Chapter 20

Therapeutic Potential of HDPs as Immunomodulatory Agents

Håvard Jenssen and Robert E.W. Hancock

Abstract

One of the most significant advances in medical history is the discovery and development of antibiotics, which in the middle of last century was flourishing and appeared to be the ultimate solution to the treatment of life-threatening human bacterial diseases. However, lately there has been a huge decline in the rate of discovery of new antimicrobial intervention strategies in parallel with an increasing incidence of multidrug-resistant pathogens; if these circumstances do not change we will continue to approach the end of the antibiotic era. Facing this dark future, scientists are considering new strategies for intervention tailored around the appropriate (selective) stimulation of the host's immune system, and particularly rapid acting innate immunity, as an alternative to direct targeting of microbial pathogens. One recent player in such an immunomodulatory strategy is the naturally occurring host defence peptides (HDP) and their synthetic innate defence regulator (IDR) analogues. In this chapter, we will discuss the potential therapeutic use of HDPs and IDRs as immunomodulatory agents.

Key words: Cationic host defence peptides, anti-infective therapy, antimicrobial peptides, innate defence regulators, immune stimulation, immunomodulator, anti-inflammatory.

1. Introduction

The initiation of the therapeutic use of penicillin during the Second World War followed by the discovery and development of other antibiotics targeting bacterial, and later also viral and fungal pathogens, has undoubtedly had a tremendous impact on human life. However, recently there has been a tremendous decline in the discovery and development of new drugs, e.g. only three new chemical classes of antibacterials have entered the market in the past 40 years (i.e., lipopeptides, oxazolidinones and streptogramins, all targeting

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Gram-positive infections), while the frequency of isolation of drug-resistant pathogens is rapidly increasing. Globally it is estimated that infectious diseases still account for one-third of all human deaths, and big players like respiratory infections, HIV, malaria, and tuberculosis are all found among the top eight killers (www.who.int/whosis/whostat/2008/en/index.html). A novel strategy for the development of new interventions is to target the host's own immediate defence system, innate immunity. However, the development of drugs targeting the innate immune system has experienced some difficulties, and sceptics have argued against the potential of this strategy, largely due to the potential downside offered by inflammation (especially if too vigorous or too prolonged) and the complexity and consequent lack of mechanistic biological understanding of this defence machinery. Despite this there are several exciting potential targets for development of new anti-infectives and a number of targets are currently being targeted by different strategies, e.g. antibodies, biologicals including proteins and peptides, and small-molecule agonists and antagonists; the success of these ventures has been reviewed elsewhere (1–6). Another promising drug class is so-called host defence peptides (HDPs; also termed cationic antimicrobial peptides – AMPs when they have direct antimicrobial activity) and synthetic derivative thereof. The therapeutic use and potential of these HDPs as potential anti-infective immunemodulators are discussed in detail in this chapter.

2. Targeting the Host Innate Immune System

To prevent and cure infections by targeting the innate immune system is an innovative but ambitious idea. This intrinsically complex and conserved signalling system has developed throughout evolution to respond and defeat all types of infections. It is highly effective, given our constant exposure to pathogens but relatively infrequent symptomatic infections; at the same time it is delicately tuned such that it seldom results in prolonged inflammation. The strategy of designing anti-infectives for selective modulation of the innate immune defence system has been made possible by the discovery of specific pathogen recognition receptors, such as Toll-like receptors, and intracellular sensors of microbial components such as the Nod-like receptors and retinoic-acid inducible gene I-like receptors (7). Exposure to pathogenic signalling molecules results in a rapid response that normally returns to homeostasis within hours to days; if unsuccessful in clearing the infection a smooth transition to adaptive (acquired immunity) occurs. However, under some circumstances this control

of inflammation can break down and/or uncontrolled so-called chronic inflammation can ensue; if the inflammatory response is too vigorous or maintained for a prolonged period of time it may lead to pathological consequences and many human disease syndromes demonstrate the symptoms of uncontrolled inflammation including excessive bleeding and chronic pain. Hence the optimal anti-infective therapy based on innate immunity should employ tailored immunomodulators that offer the potential to tip the balance back in favour of the host, by either (selectively) boosting or inhibiting selected elements of the immune response at the same time as exploiting and enhancing the efficiency of the powerful and multifaceted effector mechanisms that have evolved specifically for the purpose of pathogen clearance. It is also important to remember that there are many aspects of the pathogen-sensing, response and control mechanisms that we do not understand, giving hope that in the future even more novel targets for anti-infective therapies will be revealed to assist us in facing the increasing drug resistance challenge. New knowledge and sophisticated technological advances will help accelerate the development of novel anti-infective immunomodulators, e.g. systems biology approaches, genomic libraries for siRNA gene silencing, the high-throughput development of gene knockout mice, studies of single-nucleotide polymorphisms associated with disease and the profiling of transcriptional, micro-RNA and protein interaction networks will all promote new insights into innate immune response mechanisms (8, 9). In this regard, a recently launched powerful tool is the publicly-available bioinformatics resource, Innate DB (www.innatedb.com) (10) and IIDB (http://db.systemsbiology.net/IIDB) (11), which are innate immunity-specific databases that include data analysis resources to facilitate the functional analysis of innate immunity responses, as well as the IIPGA program (www.innateimmunity.net), which is a collaborative effort to analyse polymorphisms in human innate immunity genes.

3. Host Defence Peptides

The constant battle between the host and the pathogens through evolution has resulted in a finely tuned defence system involving innate (germ line encoded) and adaptive (antigen specific due to gene rearrangements and acquired due to pathogenic challenge) immunity. Host defence peptides have been demonstrated to play a pivotal role in the orchestration of innate immune and inflammatory responses of mammals, amphibians, and insects (12–14). Recently, increasing evidence is supporting the

involvement of these antimicrobial peptides in bridging these two immune defence strategies.

Being key players in the fundamental interplay between host and microbe makes these HDPs/AMPs signature molecules of host defence exhibiting substantial diversity even within a single host and occurring in moderate to high concentrations. Virtually all species of life produce them, with more than 1,000 natural occurring peptides having been described to date, and the majority of these are described in databases for eukaryotic host defence peptides: e.g. the site at the University of Trieste (http://www.bbcm.units.it/~tossi/pag1.htm) and the AMPer site (http://www.cnbi2.com/cgi-bin/amp.pl) (15).

These peptides generally fall into four major structural categories based on their amphiphilic conformations that often occur only after membrane interaction; namely β-structures with two to four β-strands and amphipathic α-helices, and less commonly loop and extended structures (**Fig. 20.1**). These peptides are typically short (12–50 amino acids), carrying a net positively charged (+ 2 to 9) due to excess basic arginine and/or lysine residues, and contain up to 50% hydrophobic amino acids (permitting membrane interaction and enabling membrane damage and/or cell penetration). Although their potency varies substantially, depending in part on antagonism by physiological concentrations of mono- and divalent cations and polyanions they often have broad spectra of activity that can encompass antibacterial, antifungal, antiviral and anti-parasitic activities (16). Equally important is

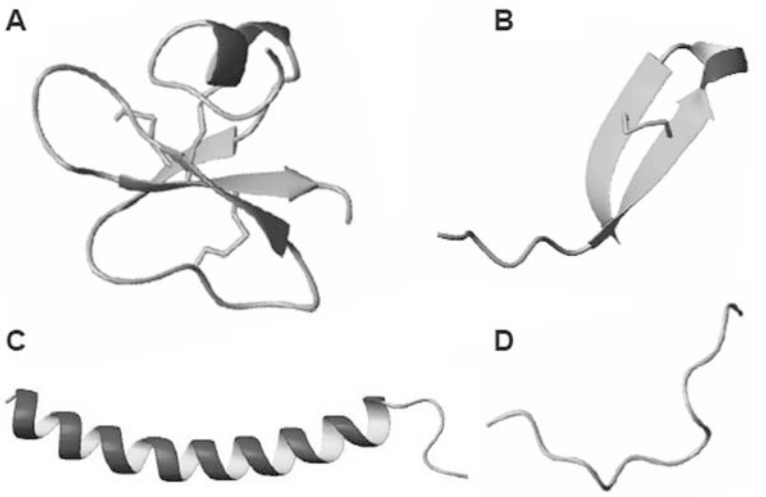

Fig. 20.1. Structural classes of antimicrobial peptides. **(A)** Mixed structure of human β-defensin-2 (HBD-2) (PDB code 1FQQ) (82), **(B)** looped thanatin (PDB code 8TFV) (83), **(C)** α-helical human cathelicidin LL-37 (PDB code 2k6o) (84), **(D)** extended indolicidin (PDB code 1G89) (85). The figures have been prepared with use of the graphic program MolMol 2 K.1 (86).

their ability to recruit and activate or modulate effectors of the innate immune system (14, 17).

4. Host Defence Peptides Mode of Action

Initially cationic peptides were investigated due to their direct antimicrobial activity. Though the peptides usually demonstrate lower potency than conventional antibiotics, one strength lies in their ability to kill multidrug-resistant bacteria. They are able to act extremely rapidly and can engage and inhibit multiple bacterial targets (18). Initially the peptides were believed to act only by perforating bacterial membranes through, e.g. "aggregate" (19), "toroidal pore" (20–23), "barrel-stave" (24) or "carpet" mechanistic models (25). Later studies also demonstrated the ability of many peptides to translocate across the cytoplasmic membrane of bacteria (26) targeting DNA/RNA synthesis (27–30), protein synthesis (28, 29, 31, 32), enzymatic activities (33, 34) and cell wall regeneration (35) among others.

In addition to this direct antimicrobial activity, there is strong evidence that a broad range of cationic peptides can stimulate the host immune system as a mode of promoting pathogen clearance, thus giving rise to the new term host defence peptides (HDPs) (**Fig. 20.2**). The terms AMPs and HDPs are to some extent interchangeable, though the term HDPs is used here for natural peptides with known immunomodulatory properties, while AMPs are known for their direct antimicrobial effects; however, a single peptide may have both features. For example, human peptides are likely directly antimicrobial when found in high concentrations (e.g. in neutrophil granules or in the crypts of the intestine) and exhibit prominent immunomodulatory properties when found at lower concentrations, e.g. after release and dissemination from these sites, e.g. at mucosal surfaces. There are several examples indicating that HDPs are key players in innate immunity. For example, inflammatory bowel disease, also known as Crohn's disease, is generally characterized by chronic inflammation of the intestine, commonly in the distal ileum and/or colon (36). Recent studies have demonstrated that patients suffering from Crohn's disease have a deficiency in the epithelial cell secretion of β-defensins (i.e. human β-defensins 2 and 3) (37, 38) as well as expression of human cathelicidin LL-37 (39). This results in an imbalance between luminal bacteria and the HDP concentrations, allowing intestinal bacteria from the microbial flora to trigger inflammation with associated adherence and invasion into the mucosa (40). HDP expression in the skin is also known to protect against invasive bacterial infections. Transgenic mice

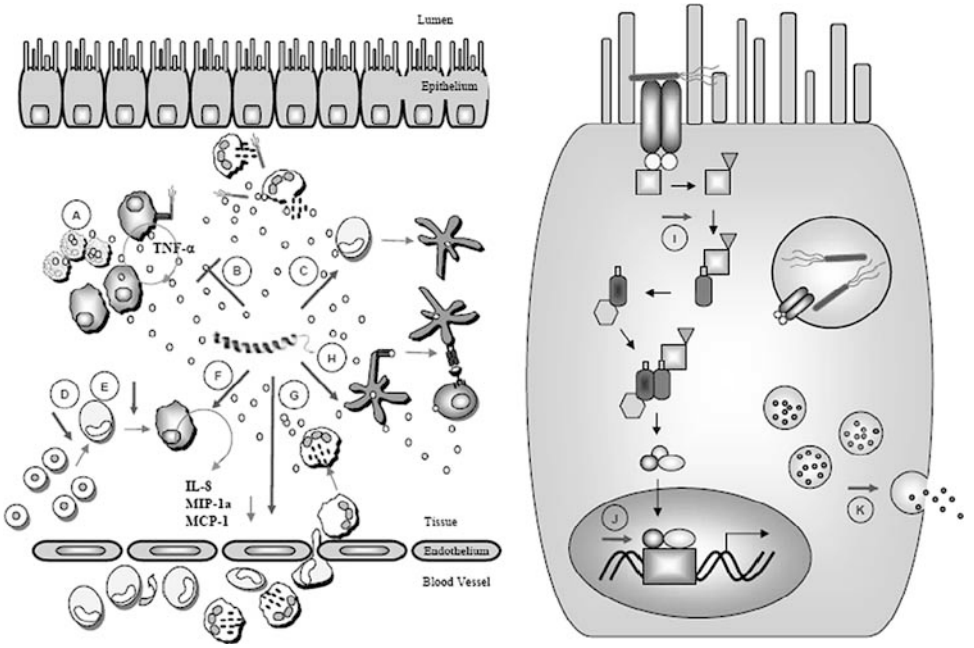

Fig. 20.2. Immunomodulatory properties of host defense peptides. The figure illustrates possible targets at the systemic level (*left*) and in a single cell (*right*). Several HDPs', e.g. LL-37, CRAMP, Indolicidin and SMAP-29 can alter toll like receptor-induced responses, e.g. suppress the expression of pro-inflammatory molecules (e.g. tumor necrosis factor α) and inhibit apoptosis of neutrophils (A & B) (47, 51, 87–91). Dendritic cell differentiation may be targeted (C) (92) as well as several steps in the maturation and differentiation of monocytes to macrophages (D & E). LL-37 activates macrophages (F), leading to release of effector molecules, primarily chemokines. These chemokines, as well as HDP's alone (e.g. CRAMP, HNP-1, HNP-2, LL-37), promotes chemotaxis and migration of leukocytes to the afflicted area (G) (93–97). LL-37 promotes the expression of co-stimulatory molecules on dendritic cell and promotes expression of T_h1 cytokine interleukin-12 (H). Several immune cells are know to be targeted by HDPs. Cell signalling may be affected at several levels in the signalling cascade (I) (73, 98), interfering with gene transcription (J), altering the cellular levels of different effector molecules and cellular degranulation (K) (47, 88, 89, 92, 99–103). The figure is reprinted and modified from Hamill et al., 2008 (1) with permission from authors and Current Opinion in Biotechnology, Copyright © 2008, Elsevier.

mutated in the gene for the mouse peptide CRAMP, a homolog of human LL-37 (41, 42), have been observed to develop significantly more severe skin infections than control mice, though the isolated leukocytes from these CRAMP-deficient mice were functionally competent and similar to wild-type leukocytes (43). Similarly, patients suffering from morbus Kostmann disease and associated severe gum inflammation have an associated deficiency in neutrophil levels of LL-37, as well as reduced concentrations of α-defensins (i.e. human neutrophil peptides-1, -2 and -3) (44). Similarly the ability of peptides to mediate the attraction of various immune cell types (either directly as chemokines or indirectly by stimulating epithelial and monocytic cells to secrete conventional chemokines) to demonstrate anti-inflammatory/anti-endotoxic activity, stimulate blood vessel growth (angiogenesis),

promote wound healing, and promote and polarize adaptive immune responses have all been demonstrated in mouse models and human or mouse tissue or cell systems (43, 45–52). Indeed it can be argued that the systemic effects found with any cationic peptide may be primarily due to the often-undescribed immunomodulatory properties of the peptide, given that the direct antimicrobial activity of peptides, but usually not their immunomodulatory activities, is strongly antagonized by physiological salt concentrations, i.e. 100 mM monovalent- and 2 mM divalent cations (as well as polyanions like glycosaminoglycans such as heparin) (46). In our view the systemic action of peptides that are deliberately added for therapeutic purposes may involve either direct (i.e. antibiotic) or indirect (i.e. immunomodulatory) antimicrobial activity. For example, the most clinically advanced peptide developed by Migenix, primarily as an antimicrobial, has recently been demonstrated to have activity in phase III clinical trails in preventing catheter-associated tunnel infections, as well as activity against the inflammatory disease Rosacea, for which there is no microbial association (**Table 20.1**).

5. Commercial Production of Host Defence Peptides

The clinical use of HDPs has been limited by issues like cost of goods. Several commercial participants have tried to circumvent this issue by moving from traditional solid-phase synthesis to solution-phase methods, and further to a hybrid methodology, e.g. a convergent strategy of synthesizing peptides, i.e., selecting peptide segments and assembling them together typically using a solution phase methodology. The best example of this is Roche (http://www.roche.com/index.htm) and Trimeris' (http://www.trimeris.com) joint success in large-scale production of the HIV fusion inhibitor, enfuvirtide (Fuzeon, T-20), by solid- and solution phase hybrid synthesis (53, 54). Enfuvirtide is a 36-amino-acid peptide that binds to a region of the HIV envelope glycoprotein gp41, thus blocking viral entry into CD^{4+} T cells. The drug is licensed for use in patients in whom other HIV medications are losing effectiveness, in part due to the limited annual production capacity (3.7 metric tons in 2005 providing treatment for ~47,000 patients). Aside from traditional peptide synthesis, other biotechnological approaches may be used for commercial production of HDPs, e.g. recombinant systems built on bacterial-, fungal- or mammalian-expression platforms. Novozymes (Bagsvœrd, Denmark, http://www.novozymes.com/en) have demonstrated great success using a fungal-based system to recombinantly

Table 20.1
Immunomodulatory and antimicrobial peptides in clinical trials or developmental stages

Drug	Sequence	Description/status/results	Company & reference
Plectasin NZ2114	$GFGC_1NGPWDEDDMQC_2HNHC_3$ KSIKGYKGGYC$_1$AKGGFVC$_2$KC$_3$Y-COOH (Plectasin)	*Preclinical*: A variant of plectasin which has demonstrated potent Gram-positive effect in systemic pneumococcal and streptococcal infections	Novozymes AS/Sanofi-Aventis (Bagsvœrd, Denmark), www.novozymes.com
PAC-113	AKRHHGYKRKFH-$CONH_2$	*Phase IIb* : A 12-mer segment present in histatin 3 and 5, which reduces oral candidiasis (0.15% mouth rinse formulation)	Pacgen (Vancouver, BC, Canada), www.pacgenbiopharm.com
XOMA 629/XMP.629	KLFR-(D-naph-A)-QAK-(D-naph-A)	*Phase IIa*: A 9-mer peptide derived from a human HDP (bactericidal/permeability-increasing protein). Developed as a topical gel for treatment of common skin disease, impetigo, with demonstrated potent antibacterial activity against several difficult-to-control bacterial strains including methicillin-resistant *Staphylococcus aureus* Note: The XOMA 629 development program was recently suspended (Press release 10 November 2008)	XOMA (Berkeley, CA, USA), www.xoma.com (104)
Omiganan® / CP-226/ MBI-226/ CLS001	ILRWPWWPWRRK-$CONH_2$	*Phase III* : Analogue of bovine AMP (indolicidin). Omiganan has been demonstrated in Phase II trials to have anti-inflammatory properties vs. Rosacea and acne *Phase IIIb*: Direct antibacterial properties for prevention of catheter related infections.	Migenix/Cutanea (Vancouver, BC, Canada), www.migenix.com, www.cutanealife.com (105)

IMX942	KSRIVPAIPVSLL-$CONH_2$ (IDR-1)	*Preclinical*: 5-mer peptide modelled on IDR-1. IDR-1 was demonstrated to work through selective stimulation of innate immunity, up-regulating protective immunity while suppressing pro-inflammatory cytokine production in response to bacterial TLR agonists	Inimex (Burnaby, BC, Canada), www.inimexpharma.com
hLF1-11	GRRRRSVQWCA-COOH	*Phase IIa*: N-terminal fragment of human HDP (lactoferrin) has indicated immunomodulatory activity. Trials address allogeneic bone marrow stem cell transplantation–associated infections and other drug resistant hospital acquired infections	AM-Pharma (Bunnvik, The Netherlands), www.ampharma.com
CZEN-002	(Ac-CKPV-$CONH_2$)$_2$	*Phase IIb*: A synthetic 8-mer peptide derived from α-melanocyte-stimulating hormone. Inhibits vulvovaginal candidiasis, commonly known as vaginal yeast infection	Zengen/Zensano (Woodland Hills, CA, USA), www.biospace.com
PTX002	SIQDLNVSMKLFRKQAKWKIIV KLNDGRELSLD-COOH	*Discovery phase*: 33-mer peptide with two distinct β-sheet folds and a 12-mer peptide derivative thereof. Has demonstrated broad-spectrum antimicrobial and antiendotoxin neutralizing activity in both in vitro and in vivo experiments	PepTx (St. Paul, MN, USA), www.peptx.com (106, 107)
PTX005/SC4	KLFKRHLKWKII-$CONH_2$		

Note: Amino-acid sequences are given in one-letter code. Cysteines forming disulfide bonds are numbered with subscripts to indicate their pairings. N- and C-terminal modifications are indicated before the hyphen in the front or after the hyphen at the end of the sequence.

produce plectasin in large scale with therapeutic purity under good manufacturing practice (GMP) conditions (55). Plectasin is a fungal defensin-like 40-amino-acid peptide, with broad-spectrum antibacterial activity (56) and no affect on cell viability or interleukin-8 production (57). A derivative of plectasin (Plectasin NZ2114) is currently in preclinical development as a very potent direct Gram-positive antimicrobial in a collaboration between Novozymes and Sanofi-Aventis (Bridgewater, NJ, USA) (**Table 20.1**).

Aside from the cost of goods, questions have been raised regarding poor pharmacokinetic properties of these peptides, in addition to their lack of oral availability (58); however, formulation and/or chemical modification, e.g. N-methylation, of peptides may rationally improve key pharmacokinetic characteristics. Multiple N-methylation has been demonstrated to significantly improve the oral bioavailability, metabolic stability and intestinal permeability of peptides (58, 59). Advances in formulation and delivery systems, e.g. implantable scaffolds, hydrogels and micro- or nano- particle systems, will also help expedite the progression of immunomodulators into clinical use (60).

Despite these solutions, the biggest challenge has probably been peptide in vivo stability. Development of a pegylated form of interferon-α for chronic hepatitis C treatment, that displays greatly enhanced stability, illustrates how technological advances in peptide derivatization can improve the clinical usefulness of biologic-based therapies (61). Another approach to increase metabolic stability is through chemically altering the peptide backbone structure, changing the traditional peptide to a so-called peptidomimetic, which can be defined as "a compound that, as the ligand of a receptor, can imitate or block the biological effect of a peptide at the receptor level" (62), or "chemical structures designed to convert the information contained in peptides into small non-peptide structures" (63). Peptidomimetics can be designed to mimic peptides and participate in protein–protein and protein–nucleic acid interactions. They often have an intrinsic ability to form stable helical conformations (64) which is important since helical motifs constitute the largest class of protein secondary structure and play a major role in mediating protein–protein interactions. At the same time these small synthetic molecules that mimic natural peptides are not susceptible to proteolytic degradation, an advantage they hold over their natural counterparts. Conversely, at the same time it can be argued that potent immunomodulatory substances should ideally be biodegradable to limit overstimulation of the immune system leading to inflammation or hyperstimulation. Thus it might be hypothesized that when moving from directly antimicrobial AMPs to host stimulatory HDPs these issues may be of lesser importance. Proteolytic degradation may in fact lead to shorter

peptide fragments with superior immunomodulatory activity, thus eliciting responses not documented through in vitro screening. Similar phenomena have been described earlier when Domagk in the early 1930s discovered that the red textile dye Prontosil effectively inhibited streptococcal and staphylococcal infections in vivo, while being inactive against the bacteria in vitro (65). It was later concluded that a metabolic degradation product of Prontosil, a sulfanilamide, possessed the antimicrobial activity (66), illustrating one of the problems of screening for active anti-infectives in a host-free system.

6. HDPs in Clinical Development

Host defence peptides are an interesting class of agents with under-explored potential as anti-infective therapeutics. Early work explored the peptides for topical applications, exploiting their direct antimicrobial properties. Biotech companies like Magainin Pharmaceuticals (now Genaera; http://www.genaera.com), Micrologix (now Migenix; http://www.migenix.com) and IntraBiotics (http://www.intrabiotics.com) started designing therapeutic peptides that only differ from their natural homologues by a few amino acids. However, despite promising results from the early clinical trials several failed at later stages, e.g. the Genaera developed Pexiganan (MSI-78) a synthetic 22-amino-acid variant of the amphibian peptide magainin-2 (67), and the IntraBiotics Pharmaceuticals developed pig protegrin analogue Iseganan (IB-367) (68–70).

Despite these disappointing early failures of cationic peptide drugs, several other new ventures have been launched, largely derived from academic labs worldwide. The first antimicrobial peptide to show statistically significant clinical effects was a bovine indolicidin homolog MX-226 (also known as Omiganan or CPI-226; originally developed by Migenix Inc, Vancouver, BC, Canada) (**Table 20.1**). In Phase III clinical trails, the topical application of MX-226 pentahydrochloride in a 1% gel (Omigard) led to a significant 21% reduction of colonization of central venous catheters and a 50% reduction in tunnel infections (www.migenix.com/prod_226.html) (71, 72). Intriguingly Omiganan (as CLS001) has also demonstrated efficacy as an anti-inflammatory peptide, in suppressing the effects of acute acne and Rosacea in Phase II clinical trials, confirming the multifaceted actions of these HDP derivatives. Oral candidiasis in immunocompromised patients is been tackled in a Phase IIb study by Pacgen (Vancouver, BC, Canada), using a short 12-mer peptide segment (PAC-113) originally isolated from

human histatin 3 and 5. Much in the line of this Xoma (Berkley, CA, USA; http://www.xoma.com) is also developing a synthetic peptide from bactericidal/permeability-increasing protein, a human host defence protein. The peptide XOMA 629 has demonstrated great direct antibacterial activity and is currently undergoing clinical Phase IIa trials for topical eradication of common skin infection pathogens in addition to methicillin-resistant *Staphylococcus aureus.*

Synthetic peptides designed based on knowledge about the immunomodulatory properties of LL-37 and other HDPs are currently being explored for their therapeutic potential (12) due to their unique ability to promote protective innate immunity while suppressing potentially harmful inflammatory responses (**Fig. 20.2**). A good example on the success of these studies is the described innate defence regulator-1 (IDR-1), an anti-infective peptide that selectively modulates the innate immune response (73). Optimization of immunomodulatory properties resulted in depletion of the peptides direct antibacterial properties, underlining the complex nature of the natural HDPs. However, despite lacking direct antibacterial activity, IDR-1 confers protection against multiple bacterial pathogens (i.e. methicillin-sensitive/-resistant *S. aureus*, vancomycin-sensitive/-resistant *Enterococcus* and *Salmonella enterica*) in in vivo mice models. Studies concluded that this protection resulted from activation of monocyte-macrophage cells, stimulation of monocyte produced chemokine expression and dampening of pro-inflammatory cytokine responses (**Fig. 20.2**). Further optimization studies on IDR-1 has led to a 5-amino-acid peptide derivative (IMX942) that currently have been launched into preclinical development by Inimex Pharmaceuticals (Burnaby, BC, Canada) (**Table 20.1**). Based on the results there are plans for continuation evaluating efficacy in targeting pneumonia, surgical site infections and chemotherapy induced neutropenia.

The human milk protein-derived peptide hLF1-11 was initially being developed by AM Pharma (http://www.ampharma.com) as a direct antimicrobial peptide. However, after the peptide demonstrated immunomodulatory properties the focus changed, and hLF1-11 is currently in clinical trials for evaluation of efficacy in protection and prevention of infection during allogeneic stem cell transplantation (**Table 20.1**).

Mimicking the structural features of endogenous host defence peptides by other chemical structures has been looked upon as an attractive way to circumvent several of the drawbacks linked to peptides and peptide chemistry. One type of HDP mimics is called Ceragenins. These are synthetically produced small-molecule compounds comprised of a sterol backbone with amino acids and/or other chemical groups attached (74). Several Ceragenins have been developed by Ceragenix

Pharmaceutical Inc. (Denver CO, USA) and have been demonstrated to possess broad-spectrum activities against multidrug-resistant clinically isolated bacteria (75, 76). However, there has been unsatisfactory safety reports for cationic steroid antibiotics (77), thus restricting the use of Ceragenins to topical application or after immobilizing at the surface of medical devices for prevention of biofilms (78). Consequently Ceragenix Pharmaceutical have used their broad-spectrum antimicrobial compounds and combined it with the Ceragenin^{TM} technology to formulate CeraShield^{TM}, antimicrobial coatings for medical devices. Preclinical studies with CeraShield^{TM} are currently ongoing for a variety of medical devices, from tubes and intravenous connectors to different catheters and orthopaedic and surgical implants (http://www.ceragenix.com/).

Another class of mimetic has been developed by PolyMedix Inc. (Radnor, PA, USA). PolyMedix has tailored small chemical molecules that mimic several of the well HDPs. On example from their portfolio is a peptide mimetic whose design was based on the structure of the well-described HDP magainin. This mimetic mPE (meta-phenylene ethynylene) has demonstrated activity against a variety of bacterial and *Candida* species down to nano-molar concentrations (79). Another of their compounds, a small non-peptide mimetic (PMX-30063), mimics the amphiphilic structure of defensines. PMX-30063 is claimed to be bactericidal against both Gram-positive and Gram-negative bacteria by disruption of the bacterial cell membrane, and the company has also other small molecule mimetic that possess similar activities (80). PMX-30063 has demonstrated efficacy in a mice model of systemic *S. aureus* infection at 2 mg/kg, and a Phase I clinical safety study with PMX-30063 has just been completed, demonstrating a safe and well-tolerated single doses with no observable adverse effects up to 24 mg/kg per day (Press release).

7. Conclusion

We are entering the "post-antibiotic era" in which many previously successful drug regimes are becoming ineffective, consequently the time has come to vigorously pursue alternative potential treatment approaches toward infectious diseases. Development of HDPs as immunomodulatory therapeutic alternatives is questionably subject to unique difficulties. The innate immune system is intrinsically complex and there is great genetic variation between individuals; additionally there is fundamental interspecies differences (81) cluttering the correlation between animal models and human trials. However, the complexity of the

innate immune system through its evolution has also resulted in huge redundancy, thus almost duplicating critical response cascades, hence creating a safety net for drug developers while also undermining the effectiveness of single targeting drug candidates.

The success of peptides as drugs, and especially HDPs as anti-infectives, has not carried significant fruits yet. However, this concept is in its infancy and with continued research and adaptation of new technology platforms especially introducing bioinformatics approaches, it may enable this strategy to solve some of the forthcoming challenges we will be facing from emerging infections diseases. Any disease is per definition caused by a pathogen's ability to overcome or circumvent the host immune response, thus we argue that development of novel anti-infectives directly targeting the host immune system has a reasonable chance of succeeding, while a reduced chance of resistance development.

Acknowledgments

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