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Host Defense Mechanisms in the Male Genital Tract: Role of Innate Immunity*

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ABSTRACT

Innate immunity is the first defence to infection. The submucosa of male genital tract is rich in lymphocytes, neutrophils, macrophages, and lymphatic tissue, which on microbial invasion produce antimicrobial peptides and proteins *viz.*, defensins, cathelicidins, lactoferrin, lysozyme and various other proteins. Innate immunity is quick responsive to microbial invasion, controls the infection in the early period of exposure to pathogenic microorganisms and is phylogenetically ancient than adaptive immune system. The innate immune system is well equipped with cellular and molecular armaments for immediate protective response against the invasion of pathogenic microorganisms. The cellular components are lymphocytes, monocytes, neutrophils, macrophages and natural killer (NK) cells. The molecular components include mucins, defensins, cathelicidins, protease inhibitors, serpins, lysozyme, α-macroglobulin, cystatins, Toll-like receptors (TLRs), lactoferrin, interleukins, complement system, bactericidal/permeability increasing protein (BPI), lipopolysaccharide binding protein (LBP), nitric oxide (NO), hydrogen peroxide (H_2O_2) and collectins. Recent studies have demonstrated the expression and localization of various peptides and proteins having antimicrobial activity against a broad spectrum of bacteria, viruses and fungi in the male genital tract. These antimicrobial peptides and proteins, with their host defense mechanisms in the male genital tract are discussed briefly in this review.

Key words: Innate Immunity, Defensins, Cathelicidins, Protease Inhibitor, Host defense proteins.

Abbreviations:

ALP, antileukoprotease; **TLR**, toll-like receptor; **LBP**, lipopolysaccharide binding protein; **CMI**, cell-mediated immunity, **ELISA**, enzyme-linked immunosorbent assay; **PCI**, protein C inhibitor; **MBL**, mannan-binding lectin; **PAMP**, pathogen associated molecular pattern; **LPS**, lipopolysaccharide; **PRR**, pattern recognition receptor; **HI**, humoral immunity; **SLPI**, secretory leukocyte protease inhibitor; **IL**, interleukin; **LAM**, lipoarrabinomannan

Mammalian genital tract is in direct communication with the external environment and is exposed to numerous bacterial, viral, fungal and parasitic invasion. Yet we co-exist with these microorganisms without chronic inflammation or tissue damage mainly due to the development of three types of immune system *viz.*, 1) innate (or natural) immune system, 2) adaptive (or acquired) immune system and 3) cell-mediated immune system in the genital tract. Histologically the submucosa of the genital tract is rich in lymphocytes, neutrophils, macrophages, and lymphatic tissue (Radicioni, 1986) which in response to microbial invasion produce antimicrobial/bactericidal peptides and proteins *viz.*, defensins (Lehrer *et. al.*, 1993), cathelicidins and numerous host defense proteins (Zanetti *et. al.*, 1997, Halls *et. al.*, 2002), which form an integral component of the innate immune system. Currently the innate immunity of the male genital tract is under active investigation due to the

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increasing incidence of Acquired Immune Deficiency Syndrome (AIDS) effect of Human Immune Deficiency Virus (HIV) infections. The innate immune system is quick responsive to microbial invasion, controls the infection in the early period of exposure to microorganisms and is phylogenitically ancient than the adaptive immune system (Hoffmann *et. al.*, 1999). When innate immune system is overwhelmed by invading microorganisms, the antibacterial peptides defensins and cathelicidins stimulate the adaptive immune system (Hoffmann *et. al.*, 1999), (Yang *et. al.*, 2002), resulting in activation of T-lymphocytes and B-lymphocytes. The former regulate cell-mediated immunity (CMI), while the latter synthesize antibodies of different isotypes of immunoglobulins *viz.*, IgG, IgA, IgM, and SIgA, which eliminate the infection through humoral immune defense mechanisms. Our present knowledge of the innate and adaptive immune defense mechanisms of the male genital tract against bacterial, viral, fungal and parasitic infection is limited. Earlier, the origin of bovine seminal plasma immunoglobulins and their role in humoral immunity of the male genital tract has been reported (Kulkarni and Dhande, 2003). A brief account of the recent advances in the innate immunity of the male genital tract is given here.

Modern Concepts of Innate Immunity

Innate immunity is the foundation of the host defense in mammalian species and is defined as "the collective functions of cells, serum proteins, cytokines and other barriers to infection in absence of specific antigen stimulated adaptive immunity" (Brown *et. al.*, 1994). During the past few years extensive studies on the mammalian innate immunity (Brown *et. al.*, 1994; Holmoskov *et. al.*, 1994; Boman 1995; Malhotra *et. al.*, 2000; Borregarred *et. al.*, 2000; Gadjeva *et. al.*, 2001; Ajaneway and Medzhilov, 2002; Beutler, 2003), have revealed that mammalian innate immunity is well equipped with various cellular and molecular armamentarium for immediate protective response against the invasion of pathogenic microorganisms. The cellular components of this armamentarium are lymphocytes, macrophages, neutrophils, monocytes and natural killer (NK) cells. The molecular components include mucins, defensins, cathelicidins, protease inhibitors, serpins, α_2-macroglobulin, secretory leukocyte proteases inhibitors (SLPI), cystatin C, lysozyme, Toll-like receptors (TLRs), lactoferrin, interleukins (ILs), complement, bactericidal / permeability increasing protein (BPI), lipopolysaccharide binding protein (LBP), nitric oxide (NO), hydrogen peroxide (H_2O_2) and collectins (Lehrer *et. al.*, 1993; Hoffmann *et. al.*, 1999; Holmoskov *et. al.*, 1994; Beutler, 2003; Adrem and Ulveitch, 2000; Armstrong, 2001; O'Neille, 2002; Mastallo and Lambris, 2002).

Innate immune system is the first line of mucosal defense in the host, which controls the infection in the early period of microbial invasion. The immediate immune response is mediated mainly by the neutrophils and macrophages, which phagocytose and kill the pathogens. In addition, a wide variety of biochemical compounds of the outer and inner membranes of the pathogenic bacteria, such as lipopolysaccharides, (LPS), peptidoglycans, lipoteichoic acids, lipopeptides, lipoarabinomannan (LAM), and bacterial DNA (Adrem and Ulveitch, 2000), stimulate the innate immune response immediately after microbial invasion, resulting in the biosynthesis of antimicrobial peptides and proteins (Table –1). Antimicrobial peptides are also known as endogenous peptide antibiotics with specificity for innate immunity of that animal (Boman, 1995). Inorganic disinfectants such as nitric oxide (NO) and hydrogen peroxide (H_2O_2) are also produced, which help to control the growth of microorganisms (Hoffmann *et. al.*, 1999; Yang *et. al.*, 2002). Recent studies (Agerberth *et. al.*, 1995; Malm *et. al.*, 2000; Liu *et. al.*, 2001; Li Peng *et. al.*, 2001) have demonstrated the expression of various antimicrobial peptides and proteins, having antimicrobial activity against a broad spectrum of bacteria, viruses, fungi, and parasites in the male genital tract. These antimicrobial peptides and proteins with their role in host

fense mechanisms of the male genital tract are cussed briefly.

ntimicrobial Peptides and Proteins

A variety of cationic antimicrobial peptides d proteins have been identified and aracterized from the mammalian genital tract, which contribute to host defense against athogenic microbial invasion and colonization. All the known antimicrobial peptides are grouped into one of the two major groups *viz*., cysteine and arginine rich α and β defensins and the heterogenous group of cathelicidins (Zanetti *et. al.*, 1997). Numerous antimicrobial peptides viz., Bac-5 and Bac-7 from bovine neutrophil granules (Gennaro *et. al.*, 1989), FALL-39 from the pig intestine and bone marrow (Agerberth *et. al.*, 1995), LL-37 from human phosopholipid membrane (Oren *et. al.*, 1999), hCAP-18 from the epithelium of human epididymis and seminal plasma (Malm *et. al.*, 2000), ESC-42 from the primate epididymis (Liu *et. al.*, 2001), Bin-1 b from the epididymis of the rat (Li Peng *et. al.*, 2001), having antimicrobial activity against a variety of pathogenic microorganisms have been identified and characterized recently. These antimicrobial peptides could contribute towards the host defense mechanisms of the innate immune system. Mammalian antimicrobial peptides and proteins in different species, with their activity and host defense peptides and proteins expressed in the male genital tract with their functions are presented in (Tables 1 and 2) respectively.

Defensins

The pioneering research of Lehrer and coworkers (Lehrer *et. al.*, 1993; 1975; 1980; 1986; 1988; 1989 and 1991) and Eisenhauer *et. al.*, (1989), on the origin, structure isolation, purification, characterization and microbicidal and cytotoxic activities of defensins in various mammalian species has contributed significantly towards the advancement of present knowledge of defensins and their role in the innate immunity. Lehrer *et. al.*, (1993) first used the term "defensins" to purified microbicidal peptides MCP-1 and MCP-2 from the rabbit lung macrophages and to six more microbicidal peptides from the rabbit polymorphonuclear leukocytes.

Defensins are a group of 2 to 6 kDa cationic microbicidal and cytotoxic peptides having three pairs of intramolecular disulfide bonds. On the basis of their size, structure and pattern of disulfide bonding, the mammalian defensins are classified in to α, β and θ defensins (Yang *et. al.*, 2002). α-defensins are produced by neutrophils, while β-defensins are mainly expressed by epithelial cells of the skin, kidneys and treachea bronchial lines and can be released after microbial invasion (Yang *et. al.*, 1999). β-defensins have been reported in all the species studied so far and are expressed in the male genital tract (Halls *et. al.*, 2002) and play an important role in the innate immunity. α-defensins have been identified in human, monkey and rodents, which are abundant in neutrophils and macrophages. Their expression in the male genital tract has not been reported. The θ defensins have been identified in granules of neutrophils and monocytes of Rhesus monkey and are microbicidal for bacteria and fungi at low molecular concentration (Tang *et. al.*, 1999). The classification, size and biological activities of defensins related to host defense are presented in (Table-3). β-defensins are expressed in human testis, prostate, Sertoli cells and Leydig cells in the mouse and their involvement in innate immune defense of the male genital tract has been reported (Halls *et. al.*, 2002).

Defensin like epididymis specific protein Bin-1 b in the caput epididymis of the rat and its implication in the innate immune defense of the epididymis against pathogenic microorganisms has been reported (Li Peng *et. al.*, 2001). Using Northern blot analysis Bin-1 b mRNA was detected only in the epididymis and not in the testis and other tissues. The expression pattern of Bin - 1 b indicated that it may be involved in sperm maturation and protection in the epididymis and also preventing the entry of microorganisms in the testis. Rat Bin-1 b is a member of HE_2 family of primate epididymal proteins (Osterhoff *et. al.*,

Table 1 : Antimicrobial peptides and proteins of mammalian neutrophils

Peptides/Proteins	Molecular Weight (kDa)	Species	Antimicrobial activity
PEPTIDES:			
Defensins	4.00	Human, rabbit, rat, guinea pig	G+, G-, F, EV, P
Cathelicidin-derived peptides α-helical	3-5	Human, pig, rabbit, cow, sheep, mouse	G+, G-, F, P
Proline and Arginine rich	4.5-9	Cow, sheep, pig	G-, G+, EV
Trp-rich	2.00	Cow	G+, G-, F
One-disulfide	1.6	Cow, sheep	G+, G-
Two-disulfide	2.00	Pig	G+, G-, F
PROTEINS:			
Lactoferrin	78.00	Human, rabbit, cow	G+, G-, F
BPI Protein	60.00	Human, rabbit, cow	G-
Lysozyme	14.5	Human, horse	G+

G bacteria (+ and – refer to Gram staining); F, fungi; EV, enveloped viruses; P, parasites.
Source: Zanetti *et. al.* (1997).

1994, Fromehlicn and Yang, 2002). HE_2 has strong antibacterial activity against ***E.coli*** indicating a role for HE_2 in the defense against retrograde bacterial invasion of the male genital tract. These observations further indicate a newly recognized role of epididymis as a protector of the testis against microbial invasion. β-defensins link innate immune system with the adaptive immune system (Yang *et. al.*, 1999). Defensins contribute to the induction of adaptive antimicrobial immune response through chemotactic mobilization of immunocompetent leukocytes (Tang *et. al.*, 1999).

Recently the expression of antimicrobial defensins in the human, mice and rat male reproductive tract and their potential role in the innate immunity has been reported (Emmanulle *et. al.*, 2003). Defensins were expressed in Sertoli cells, spermatogonia, spermatocytes, late spermatids, ejaculated spermatozoa and seminal plasma. These observations indicate the involvement of defensins in the regulation of innate immunity in the male reproductive tract.

The role of defensins in the regulation of innate immunity in the male genital tract (Emmanulle *et. al.*, 2003; Porter *et. al.*, 2005; Buck *et. al.*, 2006) has been reported recently. Using reverse transcriptase polymerase chain reaction and immunohistrochemistry, Emmanulle et al (2003) reported the expression of defensins in the male reproductive tract of rats, mice and humans. Defensins were expressed in the rat testis, epididymis and isolated testicular cells. In the mice and humans defensins were expressed in the testis and epididymis. All classes of defensins were expressed in the seminiferous tubules. Spermatogonia expressed only α – defensins, in relatively high levels. This study has established that the male genital tract produces defensins, which could play important protective role in innate immune defense mechanism against pathogens. More recently, the relationship between the structure and antimicrobial function of defensins has been reported (Nagraj, 2006).

Cathelicidins

Cathelicidins are a group of structurally heterogenous cationic antimicrobial myeloid peptides of molecular mass 3-5 kDa (Zanetti *et. al.*, 1997) and have been identified and characterized in and various other mammalian species. Recent studies have demonstrated the expression of cathelicidins in human testis and epididymis, their human (Malm *et. al.*, 2000; Liu *et. al.*, 2001; Lehérer *et. al.*, 1975), bovine (Gennaro *et. al.*, 1989) presence in the seminal plasma in high concentrations and their binding to the spermatozoa. The expression of a 39 residue human antimicrobial peptide FALL-39 of

cathelicidin family in human testis and bone marrow has been reported (Agerberth *et. al.*, 1995). These organs are rarely infected. In basal medium FALL-39 was highly active against ***E.coli*** and ***B.megaterium***. Human LL-37 is an antimicrobial peptide of cathelicidin family (Oren *et. al.*, 1999). This peptide has also been named hCAP-18. *In vitro* studies indicated that LL-37 is cytotoxic to bacterial cells and is resistant to proteolytic degradation. The expression of LL-37 / hCAP-18 an antimicrobial peptide by gene transfer in mice resulted in augmentation of the innate immune response (Bals *et. al.*, 1999). These observations support the hypothesis that mammalian antimicrobial peptides protect against pathogenic microorganisms *in vivo*. Using immunophistochemical analysis, strong expression of cathelicidin, the human antimicrobial protein (hCAP-18) in the epithelium of human epididymis has been reported. Using specific enzyme-linked immunosorbant assay (ELISA), higher levels of hCAP-18 were detected in seminal plasma of healthy donors than in the blood plasma. Flow cytometry, immunohistochemistry and ELISA measurements revealed an estimated 6.6 x 10^6 molecules of h CAP-18 were bound per spermatozoa (Malm *et. al.*, 2000). These results indicate a key potential role for h CAP-18 in the maintenance of integrity of the male genital tract against pathogenic microorganisms. The binding of h CAP-18 to spermatozoa may indicate the important role for h CAP-18 in sperm protection against microorganisms during fertilization and conception. The expression of h CAP-18 in testis, prostate or seminal vesicles was not detected in his study.

Mucins

Mucins are protective physical barrier proteins, synthesized and secreted in the mammalian male genital tract, and contribute significantly towards the host defense of the genital tract against the invasion of pathogenic microorganisms.

Structurally mucins are glycoproteins with high content of O-linked oligosaccharides.The carbohydrate content of mucins is more than 50% and there is the presence of repeating amino acid sequences, known as tendem repeats in the center of their polypeptide chains. The biosynthesis, structure, properties and functions of mucins have been reviewed recently (Murray, 2002). Both secretory and membrane bound mucins occur which form protective physical barrier on the epithelial surfaces and could prevent colonization of pathogens in the genital tract. The membrane bound mucins participate in various cell-cell interactions and are resistant to proteolytic degradation. Various bacteria and viruses have been shown to bind muscins (Cohen and Lanz, 1995) and their attachment to epithelial cells can be prevented by the dynamic processes of outward secretion and mucus shedding (Halls *et. al.*, 2002). The effects of mucins- secretory IgA complex on *S.aureus* and *P.aeruginosa* were investigated (Bisenbrock *et. al.*, 1991). The results indicated that such an interaction may facilitate microbial clearance by preventing the colonization on the mucosa.

Protease Inhibitors

For colonization and penetration of a potential host, pathogenic microorganisms should be able to cross the mucosal and integument barriers and escape safely from the immune defenses of the host. Microbial proteases are important essential virulence factors, which help these processes. Host defense inhibitors potentially contribute to innate immunity by inactivating the protease virulent factors of pathogenic microorganisms (Armstrong, 2001). Recent studies have demonstrated the expression and localization of various protease inhibitors such as α_2-macroglobulin (Cheng *et. al.*, 1990; Zhu *et. al.*, 1994), cystatin C (Trusuta *et. al.*, 1993), secretory leukocyte protease inhibitor SLPI (Ohlsson *et. al.*, 1995), antileukoprotease ALP (Hiemestra *et. al.*, 1996), serine protease inhibitor (Aravindan *et. al.*, 1997), and protein C inhibitor (Kise *et. al.*, 2000) in the male reproductive tract. More recently the important contribution of

Table 2: Antimicrobial/host defense peptides and proteins expressed in the male reproductive tract

Peptides / Proteins	Site of Expression	Known Functions
Mucins	Testis, epididymis, prostate	Mobile barrier to prevent entry of bacteria, viruses, toxins
β - Defensins	Testis, epididymis, prostate, seminal vesicles	Kill *Staphylococcus, Enterococcus, Escherichia coli,* Candida, chemotaxis
Cathelicidins	Epididymis, neutrophils	Kill *Staphylococcus, E.coli, Pseudomonas* Chemotaxis, sperm binding
Bin 1 b / HE_2	Epididymis, Sertoli cells	Non-specific inhibition of mammalian proteases
Secretory leukocyte protease inhibitor	Epididymis, prostate, seminal vesicles	Inhibition of elastase, cathepsin G, inhibition of viral infection, kill *E.coli* and *Staphylococcus* species
Cystatin C	Sertoli and Germ cells, epididymis, prostate, seminal vesicles	Inhibition of mammalian cathepsins, inhibition of *streptococcal* cysteine protease
Lactoferrin	Epididymis	Inhibits bacterial, viral and fungal infection by nutrient sequestering
Lysozyme	Testis, prostate, epididymis	Bacterial lysis
Toll-like Receptors	Testis, prostate	Cell surface mediators of intracellular responses to pathogens

Source : Halls *et. al.* (2002)

protease inhibitors in the host immune defense has been reported (Armstrong, 2001). Protein C inhibitor (PCI) is a member of plasma serine protease inhibitor family and is the major inhibitor of activated protein C (APC). PCI is present in testis (germinal cell layer and Leydig cells), epididymal glands, prostate and seminal vesicles (Kise *et. al.*, 2001). Secretory leukocyte protease inhibitor (SLPI) is a low molecular mass, acid stable protein present in fresh human seminal plasma (Ohlsson *et. al.*, 1995). SLPI is a strong inhibitor of several proteases, including leukocyte elastase and cathepsin G. SLPI could have a local protective function in the male genital tract against the proteolytic degradation during the infection by pathogenic microorganisms.

Cystatin C, a low molecular mass (14.00 kDa) is a potent inhibitor of cysteine proteases and is synthesized and secreted by the rat Sertoli cells (Bjorck *et. al.*, 1989). Western blot and immunohistochemical analysis with antiserum to human cystatin C demonstrated that cultured Sertoli cells secreted three immunoreactive forms of cystatin C. Cystatin C is present in Sertoli cells, and germ cells in the testis and also in the epididymis, prostate and seminal vesicles (Halls *et. al.*, 2002). Cystatin C inhibits cysteine proteases including the lysosomal cathapsins, B, L and H, which are expressed in the male genital tract. A synthetic peptide similar to human cystatin C has been shown to be antimicrobial for a large number of bacteria specifically all the strains of group A streptococci (Tohoen *et. al.*, 1998). In addition to cystatin C, four cystatins, *viz*; cystatin related epididymal and spermatogenic protein (CRES or cystatin 8), testatin (cystatin 9), cystatin T and CREs / cystatin related protein ESC 13 (cystatin 11) are expressed in the male genital tract (Halls *et. al.*, 2002). The function of these cystatins in the innate immune defense of the genital tract is unknown. Using a modified mRNA differential display method, a gene named testatin was isolated which was found to be related to a group of genes that encode cysteine protease inhibitors, known as testatins (Tohoen *et. al.*, 1998). Antileukoprotease (ALP) is an endogenous inhibitor of serine proteases, which is present in human lung and is a reversible inhibitor of elastase and cathepsin G. An antimicrobial peptide similar to ALP has been identified in equine neutrophils. ALP has strong *in vitro* activity against *E.coli* and *S.aureus*. Incubation of ALP with *E.coli* or *S.aureus* resulted in killing of these bacteria (Hiemestra *et. al.*, 1996).

α_2-macroglobulin is a non-specific broad spectrum protease inhibitor synthesized and secreted by sertoli cells of the testis (Cheng *et. al.*, 1990; Zhu *et. al.*, 1994). α_2-macroglobulin is a major protease inhibitor in seminiferous tubular and rete testis fluids and is a major inhibitor of proteases in the seminiferous tubule. By analogy with the action of protease inhibitors in other tissue α_2-macroglobulin in the testis could inhibit proteases that are released from the damaged spermatozoa during their movement through tubular lumen and genital tract and could protect the seminiferous epithelium and genital tract from damage by acrosomal proteases released from elongated spermatozoa during maturation process (Armstrong, 2001).

It is hypothesized that the major function of α_2-macroglobulin is to protect the host from the proteases of invading microorganisms.

Lectins

Lectins are glycoproteins other than immunoglobulins and enzymes, present in various tissues including the testis and prostate (Madson *et. al.*, 2000). Structurally and functionally the lectins are related to the first component of the complement pathway and seems to play important roles in innate immunity through opsonization and complement activation. Microbial lectins help the attachment of pathogenic microorganisms to target cells, while animal lectins are involved in intracellular communication, in the phagocytosis and destruction of pathogens (Holmskov *et. al.*, 1994). Recently, the important role of mannan – binding lectin (MBL) pathway of the innate immune response has been reported (Gadjeva *et. al.*, 2001). MBL is a member of the collectin family of glycoproteins and binds to various carbohydrate structures on the surface of bacteria, viruses, yeasts and protozoa, mediating an antimicrobial effect either by direct killing via the complement through the lytic membrane attach complex (MAC), or by phagocytosis (Gadjeva *et. al.*, 2001). Antimicrobial role for lectins in the testis and prostate has not been reported.

Lactoferrin

Lactoferrin is a host defense iron binding glycoprotein consisting of single polypeptide chain of 70 kDa, and 680 amino acids. Lactoferrin was first discovered in human milk, which is a major component of human milk protein. Lactoferrin is also present in seminal plasma, epididymal fluid, spermatozoa (Jin *et. al.*, 1997), epithelial secretions and in specific granules of the polymorphoneucular leukocytes, which is released on degradation of cells in the infected area. Lactoferrin has antimicrobial activity against broad spectrum of bacteria, viruses and fungi in body sites exposed to microbial invasion (Weinberg, 1984). The mechanism of host defense by lactoferrin is by restricting the availability of iron to invading microorganisms. In addition to this, lactoferrin can alter the permeability of bacterial membranes and disperse lipopolysaccharides through a cation mediated process, which may result bacterial death. Recently the biosynthesis of lactoferrin in the caput and cauda epididymis of the pig has been reported (Jin *et. al.*, 1997). The role of lactoferrin in innate host defense of the epididymis, spermatozoa and male genital tract await elucidation.

Lysozyme

Lysozyme is a mucolytic polysaccharide enzyme of about 15 kDa, 129 amino acid residues and consists of single polypeptide chain (Rodwel 1981; Guyton 1981). Lysozyme is widely distributed in various body fluids viz., tears, nasal mucus, gastric secretion, seminal plasma and tissues such as lysozomes, macrophages, the testis, epididymis and prostate (Tauber *et. al.*, 1976) and plays an important role in host defense against pathogenic microorganisms. Macrophages contain abundant lysozyme, which hydrolyzes link between N-acetyl muramic acid and N-acetyl D-glucosamine found in bacterial cell membranes (Murray, 2000[b]), and catalyzes the lysis of gram positive bacteria (Halls *et. al.*, 2002). In culture studies testicular macrophages were shown to secrete lysozyme for at least 8 days (Wei *et. al.*,

1988). Testicular macrophages play an important role in host defense against pathogenic bacteria by at least three different mechanisms *viz.*, 1) opsonization dependent phagocytosis, 2) the secretion of lysozyme which is involved in the lysis of the cell wall of the gram positive bacteria and 3) the production of superoxide anion which is involved in cystotoxic and bactericidal mechanisms (Wei *et. al.*, 1988). A pentadecapeptide derived from lysozyme, which lacks muraminidase activity alters the permeability of the outer bacterial membrane, inhibits the bacterial RNA and DNA synthesis, resulting in bacterial death (Pelegrini *et. al.*, 2000) protective role of lysozyme in host defense of the male genital tract against pathogenic microorganisms is unknown.

Antimicrobial Mechanisms

All cationic antimicrobial peptides *viz.*, defensins, and cathelicidins form an important and powerful component of innate immune system, which are able to kill or inactivate a wide range of bacteria, viruses and fungi in vitro and are the direct effectors of innate antimicrobial immunity (Yang *et. al.*, 2002). The mechanisms involved in antimicrobial activity of defensins (Lehrer *et. al.*, 1991; Honcock 1984; Sawyer *et. al.*, 1988; Viljenen *et. al.*, 1988) and cathelicidins (Lehrer *et. al.*, 1975; Bals *et. al.*, 1999), have been investigated. Rabbit defensins NP-1 and NP-2 bind to the surface of *P.aeruginosa* with high affinity and alter the permeability of bacterial cell membrane and form small bleb-like structures (Sawyer *et. al.*, 1988). Human defensins increase the permeability of the outer membrane of *P.aeruginosa* and *P.typhimurium* and disturb the ionic equilibrium of microorganisms (Viljenen *et. al.*, 1988). Bactericidal concentrations of HNP-1 caused increased permeability of inner and outer membranes of bacteria which resulted in ceasetion of DNA, RNA and protein synthesis and arrest of respiration resulting in bacterial death. Bacterial death was attributed to the increased permeability of inner membrane which allowed both the loss of intracellular contents and the entry of defensins and other host defense molecules in the bacterial cell, resulting in bacterial death. Members of cathelicidin family have different microbicidal mechanisms. Some members rapidly increase the permeability of bacterial cell membranes while others stop DNA, RNA and protein synthesis in gram negative bacteria resulting in bacterial death (Oren *et. al.*, 1999). Another cathelicidin antimicrobial peptide LL-37 causes bacterial lysis by covering the bacterial outer membrane in a carpet like manner. The cathelicidin molecules diffuse into the inner membrane of the bacteria and disintegrate them (Oren *et. al.*, 1999).

Innate Immune Recognition

How does the body recognize the pathogenic microorganisms from the non-pathogenic, and mount appropriate innate immune attack against the pathogens? Innate immune recognition relies on a number of receptors evolved to recognize the products of microbial cell membranes. Pathogen associated molecular patterns (PAM Ps), Toll-like receptors (TLRs) and mannan binding lectins (MBL), play an important role in innate immunes recognition of pathogens. Various microbial patterns such as lipopolysacharides (LPS) of gram negative bacteria, the glycolipids of micobacteria the lipoteichoic acids of gram positive bacteria, the mannans of yeasts and double stranded RNAs of viruses (Hoffmann *et. al.*, 1999), have been identified.

These microbial patterns are recognized by pattern recognition receptors (PRRs), which are expressed on the cell surface and are secreted in blood and tissue fluids. The major functions of PRRs are opsonization, activation of complement, phagocytosis and activation of proinflammatory signaling pathways and elimination of pathogens (Janeway and Medzhilov, 2002). Recently the role of Toll-like receptors (TLRs) (Adrem and Ulveiteh, 2000; Yang *et. al.*, 1998; Akira, 2001; Underhill and Ozinsky, 2002; Takada *et. al.*, 2003)

Table 3: Classification, size and biological activities of mammalian defensins

Mammalian defensins	Size *	Activities related to host defense
α	29 - 35	Microbicidal activityAntiviral effectChemotactic effectActivate macrophagesHistamin releaseCytokine inductionRegulate C1 activationImmunoenhancing effect
β	38 - 42	Microbicidal activityChemotactic effectActivate mast cells Immunoenhancing effect
θ	18	Microbicidal for bacteria and fungi

Size given is the number of amino acid residues. Source: Yang *et. al.* (2002)

and mannan binding lectins (MBL) (Holmskov *et. al.*, 1994; Gadjeva *et. al.*, 2001; Holmoskov *et. al.*, 2003; Rock *et. al.*, 1998) in the innate immune recognition has been reported. Toll or Toll-like receptors (TLRs) are group of proteins, which are the main censors of innate immunity, recognize the pathogens and mount rapid defensive response against them (Adrem and Ulveitch, 2000). In human and murine ten Toll-like receptors TLR-1 to TLR-10 have been identified (Janeway and Medzhitov, 2002). Toll - like receptors differ from each other in ligand specification and expression patterns and are involved in the recognition of a variety of pathogen associated molecular patterns (PAMPs). Mammalian Toll-like receptors are expressed in many tissues including the testis and prostate. However their function in the innate immune defense of the male genital tract is not clear. Mannan binding lectin (MBL) is a member of collectin family of glycoproteins. MBL binds to carbohydrate structures on the surface of microorganisms and mediate an antimicrobial effect either by killing via complement or by promoting phagocytosis. Innate immune recognition via MBL stimulate the activation of complement system, which indicates the ability of innate immune system to detect molecular patterns of microorganisms (Gadjeva *et. al.*, 2001). MBL is the only collectin known to be able to activate the complement system. Information on innate immunity of the male genital tract of farm animals and methods to enhance the same is not available and needs investigation

REFERENCES

Adrem, A. and Ulveitch R. (2000). Toll-like receptors in the induction of the innate immune response. Nature, **406** : 782-787.

Agerberth, B., Gunne, H., Odeberg, J., Kogner, P., Boman, H.G. and Gudmundsen, G.H. (1995). FALL – 39 a putative human peptide antibiotic is cystein free and expressed in bone marrow and testis. Proc. Nat. Acad. Sci., USA **92** : 195-199.

Akira, S.(2001). Toll-like receptors and innate immunity. Advances in Immunology, **78**: 1-56.

Aravindan, G.R., Murk, D., Lee, W.M. and Cheng, C.Y.(1997). Identification, isolation and characterization of a 41 kDa protein from rat sperm cell conditioned medium. Endocrinology, **138**: 3259-3268.

Armstrong, P.B. (2001). The contribution of proteinase inhibitors in immune defense. Trends in Immunology, **22** : 47-62.

Bals, R., Weiner, D.J., Moscioni, A.D., Meegalla, R.L. and Wilson, J. M. (1999). Augmentation of innate host defense by expression of a cathelicidine antimicrobial peptide. Infect. Immun., **67**:6084-6089.

Beutler, B. (2003). Innate immune response to microbial proteins : Discovery and functions of the Toll-like receptors. Annu. Rev. of Pharmacol. Toxicol., **43** : 609-628.

Bisenbrock, A.R., Reddy, M.S. and Lwevin, M.J. (1991). Interaction of salivary mucin secretory IgA complex with mucosal

pathogens. Infect. Immun., **59** : 3492-3497.

Bjorck, L., Akesson, P., Bohas, M., Trojnar, J., Abrahzamson, M., Olafsson, I. And Grubb A. (1989). Bacterial growth blocked by synthetic peptide based on the structure of human protease inhibitor. Nature, **337** : 385-386.

Boman, H. (1995). Peptide antibiotic and their role in innate immunity. Annu. Rev. Immunol, **13**:61-92.

Borregarrd, N., Elsbach, P., Ganz, T., Garred, P. and Sevigaard, A. (2000). Innate Immunity from Plants to Humans. Immunology Today, **21** : 68-70.

Brown, E., Akinson, J. and Fearon, D. (1994). Innate Immunity Overview. Current Opinion in Immunology, **6** : 73-74.

Buck, B., Dag, P.M., Thompson, C.D., Lubkowski, J., Low, D.R. and Schiller, J.T. (2006). Human alpha defensins block pepilloma virus infection, Proc. Nat. Acad. Sci., **103** : 1516-1521.

Cheng, C.Y., Grima, J., Stahler, B. and Bardin, Y.C. (1990). Sertoli cells synthesize and secrete a protease inhibitor α_2 macroglobulin. Biochemistry, **29** : 1063-1068.

Cohen, P.S. and Lanz, D.C. (1995). Bacterial adhesions to and penetration of intestinal mucus in vitro. Methods in Enzymology, **253** : 309-314.

Eisenhaur, P.W., Harwig, S.S., Szklared, D., Ganz, T., Selested, M.E. and Lehrer, R. I. (1989). Purification and antimicrobial properties of three defensins from rat neutrophils. Infect. Immun., **37** : 2021-2027.

Emmanulle, C., Bourgeon, F., Evard, B., Ganz, T. Colleu, D. Jegou, B. and Pineau, C. (2003). Expression of antimicrobial defensins in the male reproductive tract of rats, mice and humans. Biol. Reprod., **68**: 95-104.

Fromehlich, O. and Yang, L. (2002). An antimicrobial role for the EP2/HE2 gene in the epididymis, J. Androl. March/April Supplement, p.51 (Abstract).

Gadjeva, M., Thiel, S. and Jensenius, J. (2001). The mannan-binding lectin pathway of innate immune response. Current Opinion in Immunology, 13: 74-78.

Gennaro, R., Skerlvavaj, B. and Romew, D. (1989). Purification, composition and activity of two antimicrobial peptides of bovine neurophils. Infect. Immun. **57** : 3142-3146.

Guyton, A.C. (1981). Resistance of the body to infection : The leukocytes, tissue macrophage system and inflammation. Textbook of Medical Physiology, W.B.Saunder Company, London, pp.65-75.

Halls, S., Hawil, K. and French, F. (2002). Host defense proteins of the male reproductive tract. J. Androl., **23** : 585-597.

Hiemestra, P.S., Maassen, R.J., Stolk, J., Weiland, R.H., Steffen, G.J. and Dijkman, J.H. (1996). Antibacterial activity of antileukoprotease. Infection and Immunity, **64** : 4520-4525.

Holmskov, U., Malhotra, R. and Jensenius, J. (1994). The innate immune system, collectins: Collagenous C type lectins of the innate immune defense system. Immunology Today, 15: 67-73.

Holmoskov, U., Thiel, S. and Jensehius, J.C. (2003). Collectins and Fucotins : Humoral lectins of the innate immune defense. Annu. Rev. Immunol., **21** : 547-578.

Hoffmann, J., Kofatos, F., Janeway, Ç. and Kowitz, R. (1999). Phylogenetic perspective in innate immunity. Science, **284** : 1313-1317.

Honcock, R.E.W. (1984). Alteration of outer membrane permeability. Annu. Rev. Microbiol. **38** : 237-264.

Janeway C. and Medzhilov R. (2002). Innate immune recognition. Annu. Rev. Immunol., **20** : 197-216.

Jin, V.Z.; Bannai, S.; Dacheux, F.; Dechux, J.L. and Okamura, N. (1997). Direct evidence for the secretion of lactoferrin and its binding to sperm in the porcine epididymis. Mol. Reprod. Dev., 47: 490-496.

Kise, H., Nishioka, J., Satoh, K., Okunono, T., Kawamura, J. and Suzuki, K. (2000). Measurement of protein inhibitor in seminal plasma is useful for detecting of agenesis of seminal vesicles or vas. Deferens. J. Androl., 21: 207-212.

Kulkarni, B. A. and Dhande, S.G. (2003). Bovine seminal plasma immunoglobulins, in Proceedings of National Conference on Recent Advances in Reproductive Health, February 6-8, Jaipur, p.158 (Abstract).

Lehrer, R.I., Lichtrenstein, A. and Ganz, T. (1993). Defensins: Antimicrobial and cytotoxic peptides of mammalian cells. Annu. Rev. Immunol., **11**: 105-128.

Lehrer, R.I., Rosenmen, M., Hariwig, S.S., Jacksen, R. and Eisenhaur, P. (1991). Ultra sensitive assays for endogenous antimicrobial polypeptides. J. Immun. Methods, **137**: 167-173.

Lehrer, R.L., Barton, A. Daher, K.A., Harwig, S.S., Ganz, T. and Selsted, M. (1989). Interaction of human defensins with *Escherichia coli*. Mechanisms of bactericidal activity, J. Clin. Invest, **84** : 553-561.

Lehrer, R.I., Barton, a., Daher, K.A., Harwig, S.S., Ganz, T. and Selested, M. (1988). Modulation of the in vitro candidacidal activity of human neutrophil defensins by target cell metabolism and divalent cations. J. Clin. Invest. **88** : 1829-1835.

Lehrer, R.I., Szklared, D., Ganz, T. and Selrted, M.E.(1986). Synergistic activity of rabbit granulocyte peptides against candida albicans. Infect. Immun. **52** : 902-904.

Lehrer, R.I., Ferrari, L.G., Patterson, D.J., Serrell, T. (1980). Fungicidal activity of rabbit alveolar and peritoneal macrophages against candida albicans, Infect. Immun. **28**:1001-1008.

Lehrer, R.I., Ladra, K.M. and Hake, R.B. (1975). Non oxidative fungicidal mechanisms of mammalian granulocytes: demonstration of components with candidacidal activity in human, rabbit and guinea pig granulocytes. Infect. Immun. **11** : 116-123.

Li Peng, Chan, H.C., Bin, S.O., Chung, Y.W., Shang, O., Zhang, Y.D. and Zhang, Y.L. (2001). An antimicrobial peptide gene found in male reproductive tract of the rat, Science, **291** : 1783-1785.

Liu, Q., Hamil, K.G., Sivansanmugam, P., Grossman, G., Sunderajan, R., Rao, a., Richardson, R.T., Zhang, Y.L., O'Rand, M.G., Petrusz, P. and Freschch, F.S. (2001). Primate epididymis specific proteins : Characterization of ESC-42 a novel protein containing trefoil like motif in monkey and human, Endocrinology, **142** : 4529-4539.

Madson, J., Kliem, A., Thrnoc, I., Skjodt, K., Koch, C. and Holmoskov, U. (2000). Localization of Lung surfactant protein D on mucosal surfaces in human tissues. J. Immunol. **164** :5866-5870.

Malhotra, R., Mery, T. and Ray, K. (2000). Innate immunity : A premitive system in humans. Immunology Today, **21** : 534-535.

Malm, J., Sorensen, O., Persson, T., Nilsson, M.F., Johansson, B., Bjrtell, A., Lilja, H., Backhl, M.S., Borregard, N. and Egesten, A. (2000). The human cationic antimicrobial protein (hCAP-18) is expressed in the epithelium of human epididymis is present in seminal plasma at high concentration and is attached to spermatozoa. Infect. Immun. **68**, 4297-4302.

Mastallo, D., and Lambris, J.D. (2002). Complement : More than a guard against invading pathogens. Trends in Immunology, **23** : 485-491.

Murray, R.K. (2002[a]). Carbohydrates, in Haper's Biochemistry, 25th ed. Pp.675-694 (Murray,R.K. and Granner, D.K. (Eds.), Mcgraw-Hill, New York.).

Murray, R.K. (2000[b]). Red and white blood cells, in *Harper's Biochemistry*, 25th ed. (Murray,R.K. and Granner,D.K. (Eds.), McGraw-Hill, New York, pp.763-779).

Nagraj, R. (2006). Defensins : Molecular armaments of the innate immune system, in

Proceedings of International Symposium of Emerging Trends in Genomic and Proteomic Sciences, pp.5-7 (Abstract), October, 15-18, Mumbai.

Ohlsson, K., Bjrtell, L., Lilija, H. (1995). Secretory leukocyte protease inhibitor in the male genital tract, **J. Androl.**, 16 : 64-74.

O'Neille, L.A. (2002). Toll-like receptor, signal transduction and tailoring of innate immunity : a role for Mal. Trends in Immunology, **23** : 296-300.

Oren, Z., Lerman, J.C., Gudmund, G.H., Agerberth, B. and Shai, Y. (1999). Structure and organization of human antimicrobial peptide LL-37 in phospholipid membranes. Biochem. J., **341** : 501-513.

Osterhoff, C., Kirchoff, C., Krull, N., Ivell, R. (1994). Molecular clonging and characterization of a novel sperm antigen (HE_2) specifically expressed in the proximal epididymis. Biol. Reprod., **50** : 516-529.

Porter, E., Yang, S., Yavagal, G.C., Preza, O., Murillo, H., Lima, S., Greene, J., Mahoozi, M., Klein, P.G., Gulati, T., Ganz, A.r. and Qualyle, A.J. (2005). Distinct defensins profiles in *Neisseria gonorrhoeae* and *Chlamydia trachomatis urethritis* reveal novel epithelian cell neutrophile interactions. Infect. Immun. **73** : 4823-4833.

Pelegrini, A., Thomas, U., Wild, P., Schraner, E., Fellenberg, V.R. (2000). Effect of Lysozyme or modified lysozyme fragments on DNA and RNA synthesis and membrane permeability of *E.coli*. Microbiological Research, **155**: 69-70.

Radicioni, A. (1986). Immunoglobulibns in seminal plasma. J. Immunol., **11** : 71-74.

Rock, F.L., Hardiman, G., Timans, J.C., Kastolein, R.H., Bazan, J.E. (1998). A family of human receptors structurally related to *Drosophila Toll*. Proc. Nat. Acad. Sci., USA, **95**: 588-593.

Rodwel, V. (1981). Kinetic properties of enzymes, in Harper's Review of Biochemistry, 18th ed., pp.71-85 (Martin, D.W. (Ed.), Maruzen-Asian Edition, London).

Sawyer, J.G., Martin, N.L., Hancock, R.E.W. (1988). Interactions of macrophage cationic proteins with outer membranes of *P.aeruginosa*. Infect. Immun., **56**: 693-698.

Takada, K., Kaisho, T. and Akira, S. (2003). Toll-like receptors. Annu. Rev. Immunol., **21** : 335-376.

Tang, Y.Q., Yuan, J., Osapay, G., Osapay, K., Tran, D., Miller, C.J., Quell, A.J. and Selsted, M.E. (1999). Acyclic antimicrobial peptide produced in primate leukocytes by ligation of two truncate *a*-defensins. Nature, **286**: 498-502.

Tauber, P.E., Zanevel,d L.J., Propping, D., Schmacher, G.F.B. (1976). Componentsw of human split ejaculate Enzymes and proteinase inhibitor. J. Reprod. and Fertil., **46** :165-171.

Tohoen, V., Ostrulund, C., Nordqvist, K. (1998). Testatin : a cystein related gene expressed during early testis development. **Proc. Nat.** Acad. Sci. USA, **95** : 14208-14213.

Trusuta, J.K., Brien, D.A., Grswold, M.D. (1993). Sertoli cells and germ cells cystatin C : State dependent expression of two distinct mRNA transcripts in rat testis. Biol. Reprod., **49** : 1045-1054.

Underhill, D.M., and Ozinsky, A. (2002). Toll-like receptors : Key mediaters of microbe detection. Current Opinion in Immunology, **14** : 103-110.

Viljenen, P., Koski, P. and Vaara, M. (1988). Effect of small cationic leukocyte peptides (defensins) on the permeability barrer of the outer membrane. Infect. Immun., **56** : 1224-1229.

Wei, R.Q., Yee, J.B., Straus, D.C. and Hustson, J.C. (1988). Bactericidal activity of testicular macrophages. Biol.Reprod., **38**: 830-835.

Weinberg, E.D. (1984). Iron withholding : a defense against infection and neoplasma. Physiological Review, **64**: 65-102.

Yang, D., Bragym, A., Kwak, W. and

Oppenheim, J. (2002). Mammalian defensins in immunity. More than just microbicidal. Trends in Immunology, **23**: 291-295.

Yang, D., Chertov, O., Bykovskaia, S.N., Chen, Q., Buffo, M..J., Shogan, J., Anderson, M., Scheroder, J.M., Wang, J.M., Howard, M.Z. and Oppenheim, J.J. (1999). ***B***-defensins linking innate and adaptive immunity through dendrites and T-cells. **Science**, 286 : 525-528.

Yang, R.B., Mark, M.R., Gray, A., Huang, A., Xie, M.H., Zhang, M., Goddard, A., Wood, W.L., Gurney, A.L. and Godwski, P.G. (1998). Toll-like receptors – 2 mediates lipopolysaccharide induced cellular signaling. Nature, **395**: 284-288.

Zanetti, M., Gennaro, R., and Romeo, D. (1997). The cathelicidine family of antimicrobial peptide precursors: A component oxygen independent defense mechanisms of neutrophils. Ann. New York Acad. Sci. USA, **832** : 147-162.

Zhu Li-Ji, Cheng, Y.C., Phillips, D.M. and Bardin, C.W. (1994). The immunohistochemical localization of *a*-2 macroglobulin in rat testis is consistent with its role in germ cells movement and spermiation. J. Androl., **15**: 575-582.