

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Human Defensins in HIV Infection: Friends or Foes?

Rachna Shah and Theresa L. Chang

*Public Health Research Institute and Department of Microbiology and Molecular Genetics,
University of Medicine and Dentistry of New Jersey-New Jersey Medical School
United States*

1. Introduction

Defensins are antimicrobial peptides (AMPs) and play an important role in both innate and adaptive immune response (Ganz, 2002; Yang et al., 2004). Defensins display broad antimicrobial activities against bacteria, fungi and viruses (Ganz, 2003; Lehrer, 2004; Zanetti, 2004)(Yang et al., 2004). Importantly, they also play a role in inflammation, tissue repair and angiogenesis (Kruse and Kristensen, 2008; Rehaume and Hancock, 2008). Increasing evidence indicates that the AMPs can act as a double-edged sword by providing protection against invading pathogens but at the same time causing potentially harmful inflammation or facilitating pathogen invasion. This review focuses on the role of human defensins in HIV infection. We will summarize the complex mechanisms by which defensins inhibit or enhance HIV infection in vitro, clinical evidence and studies in macaques with respect to the role of defensins in HIV transmission and pathogenesis.

2. Overview of human defensins

2.1 Defensins and their regulation

Defensins are positively charged peptides with β -sheet structures stabilized by three disulfide bonds between the cysteine residues (Ganz, 2003; Selsted and Ouellette, 2005). In humans, defensins are classified into two subfamilies: α -, and β - defensins, differing in their disulfide bond pairing. The linkages of Cys residues in α -defensins are Cys¹-Cys⁶, Cys²-Cys⁴, Cys³-Cys⁵, whereas in β -defensins the linkages are Cys¹-Cys⁵, Cys²-Cys⁴, Cys³-Cys⁶ (reviewed in Ganz, 2003; Yang et al., 2004; Yang et al., 2002). Despite variation in sequences and disulfide bond linkages, both families have similar structures (Hill et al., 1991; Hoover et al., 2001; Pardi et al., 1992; Szyk et al., 2006). Neutrophil α -defensins (HNPs 1-4) are mainly synthesized as a prepropeptide in promyelocytes, neutrophil precursor cells in the bone marrow, and the mature peptide is stored in primary granules of neutrophils (Ganz, 2003). Unlike HNPs, human α -defensin-5 (HD5) is released as a propeptide that is processed extracellularly (Ghosh et al., 2002; Porter et al., 2005). An additional class of mammalian defensins is the θ -defensin, originally found in rhesus monkeys (Tang et al., 1999). Primates including human, chimpanzees and gorillas contain pseudogenes of θ -defensin mRNAs with a conserved stop codon in the upstream of the signal sequence that prevents translation (Nguyen et al., 2003). It has a circular structure with the Cys residues linking

Cys¹-Cys⁶, Cys²-Cys⁵, Cys³-Cys⁴ (Tang et al., 1999). The θ -defensins are formed by the fusion of two truncated α -defensin nonapeptides that are connected by fusion of the N- and C-termini (Leonova et al., 2001; Tang et al., 1999; Tran et al., 2002).

Human defensins are produced mainly by leukocytes and epithelial cells. HNPs 1-3 were first isolated from neutrophilic granulocytes (polymorphonucleated neutrophilic leukocytes; PMN), and account for 30-50% of total proteins in azurophilic granules of neutrophils (Ganz et al., 1985). HNP4 comprising less than 2% of defensins in neutrophils has a relatively distinct sequence but similar structure with HNPs 1-3 (Ganz, 2003; Wilde et al., 1989). While neutrophils produce the highest amount of HNPs, these peptides can be found in other immune cells including natural killer cells, B cells, $\gamma\delta$ T cells, and monocytes/macrophages, immature dendritic cells (Agerberth et al., 2000; Rodriguez-Garcia et al., 2007). In addition, cells can absorb and internalize HNPs intracellularly (Ganz, 1987; Mackewicz et al., 2003; Zaharatos et al., 2004), underlining the complexity in defining true HNP producing cells and the questions regarding the function of the up-taken defensins. HNPs have been detected in placenta, spleen, thymus, intestinal mucosa, saliva, and cervical mucus plugs (Agerberth et al., 2000; Cunliffe, 2003; Fellermann and Stange, 2001; Hein et al., 2002). HNPs can be released by chemokines, FC γ receptor cross-linking, phorbol myristate acetate (Chalifour et al., 2004; Ganz, 1987; Jan et al., 2006; Tanaka et al., 2003). Activation of toll-like receptors (TLRs) 2 and 5 by the outer membrane protein A of *Klebsiella pneumoniae* and flagellin of *Escherichia coli*, respectively, triggers the release of HNPs 1-3 by the CD3⁺CD56⁺ natural killer T cells (Chalifour et al., 2004). Additionally, direct interactions of *Mycobacterium bovis* BCG with eosinophils induces the production and release of HNPs 1-3 through TLR2 (Driss et al., 2008). Elevation of HNPs has been reported in the vaginal mucosa of women with *N. gonorrhoeae* (GC), *T. vaginalis*, or *C. trachomatis* (CT) (Simhan et al., 2007; Valore et al., 2006; Wiesenfeld et al., 2002), suggesting their role in mucosal immunity against infection *in vivo* (Heine et al., 1998; Wiesenfeld et al., 2002).

HD5, the most abundant AMPs in the small intestine, is constitutively expressed by paneth cells but can be found in other tissues such as the salivary glands, the female genital tract and the inflamed large bowel (Cunliffe et al., 2001; Fahlgren et al., 2003; Fellermann and Stange, 2001; George et al., 2008; Quayle et al., 1998; Salzman et al., 2007; Svinarich et al., 1997). Rhesus macaque, an animal model used for studying HIV pathogenesis, expresses six paneth cell defensins but their coding sequences are distinct from HD5 and HD6 (Tanabe et al., 2004). Analysis of intestinal specimens from patients with ileal Crohn's disease (CD), a chronic mucosal inflammation, suggests that NOD2 and Wnt signaling transcription factor Tcf-4 protein may modulate the level of HD5 gene expression (Wehkamp et al., 2004) (Wehkamp et al., 2007). HD5 is induced at the genital mucosa in patients with bacterial vaginosis, GC and CT infections (Fan et al., 2008; Porter et al., 2005), although the mechanism remains to be defined.

Six human β -defensins (HBD1, -2, -3, -4,-5,-6) have been identified and characterized (Pazgier et al., 2006; Yamaguchi et al., 2002; Yang et al., 2004). Although additional human β -defensins (Schutte et al., 2002) have been identified by gene-based searches. HBDs are expressed by epithelial cells and hematopoietic cells (Duits et al., 2002; Ganz, 2003; Yang et al., 2004). While HBD1 is often constitutively expressed, expression of HBD2 and HBD3 can be induced by viruses, bacteria, microbial products and pro-inflammatory cytokines, such as tumor-necrosis factor (TNF) and interleukin-1 (IL-1) (Duits et al., 2003; Ganz, 2003; Proud et al., 2004; Sorensen et al., 2005; Yang et al., 2001). HBD1, HBD2 and HBD3 have been

detected in various epithelial tissues (Fellermann and Stange, 2001; Garcia et al., 2001; Harder et al., 2001). Both human α - and β -defensins have been found in breast milk (Armogida et al., 2004; Jia et al., 2001), suggesting a role for defensins in protecting infants from infection.

The mechanisms of induction of HBD1, HBD2 and HBD3 have been shown to be distinct from each other (Pazgier et al., 2006). HBD2 can be induced by TLR2, TLR3, TLR4, TLR7, NOD1 and NOD2 signaling in various epithelial cells and keratinocytes (Hertz et al., 2003; Nagy et al., 2005; Pivarcsi et al., 2005; Uehara et al., 2007; Vora et al., 2004). Stimulation of TLR3 has been shown to induce HBD1 and HBD2 expression in uterine epithelial cells (Schaefer et al., 2005). Induction of HBD2 and HBD3 but not HBD1 in bronchial epithelial cells in response to human rhinovirus infection is mediated by activation of nuclear factor- κ B (NF- κ B) but not of IL-1 (Proud et al., 2004). As TLR3 activation also induced HBD2 and HBD3, it is possible that intracellular double-stranded RNA generated during replication of rhinovirus may be involved in the regulation of HBDs (Duits et al., 2003; Proud et al., 2004). Similarly, HBD2 and HBD3 are induced in normal human oral epithelium cells, even in the absence of HIV-1 replication (Quinones-Mateu et al., 2003). Interestingly, a recent study reported that both X4- and R5-tropic viruses cannot induce HBD2 gene expression in the MatTeck oral tissue model nor primary gingival epithelial cells (Nittayananta et al., 2009). Additionally, high concentrations of X4 virus HIV-1_{Lai} block HBD2 gene expression by 50% (Nittayananta et al., 2009). In oral epithelium, TLR2 and NOD1/2 ligands synergistically activate NF- κ B and induce HBD2 gene expression (Uehara and Takada, 2008). Cytokines such as IL-1 and IL-17 also play important roles in the regulation of HBD2 expression. Induction of HBD2 by IL-17A is mediated by PI3K pathway and MAPK pathway to activate NF- κ B in airway epithelial cells, whereas regulation of HBD2 by the activation of NF- κ B is not dependent on PI3K pathway in bronchial epithelial cell, (Huang et al., 2007; Jang et al., 2007; Kao et al., 2008), indicating that specific pathways involved in regulation of HBDs are cell type dependent.

2.2 Immunological and biological functions of defensins

Defensins have a wide range of functions in modulating innate and adaptive immunity (Yang et al., 2004) as well as biological aspects including metabolisms and angiogenesis (Coffelt and Scandurro, 2008; Joseph et al., 2008; Kruse and Kristensen, 2008; Liu et al., 2008; Rehaume and Hancock, 2008; Saraheimo et al., 2008). Both HNPs and HBDs exhibit chemotactic activity for T cells, monocytes and immature DCs and can induce production of cytokines and chemokines (Yang et al., 2004) (Chertov et al., 1996; Yang et al., 2000). HNP1 also regulates the release of IL-1 β and enhances phagocytosis (Shi et al., 2007; Tecle et al., 2007). HBDs1-3 recruit memory T cells and immature DCs through binding to CCR6, the receptor for the CC-chemokine ligand 20 (CCL20; also known as MIP3 α) (Yang et al., 1999; Zlotnik and Yoshie, 2000). HBD2 has multiple activities on mast cells, including induction of cell migration, degranulation and prostaglandin D₂ production (Niyonsaba et al., 2003). Murine β -defensin-2 can recruit bone-marrow-derived immature DCs through CCR6 and can induce DC maturation through TLR4 (Biragyn et al., 2002). HBD3 activate antigen-presenting cells such as monocytes and DCs through TLRs 1 and 2 (Funderburg et al., 2007). HBD3 activates antigen presenting cells (DCs and monocytes) via TLR1/2 (Funderburg et al., 2007). Defensins are frequently induced by pro-inflammatory cytokines or TLR activation (Ganz, 2003) (Klotman and Chang, 2006). Conversely, defensins can

induce cytokines and chemokines. HNPs upregulate the expression of CC-chemokines and IL-8 in macrophages and epithelial cells, respectively (Guo et al., 2004; Van Wetering et al., 1997). HBD2, known to be inducible in response to bacterial infection and pro-inflammatory cytokines (Ganz, 2003; Yang et al., 2004) can up-regulate IL-6, IL-8, IL-10, MCP-1, IL-1 β , MIP-1 β and RANTES in PBMCs (Boniotto et al., 2006). HD5 can induce IL-8 (Liu et al., 2007) that enhances HIV infection in cervical tissues (Narimatsu et al., 2005).

Defensins can bind to other host proteins to modulate immune or metabolic functions (Rehaume and Hancock, 2008). HNPs bind to low-density lipoprotein receptor-related proteins and interact with protein kinase C α and β , leading to decreased smooth muscle contraction in response to phenylephrine (Nassar et al., 2002). HNPs also interact with adrenocorticotrophic hormone (ACTH) receptors and heparan sulfate-containing proteoglycan (HSPGs) to modulate other biological activities (Higazi et al., 1996; Higazi et al., 2000). HNP1 has been shown to inhibit the activity of conventional PKC isoforms in a cell-free system (Charp et al., 1988). This PKC inhibitory activity appears to be important for HNP1-mediated inhibition of HIV replication in primary CD4⁺ T cells (Chang et al., 2005). As defensins display various biological functions, the roles of defensins in HIV-associated metabolic disorders or cancers in addition to HIV transmission and pathogenesis remain to be investigated.

3. Effect of defensins on HIV infection in vitro: Mechanism(s) of action

In contrary to the traditional role of defensins to defense host against pathogens, recent studies indicate that specific defensins can inhibit or enhance HIV infection. With respect to anti-HIV activities of defensins, these peptides have a dual role in antiviral activity. One aspect of antiviral activities involves direct interaction with viral envelopes possibly by disrupting virus envelopes similar to their antibacterial activity or by preventing viral entry. However, in contrast to anti-bacterial activities of defensins, there is no direct evidence supporting that defensins directly inactivate HIV virion by membrane disruption. The other involves indirect antiviral activity through interactions with potential target cells. These defensin-cell interactions are complex and at least in part mediated by interacting with cell surface glycoproteins and/or interfering with cell-signaling pathways that are required for viral replication. HD5 and HD6, induced in cervicovaginal epithelial cells in response to GC infection, enhance HIV infectivity (Klotman et al., 2008). The enhancing effect of HD5 and HD6 was more pronounced with R5 virus compared with X4 virus, indicating a potential clinical relevance as R5 virus is preferentially transmitted during primary infection. The specific mechanism is discussed as the following and activities of defensins and other antimicrobial peptides on HIV replication is summarized in Table 1.

The in vitro functions of defensins appear to be affected by factors such as the source of defensins, serum and salt. Different antiviral mechanisms of defensins may be operative in mucosal surfaces versus blood depending on the salt concentration or the presence of serum. This appears to be the case with the direct antiviral effect. Serum and salt conditions did alter the direct effect of defensins on the virion (Daher et al., 1986). (Chang et al., 2005; Quinones-Mateu et al., 2003). Some defensins (e.g. HNPs but not HD5 or HD6) at high concentrations are known to cause cytotoxicity in the absence of serum, which is associated with changes in cell membrane permeability, similar to their anti-bacterial activity. This cytotoxicity can be abolished by the presence of serum (Okrent et al., 1990 ;Van Wetering et

al., 1997) and defensin-mediated cytotoxicity may partially account for the antiviral effect (Mackewicz et al., 2003). While most defensins display potent direct antibacterial activities in conditions of low salt (Lehrer et al., 1993), neither a low concentration of salt nor the absence of serum are required for the chemotactic effects of defensins (Chertov et al., 1996; Yang et al., 1999). It is not clear whether other functions of defensins are altered depending on the environment.

Defensins	Effect	Mechanism	Reference
HNP1	Inhibit	Inactivates virion	Mackewicz et al.,2003; Chang et al.,2005
HNP1,HNP2	Inhibit	Up-regulate CC-chemokine production by macrophages	Guo et al.,2004
HNP1,HNP2	Inhibit	Bind to gp120 and CD4, block fusion	Furci et al.,2007;Wang et al.,2004
HNP1	Inhibit	Blocks viral nuclear import & transcription	Chang et al.,2005
HNP4	Inhibit	Bind to gp120 and CD4 (lectin independent)	Wang et al.,2004;Wu et al., 2005
HD5,HD6	Enhance	Enhance viral entry	Klotman et al.,2008
Cryptdin-3	Enhance	-	Tanabe et al.,2004
HBD2	Inhibit	Blocks early RT product formation	Sun et al.,2005
HBD2,HBD3	Inhibit	Down-regulate CXCR4 expression	Quinones-Mateu et al.,2003
Retrocyclin	Inhibit	Blocks viral entry	Cole et al.,2002; Munk et al.,2003
Retrocyclin	inhibit	Binds to gp120 and CD4	Cole et al.,2002; Wang et al.,2004 Munk et al.,2003;Wang et al,2003
Retrocyclin1	Inhibit	Blocks viral fusion	Gallo et al.,2006
RTD 1-3	Inhibit	Binds to gp120 and CD4	Wang et al.,2004

HNP = human neutrophil peptide; RTD = rhesus θ -defensin

Table 1. Effect of defensins on HIV infection

Inhibition of HIV replication by synthetic guinea-pig, rabbit and rat α -defensins was first reported in 1993 (Nakashima et al., 1993), when it was shown that these peptides could inhibit HIV-1 infection *in vitro* following viral entry into transformed CD4⁺ T cells in the presence of serum (Nakashima et al., 1993). HNPs1-3 block HIV infection through multiple mechanisms (Furci et al., 2007; Zhang et al., 2002) (Chang et al., 2003; Mackewicz et al., 2003). HNPs1 -3 all have similar activities against HIV primary isolates (Wu et al., 2005), in contrast to their differential chemotactic activities on monocytes, where HNP3 has no effect (Territo et al., 1989). They can inhibit HIV-1 replication by a direct interaction with the virus as well by affecting multiple steps of HIV life cycle (Chang et al., 2003; Chang et al., 2005; Furci et al., 2007; Mackewicz et al., 2003; Wang et al., 2004). In the absence of serum, HNP1 has a direct

effect on the virus prior to infection of a cell (Chang et al., 2005). In the presence of serum and at non-cytotoxic concentrations (low dose), HNP1 acts on primary CD4⁺ T cells and blocks HIV-1 infection at the steps of nuclear import and transcription by interfering with PKC signaling (Chang et al., 2005). The post-entry inhibitory effect of HIV infection occurs in primary CD4⁺ T cells and macrophages but not in several transformed T-cell lines (Chang et al., 2003; Chang et al., 2005). In the presence of serum, HNP1 did not affect expression of cell-surface CD4 and HIV-coreceptors on primary CD4⁺ T cells (Chang et al., 2005), whereas HNP2 down-regulates CD4 expression in the absence of serum (Furci et al., 2007). HNPs block HIV-mediated cell-cell fusion and the early steps of HIV infection by interacting with HIVgp120 and CD4 through their lectin-like properties (Furci et al., 2007). In macrophages, HNP1 and HNP2 upregulate the expression of CC-chemokines, which could contribute to inhibition of HIV through competition for receptors (Guo et al., 2004). CC-chemokines can also induce the release of HNPs from neutrophils by degranulation (Jan et al., 2006). Both effects could play a role *in vivo* in an innate immune response to HIV. At the mucosal surface, HNPs might work to directly inactivate the virions in the absence of serum; however, in the presence of serum, their inhibitory effect would largely be on the infected cell. HNPs1-3 have been reported to act as lectins and bind to HIV envelope glycoprotein gp120 and to CD4 with high affinity (Wang et al., 2004). The binding to gp120 is strongly attenuated by serum, thus accounting for the loss of the direct virion effect in the presence of serum. Interestingly, in contrast to HNPs1-3, HNP4 acts in a lectin-independent manner and does not bind to CD4 or HIV gp120 (Wang et al., 2004; Wu et al., 2005). However, HNP4 inhibits HIV replication more effectively than HNP1, -2 and -3 (Wu et al., 2005).

Other α -defensins, including HD5 and HD6, mouse paneth cell cryptdin-3 and cryptdin-4, and rhesus macaque myeloid α -defensin-3 (RMAD3) and RMAD4 have been tested for their ability to block HIV infection (Klotman et al., 2008; Tanabe et al., 2004). While HD5 did not exhibit any effect on X4 HIV-1_{LAI} infection of transformed CD4⁺ T cell lines (Tanabe et al., 2004), HD5 and HD6 significantly enhanced infectivity of HIV-1 R5 strains (Klotman et al., 2008). At high concentrations associated with cytotoxicity, RMAD4 blocks HIV replication, whereas, cryptdin-3 enhances viral replication. Studies on the molecular mechanism of the HIV enhancing effect of HD5 and HD6 indicate that defensins enhance HIV infection through promoting HIV attachment (Rapista et al., 2011). In addition, HD5 but not HD6 competes with heparan for binding to HIV. Importantly, these defensins have been shown to block *in vitro* anti-HIV activity of polyanionic microbicides, which have failed to protect women against HIV infection, and to interfere with anti-HIV activity of HIV entry and fusion inhibitors under specific conditions (Ding et al., 2011; Rapista et al., 2011).

The anti-HIV activities of HBD2 and HBD3 have been demonstrated under different conditions (Quinones-Mateu et al., 2003; Sun et al., 2005). Similar to HNP1 (Chang et al., 2005), HBD2 and HBD3 have dual anti-HIV activities through direct interactions with the virus and by altering the target cell. The binding of defensins to cellular membranes and HIV virions has been demonstrated by electron microscopy, although membrane disruption is not apparent (Quinones-Mateu et al., 2003). HBD2 does not affect viral fusion but inhibits the formation of early reverse transcribed HIV DNA products (Sun et al., 2005). There are conflicting reports on the downregulation of expression of HIV co-receptors by β -defensins. In studies reported by Sun *et al.* (Sun et al., 2005), HBD1 and HBD2 did not modulate cell-surface HIV co-receptor expression by primary CD4⁺ T cells, whereas Quinones-Mateu *et al.* (Quinones-Mateu et al., 2003) showed HBD2- and HBD3-mediated downregulation of

surface CXCR4 but not CCR5 expression by peripheral blood mononuclear cells (PBMCs) at high salt conditions and in the absence of serum. Interestingly, HBD2 is constitutively expressed in healthy adult oral mucosa but the level seems to be diminished in HIV-infected individuals (Sun et al., 2005).

Retrocyclins, and RTD1, -2 and -3 act as lectins and can inhibit HIV entry (Cole et al., 2002; Munk et al., 2003; Wang et al., 2003; Wang et al., 2004). Retrocyclin and RTD1,-2 and -3 inhibit several HIV-1 X4 and R5 viruses including primary isolates (Munk et al., 2003; Wang et al., 2003; Wang et al., 2004). Unlike α - and β -defensins, retrocyclin does not appear to directly inactivate the HIV virion although it is not clear whether the experiments reported to date were performed under serum-free condition (Cole et al., 2002). Retrocyclin does however bind to HIV gp120 as well as CD4 with high affinity, which is consistent with inhibition of viral entry (Cole et al., 2002)(Munk et al., 2003). This high-binding affinity to glycosylated gp120 and CD4 is mediated through interactions with their O-linked and N-linked sugars (Wang et al., 1998). Serum strongly reduces their binding to gp120 (Wang et al., 2004). RTD1 binds directly to the C-terminal heptad repeat of HIV envelope protein gp41, blocking formation of the six helix bundle required for fusion (Gallo et al., 2006). Recently, high concentrations of RTD1 and HNP1 have been shown to down-regulate CXCR4 in PBMCs in the absence of serum (Nittayananta et al., 2009). Studies on retrocyclin-1 analogues indicate that modification of this peptide can enhance its potency against HIV *in vitro* (Owen et al., 2004), suggesting their potential use as a microbicide.

4. Role of Defensins in HIV pathogenesis and transmission

Depending on the preparation of samples and analytical methods, the levels of defensins can be varied from one report to another. In addition, defensins have been found to interact with other cellular proteins in plasma (Higazi et al., 1996; Panyutich and Ganz, 1991; Panyutich et al., 1994), which may affect the measurement of defensin levels by ELISA. In healthy donors, the plasma concentration of HNPs1-3 is ranging from ~150-500 ng/ml (Mukae et al., 2002). The levels of defensins in the plasma or at the mucosa are frequently elevated in patients with infections or diseases (Coffelt and Scandurro, 2008; Ihi et al., 1997). For examples, defensin levels in plasma from patients with sepsis reach 900-170,000 ng/ml (Panyutich et al., 1993). Using liquid chromatography-tandem mass spectrometry, the levels of HNPs in the saliva from healthy donors range from 1 to 10 ug/ml, whereas the level of HBDs 1-2 range from undetectable to 33 ng/ml (Gardner et al., 2009). The level of HNPs in cervicovaginal fluid from healthy women ranges from 250 ng/ml to 5 μ g/ml depending on the laboratories (Levinson et al., 2009; Simhan et al., 2007).

HNPs1-3 The role of HNPs in HIV pathogenesis was first suggested that HNPs 1-3 were to account for the soluble anti-HIV activity of CD8⁺ T cells (CAF) isolated from patients infected with HIV but remaining free of AIDS for a prolonged period (long-term nonprogressors, LTNPs) (Zhang et al., 2002). These peptides were detected in the media of stimulated CD8⁺ T cells from normal healthy controls and LTNPs but not from HIV progressors. Subsequent studies on the cell source of defensins revealed that HNPs were probably produced by co-cultured monocytes and residual granulocytes of allogenic normal donor irradiated PBMCs that were used as feeder cells, but they were not produced by the CD8⁺ T cells themselves (Mackewicz et al., 2003; Zaharatos et al., 2004). Using similar co-culture systems, levels of HNPs1-3 were measured in CD8⁺ T-cell supernatants and cervical-vaginal mononuclear cells derived from HIV-exposed seronegative individuals, HIV-

infected patients, and normal controls (Trabattoni et al., 2004). Higher levels of HNPs were found in CD8⁺ T cells from HIV-exposed seronegative individuals and HIV patients compared to normal controls.

D'Agostino et al. recently found that HIV-infected patients have a higher level of HNPs in plasma than healthy donors (D'Agostino et al., 2009). Using a co-culture system with irradiated PBMCs, higher levels of HNPs in CD8⁺ T cells were found in patients with HIV infection compared to the healthy donors, and the intracellular HNP levels were further increased in stimulated CD8⁺ T cells. The intracellular level of HNPs in neutrophils is higher in HIV-infected patients than healthy donors. There is no significant difference in the plasma level of HNPs in HIV-infected patients with or without antiviral treatment (ART). However, reduction of HNPs in CD8⁺ T cell was found in HIV-infected patients on ART. Interestingly, this reduction in the HNP level was not found in HIV-infected patients on ART with virologic failure. In contrast to the report by D'Agostino et al., Rodriguez-Garcia et al. did not observe any association between plasma levels of HNPs and immunologic or virologic parameters (Rodriguez-Garcia et al., 2010). This report also described an increase in HNPs1-3 in dendritic cells, differentiated *in vitro*, in HIV controllers but not non-controllers compared to healthy controls. While it was suggested that increased HNPs1-3 production by dendritic cells in HIV-infected patients is associated with slower disease progression, analysis of specific immune cell subsets without further manipulation is needed to clarify the role of HNPs in HIV disease progression.

The association between production of HNPs1-3 in breast milk and transmission of HIV has also been investigated (Kuhn et al., 2005). In a case-controlled study of HIV-positive women, levels of HNPs in breast milk correlated with HIV RNA copy number in breast milk, which was a strong predictor of transmission. However, after adjusting for breast milk HIV copy number, higher levels of HNPs in breast milk were associated with a decreased incidence of intrapartum or postnatal HIV transmission. Bosire and colleagues performed similar studies to determine the correlation between the level of HNPs in breast milk and transmission risk in a cohort of 260 HIV-1-infected pregnant women in Nairobi followed for 12 months postpartum with their infants (Bosire et al., 2007). Analysis of breast milk from these women at month 1 postpartum demonstrated that women with detectable alpha-defensins and significantly higher mean breast milk HIV-1 RNA levels than women with undetectable alpha-defensins. Increased alpha-defensins concentrations in breast milk were also associated with subclinical mastitis and increased CC-chemokines in breast milk. Interestingly, in contrast to the report by Kuhn et al. (Kuhn et al., 2005), the level of defensins are not associated with vertical transmission, indicating a complex interplay between innate effectors, inflammation and HIV transmission.

There is a correlation between the abundance of several anti-HIV proteins, including HNPs1-3 and cell-associated HIV replication in lymphoid follicles compared with extrafollicular lymphoid tissue (Folkvord et al., 2005). Expression of these antiviral proteins is significantly lower in the follicular region, where HIV replication is concentrated, compared with the extrafollicular regions in lymph nodes from HIV-positive individuals.

Cationic peptides including defensins are required for anti-HIV activity of vaginal fluid from healthy women (Venkataraman et al., 2005). While it is well established that sexual transmitted infections (STIs) significantly increase the likelihood of HIV transmission (Chesson and Pinkerton, 2000; Cohen et al., 1997; Galvin and Cohen, 2004; Mabey, 2000; Plummer, 1998) and that levels of defensins including HNPs, HBDs and HD5 in genital fluid, are elevated in patients with STIs (Porter et al., 2005; Simhan et al., 2007; Valore et al.,

2006; Wiesenfeld et al., 2002), the role of defensins in HIV transmission seems to be complex. Studies using a cohort of HIV uninfected sex workers in Kenya demonstrated the association between an increase in HNPs and LL-37 levels in the IgA-depleted cervicovaginal secretions from women with bacterial STIs and increase in HIV acquisition, despite that cervicovaginal secretions with high levels of HNPs and LL-37 exhibited anti-HIV activity in vitro (Levinson et al., 2009). This study underscores the complex role of defensins in HIV transmission at the vaginal mucosa and the urgent need to define the effect of elevated innate effectors on immune responses that contribute to enhanced HIV acquisition.

HBDs Significant correlations between the single-nucleotide polymorphism (SNPs) -44C/G and -20G/A in 5' untranslated region of *DEFB1* (coding for HBD1) and a risk of perinatal transmission of HIV-1 in Italian and Brazilian populations, respectively (Braida et al., 2004; Milanese et al., 2006). The SNP -52G/G genotype is associated with reduced HIV-1 RNA in breast milk, but not in plasma in Mozabican HIV-infected women (Baroncelli et al., 2008). Interestingly, the functional analysis of promoter indicates that these SNPs suppress expression (Milanese et al., 2007). Studies on the role of HBD1 in mother-to-child transmission of HIV indicated that the -52G/G genotype and the -44/-52G haplotype exhibited a protective role against HIV infection in children, whereas the -52G/G genotype and the -44G/-52G haplotype were associated with low levels of HIV plasma viremia and a lower risk of maternal HIV transmission in mothers (Ricci et al., 2009). Although HBD1 does not exhibit any effect on HIV infection in vitro, the presence of SNP may affect HIV transmission by modulating immune response.

The role of defensins in protection against HIV infection has been studied in HIV-exposed seronegative (ESN) individuals. ESN expressed significantly greater mRNA copy numbers of HBD2 and 3 in oral mucosa than healthy controls, while no difference in mRNA copy numbers of HBD-1, 2 and 3 in vaginal and endocervical mucosa was observed between ESN and controls (Zapata et al., 2008). In addition, homozygosity for the A692G polymorphism is significantly more frequent in ESN than in seropositive individuals (Zapata et al., 2008). Sequence analysis of θ -defensin pseudogenes in ESN female sex-workers from Thailand revealed that all subjects had premature stop codons (Yang et al., 2005). Therefore, restoration of endogenous θ -defensin production does not account for the resistance to HIV-1 infection in these women.

5. Conclusion

Defensins play an important role in innate immune response. These peptides display versatile functions in modulating various immunological and biological aspects. Aberrant defensin expression has been associated with many human diseases (de Leeuw and Lu, 2007), although studies on the role of defensins in HIV pathogenesis and transmission in humans just began to reveal the complex functions of defensins in modulating HIV infection. While the innate immune system is evolutionarily conserved among multicellular organisms, it is challenging to find a suitable animal model to study the role of defensins in HIV pathogenesis and transmission due to complex diversity of defensins in mammals as well as apparent differences in mechanisms of action. Recently, increased expression of rhesus enteric α -defensins (REDs) in response to SIV infection was reported (Zaragoza et al., 2011). Additionally, decreased RED protein levels correlate with enteric opportunistic infection and advanced SIV disease. However, the primary sequences of RED and HD5

differ and it is not clear whether REDs could represent HD5. Future studies focusing on the development of a better animal model for studying innate immunity in HIV transmission and pathogenesis as well as careful assessments of immune responses in patients with reduced or elevated levels of defensins will shed light on the development of better strategies for HIV therapeutics.

6. References

- Agerberth, B., J. Charo, J. Werr, B. Olsson, F. Idali, L. Lindbom, R. Kiessling, H. Jornvall, H. Wigzell, and G.H. Gudmundsson. 2000. The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. *Blood* 96:3086-3093.
- Armogida, S.A., N.M. Yannaras, A.L. Melton, and M.D. Srivastava. 2004. Identification and quantification of innate immune system mediators in human breast milk. *Allergy Asthma Proc* 25:297-304.
- Baroncelli, S., E. Ricci, M. Andreotti, G. Guidotti, P. Germano, M.C. Marazzi, S. Vella, L. Palombi, A. De Rossi, and M. Giuliano. 2008. Single-nucleotide polymorphisms in human beta-defensin-1 gene in Mozambican HIV-1-infected women and correlation with virologic parameters. *Aids* 22:1515-1517.
- Biragyn, A., P.A. Ruffini, C.A. Leifer, E. Klyushnenkova, A. Shakhov, O. Chertov, A.K. Shirakawa, J.M. Farber, D.M. Segal, J.J. Oppenheim, and L.W. Kwak. 2002. Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. *Science* 298:1025-1029.
- Boniotto, M., W.J. Jordan, J. Eskdale, A. Tossi, N. Antcheva, S. Crovella, N.D. Connell, and G. Gallagher. 2006. Human beta-defensin 2 induces a vigorous cytokine response in peripheral blood mononuclear cells. *Antimicrob Agents Chemother* 50:1433-1441.
- Bosire, R., G.C. John-Stewart, J.M. Mabuka, G. Wariua, C. Gichuhi, D. Wamalwa, J. Ruzinski, R. Goodman, B. Lohman, D.A. Mbori-Ngacha, J. Overbaugh, and C. Farquhar. 2007. Breast milk alpha-defensins are associated with HIV type 1 RNA and CC chemokines in breast milk but not vertical HIV type 1 transmission. *AIDS Res Hum Retroviruses* 23:198-203.
- Braida, L., M. Boniotto, A. Pontillo, P.A. Tovo, A. Amoroso, and S. Crovella. 2004. A single-nucleotide polymorphism in the human beta-defensin 1 gene is associated with HIV-1 infection in Italian children. *Aids* 18:1598-1600.
- Chalifour, A., P. Jeannin, J.F. Gauchat, A. Blaecke, M. Malissard, T. N'Guyen, N. Thieblemont, and Y. Delneste. 2004. Direct bacterial protein PAMP recognition by human NK cells involves TLRs and triggers alpha-defensin production. *Blood* 104:1778-1783.
- Chang, T.L., F. Francois, A. Mosoian, and M.E. Klotman. 2003. CAF-mediated human immunodeficiency virus (HIV) type 1 transcriptional inhibition is distinct from alpha-defensin-1 HIV inhibition. *J Virol* 77:6777-6784.
- Chang, T.L., J. Vargas, Jr., A. DelPortillo, and M.E. Klotman. 2005. Dual role of alpha-defensin-1 in anti-HIV-1 innate immunity. *J Clin Invest* 115:765-773.

- Charp, P.A., W.G. Rice, R.L. Raynor, E. Reimund, J.M. Kinkade, Jr., T. Ganz, M.E. Selsted, R.I. Lehrer, and J.F. Kuo. 1988. Inhibition of protein kinase C by defensins, antibiotic peptides from human neutrophils. *Biochem Pharmacol* 37:951-956.
- Chertov, O., D.F. Michiel, L. Xu, J.M. Wang, K. Tani, W.J. Murphy, D.L. Longo, D.D. Taub, and J.J. Oppenheim. 1996. Identification of defensin-1, defensin-2, and CAP37/azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils. *J Biol Chem* 271:2935-2940.
- Chesson, H.W., and S.D. Pinkerton. 2000. Sexually transmitted diseases and the increased risk for HIV transmission: implications for cost-effectiveness analyses of sexually transmitted disease prevention interventions. *J Acquir Immune Defic Syndr* 24:48-56.
- Coffelt, S.B., and A.B. Scandurro. 2008. Tumors sound the alarmin(s). *Cancer Res* 68:6482-6485.
- Cohen, M.S., I.F. Hoffman, R.A. Royce, P. Kazembe, J.R. Dyer, C.C. Daly, D. Zimba, P.L. Vernazza, M. Maida, S.A. Fiscus, and J.J. Eron, Jr. 1997. Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. *Lancet* 349:1868-1873.
- Cole, A.M., T. Hong, L.M. Boo, T. Nguyen, C. Zhao, G. Bristol, J.A. Zack, A.J. Waring, O.O. Yang, and R.I. Lehrer. 2002. Retrocyclin: a primate peptide that protects cells from infection by T- and M-tropic strains of HIV-1. *Proc Natl Acad Sci U S A* 99:1813-1818.
- Cunliffe, R.N. 2003. Alpha-defensins in the gastrointestinal tract. *Mol Immunol* 40:463-467.
- Cunliffe, R.N., F.R. Rose, J. Keyte, L. Abberley, W.C. Chan, and Y.R. Mahida. 2001. Human defensin 5 is stored in precursor form in normal Paneth cells and is expressed by some villous epithelial cells and by metaplastic Paneth cells in the colon in inflammatory bowel disease. *Gut* 48:176-185.
- D'Agostino, C., M. Lichtner, C.M. Mastroianni, G. Ceccarelli, M. Iannetta, S. Antonucci, V. Vullo, and A.P. Massetti. 2009. In vivo release of alpha-defensins in plasma, neutrophils and CD8 T-lymphocytes of patients with HIV infection. *Curr HIV Res* 7:650-655.
- Daher, K.A., M.E. Selsted, and R.I. Lehrer. 1986. Direct inactivation of viruses by human granulocyte defensins. *J Virol* 60:1068-1074.
- de Leeuw, E., and W. Lu. 2007. Human defensins: turning defense into offense? *Infect Disord Drug Targets* 7:67-70.
- Ding, J., A. Rapista, N. Teleshova, W. Lu, M.E. Klotman, and T.L. Chang. 2011. Mucosal human defensins 5 and 6 antagonize the anti-HIV activity of candidate polyanion microbicides. *J Innate Immun* 3:208-212.
- Driss, V., F. Legrand, E. Hermann, S. Loiseau, Y. Guerardel, L. Kremer, E. Adam, G. Woerly, D. Dombrowicz, and M. Capron. 2008. TLR2-dependent eosinophil interactions with mycobacteria : role of {alpha}-defensins. *Blood*
- Duits, L.A., P.H. Nibbering, E. van Strijen, J.B. Vos, S.P. Mannesse-Lazeroms, M.A. van Sterkenburg, and P.S. Hiemstra. 2003. Rhinovirus increases human beta-defensin-2 and -3 mRNA expression in cultured bronchial epithelial cells. *FEMS Immunol Med Microbiol* 38:59-64.

- Duits, L.A., B. Ravensbergen, M. Rademaker, P.S. Hiemstra, and P.H. Nibbering. 2002. Expression of beta-defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. *Immunology* 106:517-525.
- Fahlgren, A., S. Hammarstrom, A. Danielsson, and M.L. Hammarstrom. 2003. Increased expression of antimicrobial peptides and lysozyme in colonic epithelial cells of patients with ulcerative colitis. *Clin Exp Immunol* 131:90-101.
- Fan, S.R., X.P. Liu, and Q.P. Liao. 2008. Human defensins and cytokines in vaginal lavage fluid of women with bacterial vaginosis. *Int J Gynaecol Obstet*
- Fellermann, K., and E.F. Stange. 2001. Defensins -- innate immunity at the epithelial frontier. *Eur J Gastroenterol Hepatol* 13:771-776.
- Folkvord, J.M., C. Armon, and E. Connick. 2005. Lymphoid follicles are sites of heightened human immunodeficiency virus type 1 (HIV-1) replication and reduced antiretroviral effector mechanisms. *AIDS Res Hum Retroviruses* 21:363-370.
- Funderburg, N., M.M. Lederman, Z. Feng, M.G. Drage, J. Jadlowsky, C.V. Harding, A. Weinberg, and S.F. Sieg. 2007. Human α -defensin-3 activates professional antigen-presenting cells via Toll-like receptors 1 and 2. *Proc Natl Acad Sci U S A* 104:18631-18635.
- Furci, L., F. Sironi, M. Tolazzi, L. Vassena, and P. Lusso. 2007. Alpha-defensins block the early steps of HIV-1 infection: interference with the binding of gp120 to CD4. *Blood* 109:2928-2935.
- Gallo, S.A., W. Wang, S.S. Rawat, G. Jung, A.J. Waring, A.M. Cole, H. Lu, X. Yan, N.L. Daly, D.J. Craik, S. Jiang, R.I. Lehrer, and R. Blumenthal. 2006. Theta-defensins prevent HIV-1 Env-mediated fusion by binding gp41 and blocking 6-helix bundle formation. *J Biol Chem* 281:18787-18792.
- Galvin, S.R., and M.S. Cohen. 2004. The role of sexually transmitted diseases in HIV transmission. *Nat Rev Microbiol* 2:33-42.
- Ganz, T. 1987. Extracellular release of antimicrobial defensins by human polymorphonuclear leukocytes. *Infect Immun* 55:568-571.
- Ganz, T. 2002. Epithelia: not just physical barriers. *Proc Natl Acad Sci U S A* 99:3357-3358.
- Ganz, T. 2003. Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol* 3:710-720.
- Ganz, T., M.E. Selsted, D. Szklarek, S.S. Harwig, K. Daher, D.F. Bainton, and R.I. Lehrer. 1985. Defensins. Natural peptide antibiotics of human neutrophils. *J Clin Invest* 76:1427-1435.
- Garcia, J.R., F. Jaumann, S. Schulz, A. Krause, J. Rodriguez-Jimenez, U. Forssmann, K. Adermann, E. Kluver, C. Vogelmeier, D. Becker, R. Hedrich, W.G. Forssmann, and R. Bals. 2001. Identification of a novel, multifunctional beta-defensin (human beta-defensin 3) with specific antimicrobial activity. Its interaction with plasma membranes of *Xenopus* oocytes and the induction of macrophage chemoattraction. *Cell Tissue Res* 306:257-264.
- Gardner, M.S., M.D. Rowland, A.Y. Siu, J.L. Bundy, D.K. Wagener, and J.L. Stephenson. 2009. Comprehensive defensin assay for saliva. *Anal Chem* 81:557-566.
- George, M.D., J. Wehkamp, R.J. Kays, C.M. Leutenegger, S. Sabir, I. Grishina, S. Dandekar, and C.L. Bevins. 2008. In vivo gene expression profiling of human intestinal

- epithelial cells: analysis by laser microdissection of formalin fixed tissues. *BMC Genomics* 9:209.
- Ghosh, D., E. Porter, B. Shen, S.K. Lee, D. Wilk, J. Drazba, S.P. Yadav, J.W. Crabb, T. Ganz, and C.L. Bevins. 2002. Paneth cell trypsin is the processing enzyme for human defensin-5. *Nat Immunol* 3:583-590.
- Guo, C.J., N. Tan, L. Song, S.D. Douglas, and W.Z. Ho. 2004. Alpha-defensins inhibit HIV infection of macrophages through upregulation of CC-chemokines. *Aids* 18:1217-1218.
- Harder, J., J. Bartels, E. Christophers, and J.M. Schroder. 2001. Isolation and characterization of human beta -defensin-3, a novel human inducible peptide antibiotic. *J Biol Chem* 276:5707-5713.
- Hein, M., E.V. Valore, R.B. Helmig, N. Uldbjerg, and T. Ganz. 2002. Antimicrobial factors in the cervical mucus plug. *Am J Obstet Gynecol* 187:137-144.
- Heine, R.P., H. Wiesenfeld, L. Mortimer, and P.C. Greig. 1998. Amniotic fluid defensins: potential markers of subclinical intrauterine infection. *Clin Infect Dis* 27:513-518.
- Hertz, C.J., Q. Wu, E.M. Porter, Y.J. Zhang, K.H. Weismuller, P.J. Godowski, T. Ganz, S.H. Randell, and R.L. Modlin. 2003. Activation of Toll-like receptor 2 on human tracheobronchial epithelial cells induces the antimicrobial peptide human beta defensin-2. *J Immunol* 171:6820-6826.
- Higazi, A.A., T. Ganz, K. Kariko, and D.B. Cines. 1996. Defensin modulates tissue-type plasminogen activator and plasminogen binding to fibrin and endothelial cells. *J Biol Chem* 271:17650-17655.
- Higazi, A.A., T. Nassar, T. Ganz, D.J. Rader, R. Udassin, K. Bdeir, E. Hiss, B.S. Sachais, K.J. Williams, E. Leitersdorf, and D.B. Cines. 2000. The alpha-defensins stimulate proteoglycan-dependent catabolism of low-density lipoprotein by vascular cells: a new class of inflammatory apolipoprotein and a possible contributor to atherogenesis. *Blood* 96:1393-1398.
- Hill, C.P., J. Yee, M.E. Selsted, and D. Eisenberg. 1991. Crystal structure of defensin HNP-3, an amphiphilic dimer: mechanisms of membrane permeabilization. *Science* 251:1481-1485.
- Hoover, D.M., O. Chertov, and J. Lubkowski. 2001. The structure of human beta-defensin-1: new insights into structural properties of beta-defensins. *J Biol Chem* 276:39021-39026.
- Huang, F., C.Y. Kao, S. Wachi, P. Thai, J. Ryu, and R. Wu. 2007. Requirement for both JAK-mediated PI3K signaling and ACT1/TRAF6/TAK1-dependent NF-kappaB activation by IL-17A in enhancing cytokine expression in human airway epithelial cells. *J Immunol* 179:6504-6513.
- Ihi, T., M. Nakazato, H. Mukae, and S. Matsukura. 1997. Elevated concentrations of human neutrophil peptides in plasma, blood, and body fluids from patients with infections. *Clin Infect Dis* 25:1134-1140.
- Jan, M.S., Y.H. Huang, B. Shieh, R.H. Teng, Y.P. Yan, Y.T. Lee, K.K. Liao, and C. Li. 2006. CC Chemokines Induce Neutrophils to Chemotaxis, Degranulation, and alpha-Defensin Release. *J Acquir Immune Defic Syndr* 41:6-16.

- Jang, B.C., K.J. Lim, M.H. Suh, J.G. Park, and S.I. Suh. 2007. Dexamethasone suppresses interleukin-1 β -induced human beta-defensin 2 mRNA expression: involvement of p38 MAPK, JNK, MKP-1, and NF-kappaB transcriptional factor in A549 cells. *FEMS Immunol Med Microbiol* 51:171-184.
- Jia, H.P., T. Starner, M. Ackermann, P. Kirby, B.F. Tack, and P.B. McCray, Jr. 2001. Abundant human beta-defensin-1 expression in milk and mammary gland epithelium. *J Pediatr* 138:109-112.
- Joseph, G., L. Tarnow, A.S. Astrup, T.K. Hansen, H.H. Parving, A. Flyvbjerg, and J. Frystyk. 2008. Plasma alpha-defensin is associated with cardiovascular morbidity and mortality in type 1 diabetic patients. *J Clin Endocrinol Metab* 93:1470-1475.
- Kao, C.Y., C. Kim, F. Huang, and R. Wu. 2008. Requirements for two proximal NF-kappaB binding sites and IkappaB-zeta in IL-17A-induced human beta-defensin 2 expression by conducting airway epithelium. *J Biol Chem* 283:15309-15318.
- Klotman, M.E., and T.L. Chang. 2006. Defensins in innate antiviral immunity. *Nat Rev Immunol* 6:447-456.
- Klotman, M.E., A. Rapista, N. Teleshova, A. Micsenyi, G.A. Jarvis, W. Lu, E. Porter, and T.L. Chang. 2008. Neisseria gonorrhoeae-Induced Human Defensins 5 and 6 Increase HIV Infectivity: Role in Enhanced Transmission. *J Immunol* 180:6176-6185.
- Kruse, T., and H.H. Kristensen. 2008. Using antimicrobial host defense peptides as anti-infective and immunomodulatory agents. *Expert Rev Anti Infect Ther* 6:887-895.
- Kuhn, L., D. Trabattoni, C. Kankasa, K. Semrau, P. Kasonde, F. Lissoni, M. Sinkala, M. Ghosh, C. Vwalika, G.M. Aldrovandi, D.M. Thea, and M. Clerici. 2005. Alpha-defensins in the prevention of HIV transmission among breastfed infants. *J Acquir Immune Defic Syndr* 39:138-142.
- Lehrer, R.I. 2004. Primate defensins. *Nat Rev Microbiol* 2:727-738.
- Lehrer, R.I., A.K. Lichtenstein, and T. Ganz. 1993. Defensins: antimicrobial and cytotoxic peptides of mammalian cells. *Annu Rev Immunol* 11:105-128.
- Leonova, L., V.N. Kokryakov, G. Aleshina, T. Hong, T. Nguyen, C. Zhao, A.J. Waring, and R.I. Lehrer. 2001. Circular minidefensins and posttranslational generation of molecular diversity. *J Leukoc Biol* 70:461-464.
- Levinson, P., R. Kaul, J. Kimani, E. Ngugi, S. Moses, K.S. MacDonald, K. Broliden, and T. Hirbod. 2009. Levels of innate immune factors in genital fluids: association of alpha defensins and LL-37 with genital infections and increased HIV acquisition. *Aids* 23:309-317.
- Liu, C.Y., H.C. Lin, C.T. Yu, S.M. Lin, K.Y. Lee, H.C. Chen, C.L. Chou, C.D. Huang, P.C. Chou, W.T. Liu, C.H. Wang, and H.P. Kuo. 2007. The concentration-dependent chemokine release and pro-apoptotic effects of neutrophil-derived alpha-defensin-1 on human bronchial and alveolar epithelial cells. *Life Sci* 80:749-758.
- Liu, H.Y., Q.F. Collins, F. Moukdar, D. Zhuo, J. Han, T. Hong, S. Collins, and W. Cao. 2008. Suppression of hepatic glucose production by human neutrophil alpha-defensins through a signaling pathway distinct from insulin. *J Biol Chem* 283:12056-12063.
- Mabey, D. 2000. Interactions between HIV infection and other sexually transmitted diseases. *Trop Med Int Health* 5:A32-36.

- Mackewicz, C.E., J. Yuan, P. Tran, L. Diaz, E. Mack, M.E. Selsted, and J.A. Levy. 2003. alpha-Defensins can have anti-HIV activity but are not CD8 cell anti-HIV factors. *Aids* 17:F23-32.
- Milanese, M., L. Segat, and S. Crovella. 2007. Transcriptional effect of DEFB1 gene 5' untranslated region polymorphisms. *Cancer Res* 67:5997; author reply 5997.
- Milanese, M., L. Segat, A. Pontillo, L.C. Arraes, J.L. de Lima Filho, and S. Crovella. 2006. DEFB1 gene polymorphisms and increased risk of HIV-1 infection in Brazilian children. *Aids* 20:1673-1675.
- Mukae, H., H. Iiboshi, M. Nakazato, T. Hiratsuka, M. Tokojima, K. Abe, J. Ashitani, J. Kadota, S. Matsukura, and S. Kohno. 2002. Raised plasma concentrations of alpha-defensins in patients with idiopathic pulmonary fibrosis. *Thorax* 57:623-628.
- Munk, C., G. Wei, O.O. Yang, A.J. Waring, W. Wang, T. Hong, R.I. Lehrer, N.R. Landau, and A.M. Cole. 2003. The theta-defensin, retrocyclin, inhibits HIV-1 entry. *AIDS Res Hum Retroviruses* 19:875-881.
- Nagy, I., A. Pivarcsi, A. Koreck, M. Szell, E. Urban, and L. Kemeny. 2005. Distinct strains of *Propionibacterium acnes* induce selective human beta-defensin-2 and interleukin-8 expression in human keratinocytes through toll-like receptors. *J Invest Dermatol* 124:931-938.
- Nakashima, H., N. Yamamoto, M. Masuda, and N. Fujii. 1993. Defensins inhibit HIV replication in vitro. *Aids* 7:1129.
- Narimatsu, R., D. Wolday, and B.K. Patterson. 2005. IL-8 increases transmission of HIV type 1 in cervical explant tissue. *AIDS Res Hum Retroviruses* 21:228-233.
- Nassar, T., S. Akkawi, R. Bar-Shavit, A. Haj-Yehia, K. Bdeir, A.B. Al-Mehdi, M. Tarshis, and A.A. Higazi. 2002. Human alpha-defensin regulates smooth muscle cell contraction: a role for low-density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor. *Blood* 100:4026-4032.
- Nguyen, T.X., A.M. Cole, and R.I. Lehrer. 2003. Evolution of primate theta-defensins: a serpentine path to a sweet tooth. *Peptides* 24:1647-1654.
- Nittayananta, W., F. Hladik, M. Klausner, S. Harb, B.A. Dale, and R.W. Coombs. 2009. HIV type 1 fails to trigger innate immune factor synthesis in differentiated oral epithelium. *AIDS Res Hum Retroviruses* 25:1013-1021.
- Niyonsaba, F., M. Hirata, H. Ogawa, and I. Nagaoka. 2003. Epithelial cell-derived antibacterial peptides human beta-defensins and cathelicidin: multifunctional activities on mast cells. *Curr Drug Targets Inflamm Allergy* 2:224-231.
- Okrent, D.G., A.K. Lichtenstein, and T. Ganz. 1990. Direct cytotoxicity of polymorphonuclear leukocyte granule proteins to human lung-derived cells and endothelial cells. *Am Rev Respir Dis* 141:179-185.
- Owen, S.M., D.L. Rudolph, W. Wang, A.M. Cole, A.J. Waring, R.B. Lal, and R.I. Lehrer. 2004. RC-101, a retrocyclin-1 analogue with enhanced activity against primary HIV type 1 isolates. *AIDS Res Hum Retroviruses* 20:1157-1165.
- Panyutich, A., and T. Ganz. 1991. Activated alpha 2-macroglobulin is a principal defensin-binding protein. *Am J Respir Cell Mol Biol* 5:101-106.

- Panyutich, A.V., E.A. Panyutich, V.A. Krapivin, E.A. Baturevich, and T. Ganz. 1993. Plasma defensin concentrations are elevated in patients with septicemia or bacterial meningitis. *J Lab Clin Med* 122:202-207.
- Panyutich, A.V., O. Szold, P.H. Poon, Y. Tseng, and T. Ganz. 1994. Identification of defensin binding to C1 complement. *FEBS Lett* 356:169-173.
- Pardi, A., X.L. Zhang, M.E. Selsted, J.J. Skalicky, and P.F. Yip. 1992. NMR studies of defensin antimicrobial peptides. 2. Three-dimensional structures of rabbit NP-2 and human HNP-1. *Biochemistry* 31:11357-11364.
- Pazgier, M., D.M. Hoover, D. Yang, W. Lu, and J. Lubkowski. 2006. Human beta-defensins. *Cell Mol Life Sci* 63:1294-1313.
- Pivarcsi, A., I. Nagy, A. Koreck, K. Kis, A. Kenderessy-Szabo, M. Szell, A. Dobozy, and L. Kemeny. 2005. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. *Microbes Infect* 7:1117-1127.
- Plummer, F.A. 1998. Heterosexual transmission of human immunodeficiency virus type 1 (HIV): interactions of conventional sexually transmitted diseases, hormonal contraception and HIV-1. *AIDS Res Hum Retroviruses* 14 Suppl 1:S5-10.
- Porter, E., H. Yang, S. Yavagal, G.C. Preza, O. Murillo, H. Lima, S. Greene, L. Mahoozi, M. Klein-Patel, G. Diamond, S. Gulati, T. Ganz, P.A. Rice, and A.J. Quayle. 2005. Distinct defensin profiles in *Neisseria gonorrhoeae* and *Chlamydia trachomatis* urethritis reveal novel epithelial cell-neutrophil interactions. *Infect Immun* 73:4823-4833.
- Proud, D., S.P. Sanders, and S. Wiehler. 2004. Human rhinovirus infection induces airway epithelial cell production of human beta-defensin 2 both in vitro and in vivo. *J Immunol* 172:4637-4645.
- Quayle, A.J., E.M. Porter, A.A. Nussbaum, Y.M. Wang, C. Brabec, K.P. Yip, and S.C. Mok. 1998. Gene expression, immunolocalization, and secretion of human defensin-5 in human female reproductive tract. *Am J Pathol* 152:1247-1258.
- Quinones-Mateu, M.E., M.M. Lederman, Z. Feng, B. Chakraborty, J. Weber, H.R. Rangel, M.L. Marotta, M. Mirza, B. Jiang, P. Kiser, K. Medvik, S.F. Sieg, and A. Weinberg. 2003. Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. *Aids* 17:F39-48.
- Rapista, A., J. Ding, B. Benito, Y.T. Lo, M.B. Neiditch, W. Lu, and T.L. Chang. 2011. Human defensins 5 and 6 enhance HIV-1 infectivity through promoting HIV attachment. *Retrovirology* 8:45.
- Rehaume, L.M., and R.E. Hancock. 2008. Neutrophil-derived defensins as modulators of innate immune function. *Crit Rev Immunol* 28:185-200.
- Ricci, E., S. Malacrida, M. Zanchetta, M. Montagna, C. Giaquinto, and A.D. Rossi. 2009. Role of beta-Defensin-1 Polymorphisms in Mother-to-Child Transmission of Human Immunodeficiency Virus Type 1. *J Acquir Immune Defic Syndr*
- Rodriguez-Garcia, M., N. Climent, H. Oliva, V. Casanova, R. Franco, A. Leon, J.M. Gatell, F. Garcia, and T. Gallart. 2010. Increased alpha-defensins 1-3 production by dendritic cells in HIV-infected individuals is associated with slower disease progression. *PLoS One* 5:e9436.

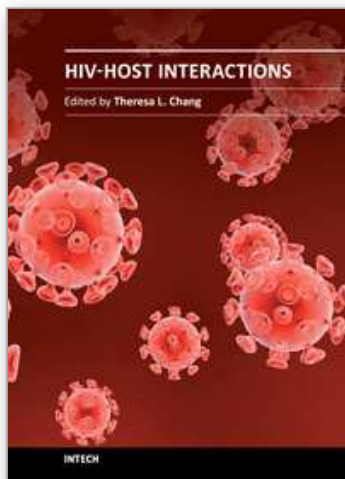
- Rodriguez-Garcia, M., H. Oliva, N. Climent, F. Garcia, J.M. Gatell, and T. Gallart. 2007. Human immature monocyte-derived dendritic cells produce and secrete alpha-defensins 1-3. *J Leukoc Biol* 82:1143-1146.
- Salzman, N.H., M.A. Underwood, and C.L. Bevins. 2007. Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa. *Semin Immunol* 19:70-83.
- Saraheimo, M., C. Forsblom, K. Pettersson-Fernholm, A. Flyvbjerg, P.H. Groop, and J. Frystyk. 2008. Increased levels of alpha-defensin (-1, -2 and -3) in type 1 diabetic patients with nephropathy. *Nephrol Dial Transplant* 23:914-918.
- Schaefer, T.M., J.V. Fahey, J.A. Wright, and C.R. Wira. 2005. Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C). *J Immunol* 174:992-1002.
- Schutte, B.C., J.P. Mitros, J.A. Bartlett, J.D. Walters, H.P. Jia, M.J. Welsh, T.L. Casavant, and P.B. McCray, Jr. 2002. Discovery of five conserved beta -defensin gene clusters using a computational search strategy. *Proc Natl Acad Sci U S A* 99:2129-2133.
- Selsted, M.E., and A.J. Ouellette. 2005. Mammalian defensins in the antimicrobial immune response. *Nat Immunol* 6:551-557.
- Shi, J., S. Aono, W. Lu, A.J. Ouellette, X. Hu, Y. Ji, L. Wang, S. Lenz, F.W. van Ginkel, M. Liles, C. Dykstra, E.E. Morrison, and C.O. Elson. 2007. A novel role for defensins in intestinal homeostasis: regulation of IL-1beta secretion. *J Immunol* 179:1245-1253.
- Simhan, H.N., B.L. Anderson, M.A. Krohn, R.P. Heine, B. Martinez de Tejada, D.V. Landers, and S.L. Hillier. 2007. Host immune consequences of asymptomatic *Trichomonas vaginalis* infection in pregnancy. *Am J Obstet Gynecol* 196:59 e51-55.
- Sorensen, O.E., D.R. Thapa, A. Rosenthal, L. Liu, A.A. Roberts, and T. Ganz. 2005. Differential regulation of beta-defensin expression in human skin by microbial stimuli. *J Immunol* 174:4870-4879.
- Sun, L., C.M. Finnegan, T. Kish-Catalone, R. Blumenthal, P. Garzino-Demo, G.M. La Terra Maggiore, S. Berrone, C. Kleinman, Z. Wu, S. Abdelwahab, W. Lu, and A. Garzino-Demo. 2005. Human {beta}-Defensins Suppress Human Immunodeficiency Virus Infection: Potential Role in Mucosal Protection. *J Virol* 79:14318-14329.
- Svinarich, D.M., N.A. Wolf, R. Gomez, B. Gonik, and R. Romero. 1997. Detection of human defensin 5 in reproductive tissues. *Am J Obstet Gynecol* 176:470-475.
- Szyk, A., Z. Wu, K. Tucker, D. Yang, W. Lu, and J. Lubkowski. 2006. Crystal structures of human alpha-defensins HNP4, HD5, and HD6. *Protein Sci* 15:2749-2760.
- Tanabe, H., A.J. Ouellette, M.J. Cocco, and W.E. Robinson, Jr. 2004. Differential effects on human immunodeficiency virus type 1 replication by alpha-defensins with comparable bactericidal activities. *J Virol* 78:11622-11631.
- Tanaka, S., J.C. Edberg, W. Chatham, G. Fassina, and R.P. Kimberly. 2003. Fc gamma RIIb allele-sensitive release of alpha-defensins: anti-neutrophil cytoplasmic antibody-induced release of chemotaxins. *J Immunol* 171:6090-6096.
- Tang, Y.Q., J. Yuan, G. Osapay, K. Osapay, D. Tran, C.J. Miller, A.J. Ouellette, and M.E. Selsted. 1999. A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha-defensins. *Science* 286:498-502.

- Tecle, T., M.R. White, D. Gantz, E.C. Crouch, and K.L. Hartshorn. 2007. Human neutrophil defensins increase neutrophil uptake of influenza A virus and bacteria and modify virus-induced respiratory burst responses. *J Immunol* 178:8046-8052.
- Territo, M.C., T. Ganz, M.E. Selsted, and R. Lehrer. 1989. Monocyte-chemotactic activity of defensins from human neutrophils. *J Clin Invest* 84:2017-2020.
- Trabattoni, D., S.L. Caputo, G. Maffei, F. Vichi, M. Biasin, P. Pierotti, F. Fasano, M. Saresella, M. Franchini, P. Ferrante, F. Mazzotta, and M. Clerici. 2004. Human alpha Defensin in HIV-Exposed But Uninfected Individuals. *J Acquir Immune Defic Syndr* 35:455-463.
- Tran, D., P.A. Tran, Y.Q. Tang, J. Yuan, T. Cole, and M.E. Selsted. 2002. Homodimeric theta-defensins from rhesus macaque leukocytes: isolation, synthesis, antimicrobial activities, and bacterial binding properties of the cyclic peptides. *J Biol Chem* 277:3079-3084.
- Uehara, A., Y. Fujimoto, K. Fukase, and H. Takada. 2007. Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce anti-microbial peptides, but not proinflammatory cytokines. *Mol Immunol* 44:3100-3111.
- Uehara, A., and H. Takada. 2008. Synergism between TLRs and NOD1/2 in oral epithelial cells. *J Dent Res* 87:682-686.
- Valore, E.V., D.J. Wiley, and T. Ganz. 2006. Reversible deficiency of antimicrobial polypeptides in bacterial vaginosis. *Infect Immun* 74:5693-5702.
- Van Wetering, S., S.P. Manneke-Lazeroms, J.H. Dijkman, and P.S. Hiemstra. 1997a. Effect of neutrophil serine proteinases and defensins on lung epithelial cells: modulation of cytotoxicity and IL-8 production. *J Leukoc Biol* 62:217-226.
- Van Wetering, S., S.P. Manneke-Lazeroms, M.A. Van Sterkenburg, M.R. Daha, J.H. Dijkman, and P.S. Hiemstra. 1997b. Effect of defensins on interleukin-8 synthesis in airway epithelial cells. *Am J Physiol* 272:L888-896.
- Venkataraman, N., A.L. Cole, P. Svoboda, J. Pohl, and A.M. Cole. 2005. Cationic polypeptides are required for anti-HIV-1 activity of human vaginal fluid. *J Immunol* 175:7560-7567.
- Vora, P., A. Youdim, L.S. Thomas, M. Fukata, S.Y. Tesfay, K. Lukasek, K.S. Michelsen, A. Wada, T. Hirayama, M. Arditi, and M.T. Abreu. 2004. Beta-defensin-2 expression is regulated by TLR signaling in intestinal epithelial cells. *J Immunol* 173:5398-5405.
- Wang, S.Z., P.K. Smith, M. Lovejoy, J.J. Bowden, J.H. Alpers, and K.D. Forsyth. 1998. The apoptosis of neutrophils is accelerated in respiratory syncytial virus (RSV)-induced bronchiolitis. *Clin Exp Immunol* 114:49-54.
- Wang, W., A.M. Cole, T. Hong, A.J. Waring, and R.I. Lehrer. 2003. Retrocyclin, an antiretroviral theta-defensin, is a lectin. *J Immunol* 170:4708-4716.
- Wang, W., S.M. Owen, D.L. Rudolph, A.M. Cole, T. Hong, A.J. Waring, R.B. Lal, and R.I. Lehrer. 2004. Activity of alpha- and theta-defensins against primary isolates of HIV-1. *J Immunol* 173:515-520.
- Wehkamp, J., J. Harder, M. Weichenthal, M. Schwab, E. Schaffeler, M. Schlee, K.R. Herrlinger, A. Stallmach, F. Noack, P. Fritz, J.M. Schroder, C.L. Bevins, K. Fellermann, and E.F. Stange. 2004. NOD2 (CARD15) mutations in Crohn's disease

- are associated with diminished mucosal alpha-defensin expression. *Gut* 53:1658-1664.
- Wehkamp, J., G. Wang, I. Kubler, S. Nuding, A. Gregorieff, A. Schnabel, R.J. Kays, K. Fellermann, O. Burk, M. Schwab, H. Clevers, C.L. Bevins, and E.F. Stange. 2007. The Paneth cell alpha-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. *J Immunol* 179:3109-3118.
- Wiesenfeld, H.C., R.P. Heine, M.A. Krohn, S.L. Hillier, A.A. Amortegui, M. Nicolazzo, and R.L. Sweet. 2002. Association between elevated neutrophil defensin levels and endometritis. *J Infect Dis* 186:792-797.
- Wilde, C.G., J.E. Griffith, M.N. Marra, J.L. Snable, and R.W. Scott. 1989. Purification and characterization of human neutrophil peptide 4, a novel member of the defensin family. *J Biol Chem* 264:11200-11203.
- Wu, Z., F. Cocchi, D. Gentles, B. Ericksen, J. Lubkowski, A. Devico, R.I. Lehrer, and W. Lu. 2005. Human neutrophil alpha-defensin 4 inhibits HIV-1 infection in vitro. *FEBS Lett* 579:162-166.
- Yamaguchi, Y., T. Nagase, R. Makita, S. Fukuhara, T. Tomita, T. Tominaga, H. Kurihara, and Y. Ouchi. 2002. Identification of multiple novel epididymis-specific beta-defensin isoforms in humans and mice. *J Immunol* 169:2516-2523.
- Yang, C., L. Boone, T.X. Nguyen, D. Rudolph, K. Limpakarnjanarat, T.D. Mastro, J. Tappero, A.M. Cole, and R.B. Lal. 2005. theta-Defensin pseudogenes in HIV-1-exposed, persistently seronegative female sex-workers from Thailand. *Infect Genet Evol* 5:11-15.
- Yang, D., A. Biragyn, D.M. Hoover, J. Lubkowski, and J.J. Oppenheim. 2004. Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. *Annu Rev Immunol* 22:181-215.
- Yang, D., A. Biragyn, L.W. Kwak, and J.J. Oppenheim. 2002. Mammalian defensins in immunity: more than just microbicidal. *Trends Immunol* 23:291-296.
- Yang, D., Q. Chen, O. Chertov, and J.J. Oppenheim. 2000. Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells. *J Leukoc Biol* 68:9-14.
- Yang, D., O. Chertov, S.N. Bykovskaia, Q. Chen, M.J. Buffo, J. Shogan, M. Anderson, J.M. Schroder, J.M. Wang, O.M. Howard, and J.J. Oppenheim. 1999. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science* 286:525-528.
- Yang, D., O. Chertov, and J.J. Oppenheim. 2001. Participation of mammalian defensins and cathelicidins in anti-microbial immunity: receptors and activities of human defensins and cathelicidin (LL-37). *J Leukoc Biol* 69:691-697.
- Zaharatos, G.J., T. He, P. Lopez, W. Yu, J. Yu, and L. Zhang. 2004. alpha-Defensins Released Into Stimulated CD8+ T-Cell Supernatants Are Likely Derived From Residual Granulocytes Within the Irradiated Allogeneic Peripheral Blood Mononuclear Cells Used as Feeders. *J Acquir Immune Defic Syndr* 36:993-1005.
- Zanetti, M. 2004. Cathelicidins, multifunctional peptides of the innate immunity. *J Leukoc Biol* 75:39-48.

- Zapata, W., B. Rodriguez, J. Weber, H. Estrada, M.E. Quinones-Mateu, P.A. Zimmermann, M.M. Lederman, and M.T. Rugeles. 2008. Increased levels of human beta-defensins mRNA in sexually HIV-1 exposed but uninfected individuals. *Curr HIV Res* 6:531-538.
- Zaragoza, M.M., S. Sankaran-Walters, D.R. Canfield, J.K. Hung, E. Martinez, A.J. Ouellette, and S. Dandekar. 2011. Persistence of gut mucosal innate immune defenses by enteric alpha-defensin expression in the simian immunodeficiency virus model of AIDS. *J Immunol* 186:1589-1597.
- Zhang, L., W. Yu, T. He, J. Yu, R.E. Caffrey, E.A. Dalmasso, S. Fu, T. Pham, J. Mei, J.J. Ho, W. Zhang, P. Lopez, and D.D. Ho. 2002. Contribution of human alpha-defensin 1, 2, and 3 to the anti-HIV-1 activity of CD8 antiviral factor. *Science* 298:995-1000.
- Zlotnik, A., and O. Yoshie. 2000. Chemokines: a new classification system and their role in immunity. *Immunity* 12:121-127.

IntechOpen



HIV-Host Interactions

Edited by Dr. Theresa Li-Yun Chang

ISBN 978-953-307-442-9

Hard cover, 364 pages

Publisher InTech

Published online 02, November, 2011

Published in print edition November, 2011

HIV remains the major global health threat, and neither vaccine nor cure is available. Increasing our knowledge on HIV infection will help overcome the challenge of HIV/AIDS. This book covers several aspects of HIV-host interactions in vitro and in vivo. The first section covers the interaction between cellular components and HIV proteins, Integrase, Tat, and Nef. It also discusses the clinical relevance of HIV superinfection. The next two chapters focus on the role of innate immunity including dendritic cells and defensins in HIV infection followed by the section on the impact of host factors on HIV pathogenesis. The section of co-infection includes the impact of Human herpesvirus 6 and *Trichomonas vaginalis* on HIV infection. The final section focuses on generation of HIV molecular clones that can be used in macaques and the potential use of cotton rats for HIV studies.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Rachna Shah and Theresa L. Chang (2011). Human Defensins in HIV Infection: Friends or Foes?, HIV-Host Interactions, Dr. Theresa Li-Yun Chang (Ed.), ISBN: 978-953-307-442-9, InTech, Available from: <http://www.intechopen.com/books/hiv-host-interactions/human-defensins-in-hiv-infection-friends-or-foes->

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen