozoa in the vagina, permitting their migration to the Fallopian tubes, and facilitating implantation of the semi-allogeneic fertilized ovum [2].

The ultimate goal of the lower female genital tract immune system is thus to effectively combat pathogens while at the same time modulate immune function in order to optimize fertility [3]. This delicate balance is accomplished by a complex interplay of local humoral, cell-mediated, and innate immunity [3].

Innate immune system defenses do not recognize single microbial epitopes and can therefore eliminate microorganisms rapidly, without the 3 to 5-day delay involved in mounting an antigen-specific response [4]. Bacterial cells or components of bacterial cell walls are nonspecifically recognized by innate immune factors which then eliminate the potential pathogens by multiple mechanisms, including antimicrobial factors secreted by local epithelial cells, ingestion by resident phagocytes, and activation of an inflammatory response [4].

Innate defenses, while common to all women, can nonetheless vary. Genetic polymorphisms specific to individual hosts can compromise the ability to mount an effective innate immune response in the vagina and thus increase susceptibility to sexually transmitted diseases [5]. For example, variations in the ability to produce adequate levels of interleukin-1 receptor antagonist and Toll-like receptor (TLR)-4 have been shown to influence the bacterial composition of the vagina [6,7]. Observed racial differences in composition of the endogenous microflora may also be attributable to the incidence of genetic polymorphisms in a given population [8].

Adaptive immune processes in the lower genital tract involve both B and T lymphocytes and their products, which recognize specific components of individual microorganisms [9].

Humoral immunity consists of B cells that secrete antibodies. Antibodies bind to microbial cells (thus preventing their entry into host cells), coat pathogens to induce phagocytosis, and stimulate other immune responses such as the complement pathway [10]. Components of humoral immunity are displayed in Table 1.

Cell-mediated immunity consists of various subsets of T lymphocytes which act in conjunction with other cytotoxic cells. Cell-mediated immunity in the lower genital tract eliminates primarily intracellular bacteria as well as virus-infected and tumor cells either by stimulating phagocytosis or by the direct action of cytotoxic, or natural killer (NK) and antibody-dependent killer (K) cells. Cytotoxic T cells (CD8+) kill virus-infected cells; stimulatory cells (CD4+) activate other types of cells including macrophages and B cells [9]. Components of cell-mediated immunity are displayed in Table 2.

Mucosa of the Female Reproductive Tract

The female reproductive tract is subdivided into three major compartments: the lower genital tract (comprised of the vagina and cervix), the transitional endocervix, and the upper genital tract (comprised of the endometrium and the Fallopian tubes) [11]. The lower reproductive tract is populated by a rich commensal microflora, abundant in anaerobic microbes [12], which blanket the surface of the vagina and ectocervix and assist in limiting the growth of more virulent microorganisms [1]. In most healthy reproductive age women, this population is dominated by *Lactobacillus* or other acid-producing species [8,11,13,14].

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Antibody	Source	Action
IgA	Secretory IgA produced by mucosal tissues and fallopian tubes [67]	Inhibits microbial adherence to surfaces Agglutinating resident microbes [68,69] Reducing the hydrophobic nature of the microbes [70] Blocking microbial adhesions [71]
		Rendering viruses ineffective. Aggregating virus particles [72]
		Neutralizing microbial toxins and enzymes. Blocking binding to target cells [73]
		Inhibiting penetration of antigen into the mucosa. Binding soluble antigens and facilitating removal by mucus flow [68]
		Opsonization of microbes for mucosal phagocytes. Coating pathogen with IgA [68]
		Stimulating antibody-assisted cell-mediated immunity. Inducing complement-independent antibacterial action of monocytes [74] Augmenting T-cell antimicrobial activity (specifically against T cells) [75]
		Promoting activity of innate immunity [76] Increasing microbe trapping of mucin by mimicking microbe receptor sites [77]
IgG	Transudate from blood stream [67] Actively transported [78] Locally produced [79]	Direct action against bacteria and viruses. Immune exclusion of HIV particles [80]; binding, agglutination of bacteria, complement activation [81]

Ig = immunoglobulin

Table 1: Components of Humoral Immunity in the Lower Female Reproductive Tract.

Cell Type	Distribution	Action
Langerhans/ dendritic cells	Abundant in vaginal and cervical mucosa	Present antigen to T cells (adaptive); phagocytize bacteria or virus particles (innate) [11]
Neutrophils	Abundant throughout female genital tract	Produce antipathogenic chemokines and cytokines (innate) [50]
T cells	Abundant within mucosal-associated lymphoreticular tissue within the lamina propria of cervix	Direct cytotoxic action(innate) or stimulation of other immune responses (adaptive) [11]
• CD8+	Most common epithelial T cells in FGT, cytotoxic	Kill virus- infected cells (adaptive) [82]
• CD4+	Less common, Stimulatory	Activate macrophages, B cells (adaptive) [82]
NK cells	Throughout FGT	Kill virus-infected host cells (adaptive and innate mechanisms) [83]
Macrophages	Abundant within mucosal-associated lymphoreticular tissue within lamina propria of cervix; most abundant phagocytes	Present antigen to T cell (adaptive) [11]

FGT = female genital tract; NK = natural killer

Table 2: Components of Cell-Mediated Immunity in The Female Lower Reproductive Tract.

The mucosal surface of the female genital tract is an intricate and dynamic biosystem containing multiple innate and acquired immune system components that provide an effective barrier to external pathogens and is therefore adapted in each area of the genital tract to the particular local needs. The endometrium as well as the endocervix is lined by a single-cell layered columnar epithelium which secretes mucus. Endocervical tubular glands and deep invaginations of the surface epithelium increase the surface area available for mucus-producing cells [15]. The secretory activity of the endocervical glands is regulated by estrogens [16].

The endocervix is a transition zone, where the columnar epithelium of the endometrium and endocervix meets the squamous epithelium of the cervix, the entry to the lower genital tract. The cervix as well as the ectocervix is lined by non-keratinized, stratified, squamous epithelium that sits atop a thick lamina propria and vascular submucosa and that continues into the vaginal epithelial layer.

The vaginal wall consists of three layers, an inner layer of fibrous connection tissue, a middle muscular layer, and an outer layer of mucosa, lubricated by secretions of the cervical glands.

The vaginal mucosa, in contrast to other mucosal surfaces that contain squamous epithelia that began as columnar in the developing fetus, lacks subdermal secretory glands; nevertheless it maintains significant secretory capacity through a network of intercellular pathways, making the entire vaginal surface a secretory structure [17]. The epithelial cells of the vagina also contain large numbers of estrogen receptors which respond to estrogen stimulation [16]. The ectocervix is structurally and immunologically similar to the vagina.

Throughout the female genital tract a mucus blanket coats the nonkeratinized epithelia, providing a formidable semi-permeable protective barrier to the exposed epithelial surface [18].

Components of Innate Immunity in the Female Genital Tract

Epithelial cells barrier

Organisms which manage to navigate their way through the mucus blanket that covers the vaginal surface will eventually reach the vaginal epithelia. The maturation and proliferation of the epithelium is under hormonal control, with maximum thickness occurring during peak levels of circulating estrogen [19]. In addition, the lower reproductive tract mucosa is unique in that it is responsive to both the direct and indirect effects of sex hormones. It responds directly to estrogen stimulation as well as to the cytokines and growth factors also stimulated by estrogen and produced by fibroblasts and migratory cells in the reproductive tract, enabling the lower female genital tract to exquisitely balance both immune and reproductive functions [20].

The cervicovaginal epithelium, together with its tissue-associated phagocytes (macrophages and neutrophils), represent the first line of cellular microbial defense to provide a physical and chemical barrier and act as sentinels, inducing other immune responses through the production of cytokines and chemokines [9]. Epithelia play an

important role in innate immunity by 1) providing a mechanical barrier to pathogen entry, 2) inducing death of infected cells by necrosis, apoptosis, or phagocytosis, 3) releasing protective cytotoxic substances, 4) producing a wide variety of signals of cell injury (e.g., chemokines, cytokines, prostaglandins, heat shock proteins) that both attract and activate leukocytes, 5) initiating and amplifying an acute inflammatory reaction, and 6) activating both humoral and cell-mediated immunity [12].

Rapid innate defenses against microbial infection necessitate a broadly specific recognition of invading bacteria, fungi, parasites, and viruses as well as endogenous ligands associated with cell damage [12]. Epithelial cells recognize specific patterns in the arrangement of conserved key molecules on the surface called microbe-associated molecular patterns (MAMPs) which include lipopolysaccharide (LPS), lipoproteins, peptidoglycans, lipoarabinomannans and oligosaccharides.

The MAMPs are recognized by pattern recognition receptors (PRRs) which are widespread on a wide variety of immune cells, particularly those involved in innate immunity (macrophages, neutrophils, dendritic cells) [4], but are also found throughout the mucosal epithelium [21]. One family of PRRs called Toll-like receptors (TLRs) play an important role in innate immunity [12]. Ligation of TLR by microbial products results in induction of an inflammatory immune response characterized by the production of cytokines and antimicrobial factors and resulting in facilitation of adaptive immune responses [22].

Toll-like receptors are transmembrane proteins which have in common similar structures All TLRs include first a cytoplasmic signaling domain. This domain is separated by a single, membrane-spanning domain from a third domain, a ligand-recognition domain. The ligand-recognition domain contains 19 to 25 copies of leucine-rich repeats, and provides a highly specific binding surface for the cognate ligand [23].

Ten different TLR receptors have been identified in the lower genital tract of human females; each responds specifically to a distinct MAMP [24]. TLRs are designed to recognize, with high specificity, various proteins, lipids, carbohydrates, and nucleic acids of invading microorganisms and are typically located on either plasma membranes or intracellularly. (Table 3) Recognition produces a rapid response by activating signaling cascades that trigger immune and inflammatory responses involving the production of pro-inflammatory mediators [25] and activation of the acquired immune response (both humoral and cell-mediated) [26].

Expression of TLRs, however, vary widely at different sites in the lower genital tract. Endometrial epithelial cells express TLRs 1-6, and 9; endocervical cells express TLRs 1-3, and 6 but not 4 or 5. TLRs 2 and 4 levels of expression vary widely. TLR 4 is not expressed by cervical or vaginal epithelial cells [27]. These studies suggest that the differential expression of Toll-like receptors in the female reproductive tract may be distributed in such a way as to maintain commensal microbial populations [28].

TLR expression is modulated by estrogen levels, with higher levels of expression of TLRs 2, 3, 4, 5, 6 , and 9 during the secretory phase as compared to other phases [12,29]. It has also been observed that the declining levels of estrogen after menopause are associated with a loss of TLR expression [30].

Although each TLR has a specific ligand, together they are collectively able to respond to a wide variety of bacterial, viral, fungal, and parasitic components. TLRs mediate the activation of epithelial cells by microbial products and may also regulate expression of antimicrobial peptides by epithelial cells [31]. The cervicovaginal

Receptor Number	Expression	Recognition site	Target Organism
TLR 1	Constitutive expression in epithelial cells of fallopian tubes, endometrium endocervix, ectocervix, vagina, uterine NK cells, vascular endothelial cells, and smooth muscle cells in cervical stroma as well as uterus [12]	LPS, PGN, flagellin	Bacteria [60,84]
TLR 2	Constitutive expression in epithelial cells of fallopian tubes, endometrium, cervix, vagina, smooth muscle cells of cervix and vagina, endometrial stromal cells, uterine NK cells. Highest levels in fallopian tubes and cervix [12]	LPS, PGN, flagellin (heterodimers in TLR1 or TLR6 complexes recognize microbial components)	Bacteria [85,86]
TLR 3	Constitutive expression in tissue samples from fallopian tubes, endometrium, cervix, and vagina. Other expression in epithelial cells of fallopian tubes, endometrium endocervix, ectocervix, and vagina. Also in stromal fibroblasts of vagina, endocervix and in uterine NK cells [12]	Nucleic acids (ds RNA)	Virus[60,87]
TLR 4	Constitutive expression in fallopian tubes, endometrium cervix, vagina, declines from fallopian tubes to vagina, although presence in epithelial cells debated [12]	LPS, heat shock protein 60, glycoinositolphospholipids of protozoa, viral envelope proteins, activates NK and other immune response [84]	Bacteria, Virus [84]
TLR 5	Constitutive expression in epithelial cells of fallopian tubes, endometrium, vagina, endocervix [12]	flagellin	Bacteria [88]
TLR 6	Constitutive expression in epithelial cells of fallopian tubes, endometrium, endocervix, ectocervix, vagina, uterine NK cells and stroma fibroblasts in vagina [12]	LPS, PGN, flagellin	Bacteria [12] Fungi [60]
TLR 7	Constitutive expression in epithelial cells of fallopian tubes, endometrium, cervix, vagina, uterine NK cells and endometrial stroma [12]	Nucleic acid (ss RNA)	Virus [23,89]
TLR 8	Constitutive expression in epithelial cells of fallopian tubes, endometrium, cervix, vagina, and endometrial stroma [12]	Nucleic acid (ss RNA)	Virus [23]
TLR 9	Constitutive expression in epithelial cells of fallopian tubes, endometrium, cervix, vagina, and endometrial stroma [12]	Nucleic acid (unmethylated deoxytidyl-phosphate-deoxyguanosine components of both bacterial and viral genomes)	Bacteria and viruses [90]
TLR 10	Constitutive expression in fallopian tubes, human NK cells; possibly in endometrial epithelia and stroma [12,91]	Ligand unknown	Recognition unknown [12]

ds = double-stranded; LPS = lipopolysaccharide; NK = natural killer; PGN = peptidoglycan; RNA = ribonucleic acid; ss = single-stranded; TLR = toll-like receptor

Table 3: Role of Toll-Like Receptors in the Lower Female Reproductive Tract.

environment has an ongoing need to appropriately respond to pathogens yet avoid a continual immune response to commensal organisms which would result in chronic inflammation. TLRs , with their specific ligand recognition across a wide variety of epithelial and immune cells provide a unique and diverse mechanism for pathogen recognition, allowing cells to recognize a wide range of MAMPs and create an immunological tolerance of commensal organisms in the lower genital tract but a nearly absolute intolerance of commensal flora in the endometrium and Fallopian tubes, thereby keeping the uterine environment sterile and avoiding immune responses which would jeopardize reproductive function [12].

The function of other intracytoplasmic PRRs, NOD-like receptors (NLRs), and RIG-I-like receptors (RLRs) remain to be characterized in the female genital tract.

Mucus

Apical epithelial cells also produce a hydrophilic layer of glycoprotein called glycocalyx that hydrates the luminal surface and covers it with a mucus blanket [1,11]. This mucus acts as a protective barrier, not only blocking the spread of microbes from the vagina into the endometrial cavity, but also by concentrating a variety of pathogen-fighting products [32]. Commensal organisms often use "hold fast" mechanisms that operate well in viscous fluids in order to remain in the tissue surface ecosystem; successful pathogens find ways to disrupt the mucus blanket and gain access to the epithelial cell surface [33].

One property of the mucus layer that contributes to defense is the ability to maintain an undisturbed layer of mucus at epithelial surface, even under provocation such as copulation [34]. Viscoelasticity prevents nearly all bacteria from reaching the epithelial surface, although some small viruses move readily through [34]. The glycocalyx, a highly viscous layer composed of cell-surface mucins anchored in the cell membrane and combined with secreted mucin fibers, is packed more closely and forms a final imposing barrier to prevent pathogen adherence [34].

The thickness of the mucus blanket is determined by the balance between the rate of secretion and rate of degradation and shedding. The gastrointestinal (GI)-tract mucus blanket varies between 50 and 450 μm in thickness [34]. Most foreign particulates, including conventional particle-based drug delivery systems, are efficiently trapped in human mucus layers by stearic obstruction and/or adhesion. Bacteria have great difficulty in accessing mucus-covered tissues, especially if the mucus blanket is thick and is moving at a considerable speed across the tissue surface [34].

Cervical mucus provides a physical barrier by creating a concrete interface between the internal and external environment, serving as the outermost fortification against foreign pathogens, toxins, and environmental particles [18]. Mucus contains largely water and glycoproteins called mucins [11] which create a heterogeneous mesh network of cross-linked bundles and entangled mucin fibers [18] in a watery interstitial fluid [35]. Though long believed to provide primarily a steric barrier, it has been recently recognized that mucus actually contains pores much larger than the diameter of most viral pathogens (pores as big as 1800 nm in diameter versus 100 to 200 nm for the virus particle) [35], with entrapment of the potential pathogens more a function of microadhesion than steric obstruction [35]. Entrapment by microadhesion provides rapid selective passage of some proteins and particles [18]. The capacity of bacteria to degrade mucin molecules is a substantial predictor of its invasive potential [35].

The mucus layer is continually secreted and shed [35], and most of the time is about 2000 times more viscous than water. As ovulation approaches each month, however, its viscosity decreases by about 95% [18], a substantial modulation of the cervicovaginal environment reflecting its hormonal control [36].

Commensal microflora

A primary component of the vaginal fluid are commensal microorganisms [17]. The microflora of most women is dominated by Lactobacilli or other acid producers; other common commensals include *Gardnerella vaginalis*, coagulase-negative Staphylococci, *Enterococcus* spp, *Ureaplasma urealyticum*, and *Escherichia coli* [37]. The presence of commensal microflora is known to have inhibitory effects with regard to significant growth of non-commensal organisms. This can be traced to at least four aspects of commensal populations: production of an acidic vaginal milieu, production of hydrogen peroxide, competition by commensal bacterial for adherence to the vaginal epithelia and production of antimicrobial products.

During the reproductive years, desquamated vaginal epithelial cells release glycogen which is commonly degraded by *Lactobacillus*, creating an acidic milieu which acts to restrict the growth of pathogenic microorganisms [38]. Lactobacilli metabolize glycogen, released by vaginal epithelial cells, into lactic acid, which in turn renders vaginal fluid acidic (pH 3.5 to 4.7) [11]. Lactic acid and low pH of vaginal fluid has been shown to exert selective antimicrobial activity against nonresident species of bacteria while sparing the commensal microbiota [39]. It was also observed that acidic cervicovaginal mucus (CVM) (acidified to approximately pH 4 by lactic acid produced continuously by anaerobic metabolism of Lactobacilli) trapped human immunodeficiency virus (HIV) while neutral CVM did not [40]. The trapping of HIV particles by mucoadhesion was also shown to be specifically associated with lactic acid [40]. The acidic milieu common to the vaginal vault of the adult female is most commonly attributed to the presence of *Lactobacillus acidophilus*.

For a long time, acidification of the vaginal vault by *Lactobacillus* and other acid-producing microbes was believed to be main effector of vaginal immunity. Lactic acid, however, is not produced only by Lactobacilli but also by vaginal mucosa [41], a significant source of lactic acid in the vaginal fluid [17]. There is no correlation between the number of Lactobacilli present and the pH of the vagina, which is not noticeably affected when *Lactobacillus* is absent in any significant numbers [17]. In addition, the vaginal vault of the newborn female, while sterile, contains substantial lactate and is acidic [42].

In addition, the use of non culture -dependent gene amplification techniques capable of producing molecular identification of component species has revealed that the traditional view of *L. acidophilus* as the obligatory foundation of an acidic vaginal environment is an oversimplification. Recent molecular studies have demonstrated that other *Lactobacillus* species, including *L. crispatus, L. gasseri, L. iners, L. gallinarum and L vaginalis*, are capable of providing a vaginal environment rich in lactic acid as well. In addition, other acid-producing species have been identified in some women lacking a dominance of Lactobacilli, particularly *Atopobium, Megasphaera*, and *Leptotrichia*, which are also associated with an acidic vaginal fluid [8,13,14].

Some have suggested that hydrogen peroxide production by Lactobacillus species is the primary effector of acidity in the vagina. Hydrogen peroxide (H_2O_2) is a broad-spectrum disinfectant and cervicovaginal fluid (CVF) is known to contain myeloperoxidase that

enhances pathogen inactivation by H_2O_2 [43]. Women with vaginal microbiota predominantly colonized with H_2O_2-producing lactobacilli may be less likely to be infected by a number of nonresident pathogens, including HIV-1, herpes simplex virus-2 (HSV-2), *Trichomonas vaginalis*, *Gardnerella vaginalis*, and Gram-negative microorganisms associated with bacterial vaginosis (BV) [43]. In fact, the incidence of BV was observed by multiple authors to be inversely related to colonization by H_2O_2-producing bacteria [44-47].

Hydrogen peroxide-producing lactobacilli have also been shown to be specifically associated with homeostasis of the vaginal mucosa [37]. Biological concentrations of H_2O_2 measured in vaginal fluid are toxic to many nonresident microbiota, which suggests these Lactobacilli may be more beneficial to the host than Lactobacilli that do not generate H_2O_2. However, the above studies were performed using in vitro cultures. A recent study has demonstrated that CVF or semen inactivates H_2O_2 bringing into question whether it has any physiological role in the female genital tract [43].

Lactobacillus spp. have also been shown to compete for adherence to the vaginal epithelium, thereby interfering with colonization by pathogenic organisms [48]. Finally, Lactobacilli are also known to produce bacteriocins, broad-spectrum antimicrobial peptides [49].

Vaginal secretions

The vaginal fluid, secreted at about 2 mL a day [17], participates in mechanical defense of the mucosal surface as secretions continually wash pathogens toward the vaginal opening. Vaginal secretions also trap potential pathogens [17]. The vaginal fluid contains epithelial cells and stromal cells, as well as immune cells that migrate into the uterus, cervix, and vagina [50]. It is also replete with antibodies. Immunoglobin A (IgA) and IgG are produced by local B cells or transduced into the vaginal fluid from the systemic circulation [20]. Secretory IgA (sIgA) is produced by plasma cells adjacent to submucosal glands [11].

The vaginal fluid also contains mucus as well as fluids from the endometrium, Fallopian tubes, and vestibular glands [51]. Concentrations of vaginal fluid components vary depending on sexual stimulation and the presence or absence of secretory inducers [52]. There are also hormonal influences on vaginal secretions, as estrogen stimulates the glycogen-rich intermediate cell layer of the mucosa which has the greatest metabolic and secretory activity [17], including variations in the numbers of exfoliated cells [52].

There are a wide variety of organic molecules in the vaginal secretions. Lactic acid is a main component, but the vaginal fluid also contains multiple aliphatic acids, alcohols, glycols, and aromatic compounds [53], as well as urea and at least 339 proteins [54], many of which are the products of the innate and adaptive immunity systems in the vaginal environment.

Neutrophils, macrophages, and NK cells contribute numerous defense effector molecules, including cathelicidin, TLKs, calprotection, defnesisns, and SLP1 [11,12,50] Epithelial cells, when confronted by a pathogenic invader, secrete a variety of defense-effector molecules [32]. Cervicovaginal epithelial cells secrete numerous cytokines, chemokines, and other peptides which, with other components of the cervicovaginal milieu, comprise the vaginal fluid. It is now known that the vaginal secretions contain numerous antimicrobial substances, such as defensins, cathelicidin, lactoferrin, lysozyme, calprotectin, elafin, and secretory leukoprotease inhibitor (SLPI), and chemokines secreted from serous cells in submucosal glands [11], whose contributions to cervicovaginal immunity are only recently being understood.

Antimicrobial molecules

These cationic peptides of innate immunity are slowly being recognized as the principal effector molecules of cervicovaginal immunity. Most antimicrobial peptides and proteins are broad-spectrum microbicides that target Gram-positive and Gram-negative bacteria as well as fungi and some enveloped viruses by a variety of different mechanisms [55]; there is evidence that these secretory products may help regulate both innate and adaptive immunity by acting as both signal molecules and effectors [32]. Although a plethora of antimicrobial products are produced by the innate immune constituents in the cervical-vaginal tract, they have a tendency to share several chemical properties, including amphipathicity (spatial separation of polar and nonpolar residues), as well as cationicity (maintenance of a positive charge at physiological pH), facilitating insertion into microbial membranes. Thus, they appear to play multiple roles in host defense [11].

Antimicrobial peptides are constitutively expressed by epithelial cells and regulated by various inflammatory mediators and bacterial products [32]. Epithelial cells and granulocytes synthesize additional antimicrobial peptides [56]. Epithelial cells produce functional antimicrobials; granulocytes in early stages of differentiation produce antimicrobials which are packaged into granules, with antimicrobials later released during activation [57]. The primary antimicrobial components of innate immunity are discussed briefly below and elaborated in more detail in Table 4.

Defensins are an important component of innate immunity at the mucosal surface in the lower genital tract. They are small, positively charged peptides that bind to the negatively charged bacterial surface and disrupt bacterial membranes resulting in lysis [58]. Defensins are broad spectrum antimicrobials, with efficacy against both Gram-negative and Gram-positive bacteria, as well as fungi, protozoa. and enveloped viruses and contain six cysteine residues forming three sulfide bridges [1].

Human alpha-defensins (HNP 1-4) are produced by neutrophils [59], while alpha human defensins (HD) HD 5 and 6 are expressed by epithelial cells of the female genital tract [56,59]. Human beta defensins, are produced by various epithelial cells of the female reproductive tract [56,60]; some constitutively, some are induced by microbial components or by pro-inflammatory cytokines [32].

Secretory leukoprotease inhibitor is produced by macrophages and epithelial cells and inhibits proteolytic activity of neutrophil elastase, cathepsin G, trypsin and chymotrypsin and also exhibits antimicrobial activity [32]. High levels of SLPI in vaginal fluid have been associated with reduced rates of perinatal HIV-1 transmission [61]; low levels are associated with the presence of genital tract infections [62].

Mannose binding lectin (MBL) recognizes carbohydrate patterns on the surface of a variety of pathogenic microorganisms, including bacteria, viruses, protozoa and fungi. Binding of MBL to a microorganism results in activation of the complement system as well as opsonization [63].

Elafin is expressed by epithelial cells in the CVM and inhibits activity of neutrophil elastase and proteinase 3 (PRTN3) [32]. Cathelicidins (LL-37) are components of neutrophils, but are also found in various squamous epithelia as well as keratinocytes in inflamed skin (inflammatory mediators implicated as the regulatory of LL-37 expression [64]. A specific cathelicidin LL-37, found in the vaginal fluid, is the end result of processing induced by the act of sexual

Effector Molecule	Source	Activity	Target Organisms
Lysozyme 13 µg/mL in vaginal fluid, 1 mg/mL in mucus plug [92]	Secreted by serous cells in submucosal glands [11]	Cleaves bonds in peptidoglycan component of cell walls, cationic disruption of microbial membranes [93]	Gram-negative bacteria [93] Weak at normal concentrations against bacteria, some antiviral (HIV) [94]
Lactoferrin 1 µg/mL in vaginal fluid, 100 µg/mL in cervical plug [92]	Secreted by serous cells in submucosal glands [11]*	Sequesters iron, also disrupts microbial membranes [11] Inhibits cellular fusion and entry by virus [95]	Gram negative bacteria [93] , virus [95]
Calprotectin 34 µg/mL [11]	Component of neutrophils, monocytes and keratinocytes [95]	Sequesters zinc [11]	Inhibits growth of fungi and yeast [96]
Human Alpha Defensins HPN 1,2,3,4 about 2 µg/mL [59]	Synthesized in bone marrow [97]	Comprise contents of phagocytic vacuoles that effect microbicidal activity [98] increase the production of TNF and IL-1	Bacteria, fungi, viruses [11]
5 HD5 10 to 40 ng/mL	Columnar epithelium of endocervix [99]	Binds electrostatically to negatively charged microbial particle, forming pores in cell membrane and eventually causing lysis [100]	Bacteria, fungi, viruses [11]
Human Beta Defensins	vaginal epithelial cells leukocytes [11] HBD-1 is produced constitutively in mucosa [11] Human defensins HBD2 and 3 are induced at inflamed sites [11]	Antimicrobial activity, inhibition of HIV-infectivity of immunocompetent cells, chemoattraction of T cells immature dendritic cells B cells neutrophils and macrophages [101,102]	Gram-positive or Gram- negative bacteria, mycobacteria. yeast, enveloped viruses viral (in vitro) [103]
SLPI 10 to 100 µg/mL in vaginal secretions, as high as 1000 µg/mL in cervical mucus plug [11]	Found in epithelial secretions, also produced by macrophages [11]	Blocks action of hostile enzymes released by invading organisms [3] Suppresses central transcription factor of inflammatory response [104]	Bacteria and fungi (weak at normal concentrations) [11] Antiviral (HIV-1) [61]
Surfactin Protein A	In vaginal secretions [105,106]	Facilitates phagocytosis of microbes, increases chemotaxis, increases oxidative burst by phagocytes, modulates pro- inflammatory cytokine production by immune cells [105-107]	Bacteria, viruses [105,106]
Surfactin Protein D	In vaginal secretions [108]	Increases permeability of bacterial cell membranes [107] stimulates oxygen radical release, contributing to destruction of virus [109]	Bacteria [107], viruses [108]
MBL	Transudate from liver	Facilitates complement activation and opsonization by binding to pathogenic microbes [63]	
Elafin	Epithelial cells	Antimicrobial , inhibits inflammation-related tissue damage by blocking elastase [3]	
Cathelicidin (LL-37) 1 µg/mL[11]	Components of neutrophils[11]	Postcoital processing from hCAP18 precursor to functional form, targeting specifically microbes which may have been introduced by intercourse [110]	Bacteria and fungi [11]

HBD= human beta defensin; hCAP-18 = human cationic antimicrobial protein; HD5 = human alpha defensin 5; HIV = human immunodeficiency virus; HPN = human alpha defensin; IL-1 = Interleukin-1; MBL = mannose binding lectin; SLPI = secretory leukocyte protease inhibitor; TNF = tumor necrosis factor

Table 4: Defense Effector Molecules in Innate Immunity.

intercourse and is characterized by a broad spectrum of activity against both bacteria and fungi [11].

Cystatins are inhibitors of microbial cysteine proteases [65]. Cystatins A, C, and S have also been observed to have antimicrobial activity against bacteria and viruses [32].

Calprotectin is a heterodimeric protein component of neutrophils, monocytes and keratinocytes, also known as leukocyte protein L1 and calgranulin. At biological concentrations calprotectin inhibits the growth of fungi and bacteria in vitro by sequestering zinc [11].

Lysozyme cleaves the bonds in peptidoglycan, a molecules ubiquitous in microbial cell walls, allowing it to disrupt microbial membranes. It may act in synergy with other antimicrobial components of epithelia secretions [11].

Lactoferrin is an abundant component of some epithelia as well as neutrophil granules which eliminate microbes directly as well as by sequestration of iron [11].

Virtually all of the secretory products of the innate immune system are considered to be estrogen dependent. Menopause, with its declining levels of estrogen, is characterized by a parallel decline in the levels of secretory products in the vaginal fluid [3].

Discussion

Innate immunity in the female reproductive tract is of much current interest as a fascinating and interconnected array of multifunctional components of immunity are being revealed. The abundance and diversity of antimicrobial factors in the vaginal milieu would suggest a synergistic effect. Products of both host cells as well as the resident microflora, each molecule contributes a highly specific immunological function. As a family of immune effectors, however, together the molecules produce a formidable, barrier. An intact mucosal epithelium, for example, in conjunction with innate immune mechanisms and adaptive immune functions prevents 99% of HIV exposures from producing infection--a success factor far more impressive than any HIV vaccine currently in production [66].

The highly developed innate immune complex in the unique CVM, in addition, permits preservation of immune surveillance and anti-pathogen functions while maintaining a favorable environment for reproduction.

Epithelial cells, responding to both estrogen and the multiple effect or molecules that are simultaneously stimulated by estrogen, enable bidirectional communication between epithelial cells that effectively regulate both reproductive and immune function in order

to maintain the delicate balance between immunity and reproduction, between pathogenic and commensal microorganisms and sperm and the developing fetus [20]. Further understanding of the regulation of expression and activity of the multitude of microbial peptides that make up a significant part of innate immunity in the lower genital tract will facilitate strategies to further improve female reproductive tract health.

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