Special Issue Review

Journal of PeptideScience

Received: 27 August 2014 Revised: 22 September 2014 Accepted: 22 September 2014 Published online in Wiley Online Library: 31 October 2014

(wileyonlinelibrary.com) DOI 10.1002/psc.2708

Peptide IDR-1018: modulating the immune system and targeting bacterial biofilms to treat antibiotic-resistant bacterial infections‡

Sarah C. Mansour,§ César de la Fuente-Núñez§ and Robert E. W. Hancock*§

Host defense (antimicrobial) peptides, produced by all complex organisms, typically contain an abundance of positively charged and hydrophobic amino acid residues. A small synthetic peptide termed innate defense regulator (IDR-)1018 was derived by substantial modification of the bovine neutrophil host defense peptide bactenecin. Here, we review its intriguing properties that include anti-infective, anti-inflammatory, wound healing, and anti-biofilm activities. It was initially developed as an immune modulator with an ability to selectively enhance chemokine production and polarize cellular differentiation while suppressing/balancing the pro-inflammatory response. In this regard, it has demonstrated *in vivo* activity in murine models including enhancement of wound healing and an ability to protect against *Staphylococcus aureus*, multidrug resistant *Mycobacterium tuberculosis*, herpes virus, and inflammatory disorders, including cerebral malaria and neuronal damage in a pre-term birth model. More recently, IDR-1018 was shown, in a broad-spectrum fashion, to selectively target bacterial biofilms, which are adaptively resistant to many antibiotics and represent the most common growth state of bacteria in human infections. Furthermore, IDR-1018 demonstrated synergy with conventional antibiotics to both prevent biofilm formation and treat pre-existing biofilms. These data are consistent with a strong potential as an adjunctive therapy against antibiotic-resistant infections. Copyright © 2014 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: immunomodulatory; anti-biofilm; anti-infective; peptide

Introduction

Host defense peptides (HDP) are evolutionarily conserved molecules that serve in host defenses defense against insults including microbial infections and inflammation. Present in all living organisms, these peptides generally vary in size from 12 to 50 (or more) amino acids and are cationic due to the presence of excess lysine and arginine residues [1]. Moreover, HDPs contain about 50% hydrophobic amino acids, which allow them to interact with and penetrate cell membranes [1,2]. Some HDPs are able to freely translocate into both host and bacterial cells, which enable their diverse antimicrobial, immunomodulatory, and anti-biofilm activities [3–6], with the latter two being especially dependent on cell penetration.

Initially, HDPs were studied for their antimicrobial properties against an array of pathogens including bacteria (in their planktonic/free-swimming state), viruses, fungi, and protozoa [1]. However, these antimicrobial activities of HDPs are severely dampened by divalent cations and polyanions found in the mucosa, cellular interstitial spaces and body fluids, including blood [7]. For example, LL-37, a human cathelicidin produced in mucosal secretions, loses its antimicrobial activity at physiological salt conditions (~100–200 nM) [8]. These findings suggest that antimicrobial activity may not be the sole biological function of HDPs, and recently, there has been an increasing appreciation for their immunomodulatory activities. For example, despite its modest antimicrobial activity at physiological conditions, LL-37 exhibits a multitude of immunomodulatory roles including cellular recruitment [9], modulation of inflammatory responses [10], and promotion of cellular maturation [11], as well as enhanced wound repair and increased angiogenesis [11]. These various findings have suggested that the most relevant biological function of HDPs in the context of microbial infections might be their immunomodulatory activity [7].

Another newly identified role of HDPs is anti-biofilm activity [6]. Biofilms are structured aggregations of microorganisms associated with surfaces that have been widely studied over the past few decades in part because they are estimated to cause 65% or more of all infections, being particularly prevalent in device-related infections, infections on body surfaces (skin and soft tissue, lung, bladder, endocarditis, etc.) and chronic infections [12–14]. Because of their high adaptive resistance (which reverts upon removal of the inducing condition – in this case the biofilm growth state), altered phenotype, and physiology, biofilms are extremely recalcitrant to conventional antimicrobial therapy, which substantially hinders their treatment in the clinic [12–16]. More recently, cationic peptides have demonstrated anti-biofilm activity against multiple bacterial species [5,7]. This was an important finding particularly because of the lack of currently available drugs that are effective against biofilms. Interestingly, these studies showed that in some cases,

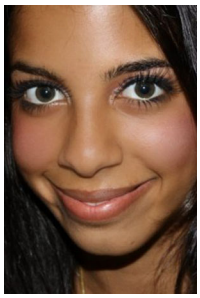
* *Correspondence to: Robert E.W. Hancock, Microbiology and Immunology, University of British Columbia, 232-2259 Lower Mall Research Station, Vancouver, BC, V6T 1Z4, USA. E-mail: bob@hancocklab.com*

‡ *Special issue of contributions presented at the 14th Naples Workshop on Bioactive Peptides "The renaissance era of peptides in drug discovery", June 12-14, 2014, Naples*

§ *These authors contributed equally to this work.*

Department of Microbiology and Immunology, University of British Columbia, 232-2259 Lower Mall Research Station, Vancouver, BC, V6T 1Z4, Canada

Biography



Sarah Mansour obtained her Bachelors of Science at the University of Waterloo in Ontario, Canada. She is currently working under Dr. Robert Hancock as a PhD candidate studying anti-staphylococcal biofilm agents with combined immunomodulatory properties. Moreover, she is currently testing these agents in a variety of murine biofilm infection models.

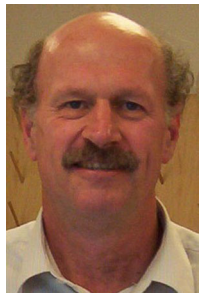
Biography

César de la Fuente-Núñez is a PhD candidate in Microbiology & Immunology at the University of British Columbia. He was born in A Coruña, Spain in 1986. In 2009, he obtained his Licentiate (BSc, MSc) in Biotechnology at Universidad de León (Spain). His research interests include small cationic peptides as novel anti-biofilm agents and modulators of innate immunity, and the development of these novel drugs for the treatment of antibiotic-resistant infections. He has published eight papers and reviews, and presented more than 15 posters at conferences. He is, with Dr. Bob Hancock, a co-inventor of a provisional patent application on the use of cationic anti-biofilm and innate defense regulator peptides (US Patent Application No. 61/870,655). He has been awarded a prestigious doctoral scholarship from the Fundación 'la Caixa' and Fundación Canadá (Spain).

the anti-biofilm properties of peptides are independent of their direct antimicrobial activity, as peptides with excellent anti-biofilm that lack antimicrobial activity have been isolated and vice versa.

The vast array of functions of natural HDPs has triggered considerable efforts to exploit the activity of these peptides by generating a new group of synthetic analogs coined innate defense regulatory peptides (IDRs) [2,4]. Using the sequences of natural HDPs as templates, synthetic peptides were generated with enhanced biological activity and minimal cytotoxicity against mammalian cells [17]. These initiatives led to the design by our lab of synthetic cationic peptide IDR-1018, a peptide that was initially selected because of its potent ability (relative, e.g., to natural peptide LL-37) to induce chemokines and attenuate pro-inflammatory responses *ex vivo* [17]. In this review, we overview these recent developments and describe the therapeutic progress of IDR-1018, particularly describing its activity in murine models. We will also overview the most recently discovered function of IDR-1018, anti-biofilm activity. In this context, we will describe its broad-spectrum inhibition and eradication of biofilms and synergy with conventional antibiotics, and outline its novel mechanism of action. Overall, we suggest that the combined approach of targeting bacterial biofilms and favorably modulating the immune system might serve as a novel therapeutic strategy to treat drug-resistant bacterial infections.

Biography



Robert E. W. Hancock He is a Professor of Microbiology and Immunology, UBC, an Associate Faculty Member of the Wellcome Trust Sanger Institute and a Canada Research Chair in Health and Genomics. His research interests include small cationic peptides as novel antimicrobials and modulators of innate immunity, the development of novel treatments for antibiotic-resistant infections, the systems biology of innate immunity, inflammatory diseases, and *Pseudomonas aeruginosa*, and antibiotic uptake and resistance. He has published more than 600 papers and reviews, has 44 patents awarded, and is an ISI (The Institute for Scientific Information) highly cited author in Microbiology with more than 51,000 citations and an h-index of 118. He has won several awards including the Aventis Pharmaceuticals Award, the leading award for research on antimicrobials, and Canada's three top prizes for Health Research, and is an Officer of the Order of Canada. He was a co-founder of Migenix, Inimex Pharmaceuticals and the Centre for Drug Research and Development.

The Origins of Peptide IDR-1018: Discovery and Initial Characterization

The amino acid sequence of peptide IDR-1018 (VRLIVAVRIWRR-NH_2) is based loosely on that of bovine HDP bactenecin derivative Bac2a (RLARIVVIRVAR-NH_2). Using this sequence as a template, a large library (>100) of 12-amino acid long peptides was generated through point substitutions, scrambling, and deletions [17]. The goal of this screen was to derive peptides with superior immunomodulatory activity compared to Bac2a. Thus, the immunomodulatory potential of each peptide was assessed by measuring the extent of induction of monocyte chemotactic protein 1 (MCP-1) in human peripheral blood mononuclear cells *ex vivo*. Among all the peptides tested, IDR-1018 had the most potent effect on induction of MCP-1 (and MCP-3), chemokines that are important for protection against infections [17]. More specifically, IDR-1018 stimulation resulted in a >50-fold increase in the tested chemokines as compared to parent peptide Bac2a [17] and the human peptide LL-37. Moreover, IDR-1018 exhibited a more than tenfold increased induction of chemokines compared to IDR-1, a synthetic peptide that had previously demonstrated therapeutic efficacy in an invasive *Staphylococcus aureus* murine model [18]. The significant elevation of chemokine production was not attributed to stress-induced toxicity as IDR-1018 did not cause membrane permeability as measured by the release of lactate dehydrogenase from human peripheral blood mononuclear cells [17].

Another major hallmark of IDRs is their ability to balance pro-inflammatory responses by attenuating pro-inflammatory cytokine [e.g., tumor necrosis factor alpha (TNF-α)] production in response to lipopolysaccharide (LPS). At 50 μg/ml peptide, IDR-1018 reduced the production of TNF-α by 89%. This was likely due to perturbation of the TLR to NF-κB pathway rather than direct neutralization of LPS, because in a dansyl polymyxin displacement assay, 1018 did not show any LPS binding [18]. The mechanisms used by IDR peptides generally involve modulation

Journal of PeptideScience

of a variety of pathways inside cells, analogous to LL-37 [19] as investigated for various IDR peptides [18,20].

Recently, it was shown that IDR-1018 was able to skew cellular responses by influencing macrophage differentiation. Macrophages play a critical role in host defenses by serving as a first line of defense during microbial invasion. Through bacterial recognition via toll-like receptors, they execute several phagocytic and antibacterial functions, as well as produce high levels of pro-inflammatory mediators and chemokines required for immune cell recruitment [21]. Recently, Peña *et al.* demonstrated that IDR-1018 drives macrophage differentiation toward an intermediate M1–M2 state [22]. M1 macrophages are 'classically activated' macrophages, which effectively kill a variety of pathogens as well as produce a number of inflammatory mediators. On the other hand, M2 'alternatively activated' macrophages have an anti-inflammatory function and as such produce a number of anti-inflammatory mediators to regulate excessive harmful inflammation as well as mediating wound repair [23]. The unique IDR-1018-mediated signature was characterized by an upregulation of many M2 markers, including anti-inflammatory cytokines (i.e., IL-10), wound healing-associated genes, and enhanced phagocytic activity toward apoptotic cells [22]. Critically however, IDR-1018-treated macrophages maintained plasticity and could return back to a pro-inflammatory M1 state after treatment with interferon-γ, a property that is crucial for being able to respond dynamically to infection while maintaining regulatory functions to return the immune system to homeostasis [22].

Investigations with IDR-1 [18] that had absolutely no direct antimicrobial activity proved that immunomodulatory and antibiotic activities were independently determined. Similarly, IDR-1018 exhibited rather weak direct antimicrobial activity against both gram-negative and gram-positive bacteria, comparable to parent peptide Bac2a [17], but much lower than the nine amino acid optimized peptide HHC10 [24] (Table 1). Because of its ability to potently stimulate chemokines induction and suppress the induction of pro-inflammatory cytokines, peptide IDR-1018 was selected for detailed *in vivo* studies.

Immunomodulatory Activity

The first major *in vivo* finding was that IDR-1018 possessed the ability to enhance wound healing. In mice with full-thickness wounds, IDR-1018 treatment resulted in faster wound closure in a concentration-dependent manner compared to a vehicle control and was also superior to two previously characterized peptides, LL-37 and HB-107 [25]. In an *S. aureus* porcine wound model, IDR-1018 also enhanced re-epithelialization, in part by promoting keratinocyte proliferation as seen in Figure 1; however it did not have any effect on bacterial colonization in the wound tissue thus ruling out the possibility that the results observed were due to removal of bacteria that would delay wound healing [25]. Interestingly, IDR-1018 showed no improvement on wound closure in diabetic mice, suggesting that IDR-1018 worked by modulating immune responses that are highly impaired in diabetic animals.

IDR-1018 was also shown to be effective, in animal models, as an adjunctive therapy for the treatment of cerebral malaria, which is a human-relevant condition in which the induction of death is thought to be due to massive inflammation in the brain. In an experimental cerebral malaria model with 100% lethality between days 6 and 7, IDR-1018 (and to a lesser extent IDR-1) is protected when administered over the course of infection [26]. However, when administration was delayed (starting at day 4) to mimic clinical treatment, only co-administration with anti-malarial treatments increased the survival of *Plasmodium*

Table 1. IDR-1018 demonstrated weak antimicrobial activity, comparable to parent sequence Bac2a based on assessments of minimal inhibitory concentrations (MIC). Peptide 1019 is a peptide with no effective antimicrobial and immunomodulatory activity, obtained from the same comprehensive Bac2a-derived peptide screen that led to isolation of 1018

Name	Sequence	MIC (μg/ml) *Pseudomonas aeruginosa*	MIC (μg/ml) *Staphylococcus aureus*	Reference
Bac2a	RLARIVVIRVAR-NH_2	50	17	[17]
1018	VRLIVAVRIWRR-NH_2	19	5	[17]
1019	IVVWRRQLVKNK-NH_2	>300	>300	[17]
HHC10	KRWWKWIRW-NH_2	0.8	1.5	[24]

IDR, innate defense regulator.

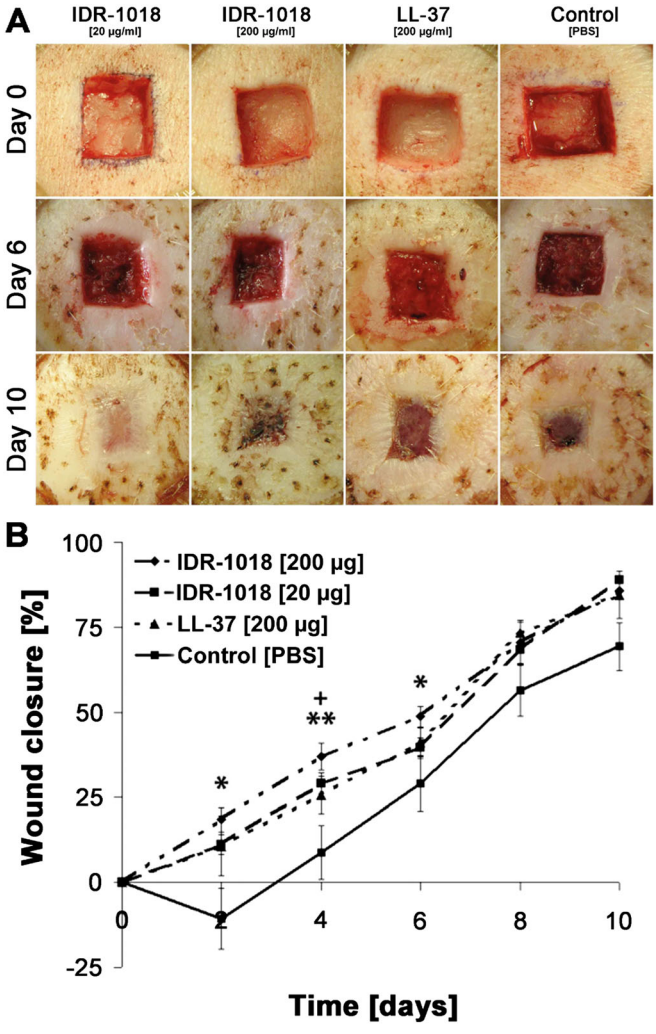


Figure 1. Wound healing activity of IDR-1018. (A) In a porcine wound healing model, *Staphylococcus aureus* infected wounds were treated every 48 h with IDR-1018 (20 or 200 μg/ml), LL-37, and vehicle control phosphate buffered saline (PBS) administered every. (B) Re-epithelialization was quantified after treatment with IDR-1018 (20 or 200 μg/ml), LL-37, and PBS control. Taken from [25].

berghei infected mice compared to anti-malarial treatment on its own (68% compared to 41% survival; $p = 0.034$), and IDR-1 was completely ineffective. Interestingly, protection was not associated with lowered parasitemia. A comprehensive microarray analysis was performed on the harvested spleens and brains of infected compared with non-infected mice administered with IDR-1018 prophylactically or given saline as a control. Infected mice that were treated with IDR-1018 produced a distinct transcriptional profile, which indicated that increased survival was due to the downregulation of inflammatory cytokine expression [26]. This dampened inflammatory response resulted in reduced brain pathology and slower progression of disease. Moreover, IDR-1018 treatment resulted in an upregulation of genes associated with erythropoiesis, which is also involved in inhibiting the production of pro-inflammatory mediators such as TNF-α and nitric oxide synthase [26]. The supplemental data to this paper also demonstrated clear protection in an invasive *S. aureus* model.

The immunomodulatory activity of this peptide was also shown to be protective in an experimental model of pulmonary tuberculosis. In this model, intra-tracheal administration of peptide IDR-1018 at 2 day intervals after 2 months of infection led to a reduction of bacillary loads for both the H37rv and a multidrug resistant *Mycobacterium tuberculosis* isolate, as well as reduced lung inflammation associated with pneumonia [27]. This phenomenon was attributed to an increased induction of chemokines that recruit cells important for controlling bacilli growth, differentiation of macrophages into more effective bacterial-killing cells, and suppression of harmful pro-inflammatory responses [27].

Consistent with these effects on brain inflammation in cerebral malaria, IDR-1018 also demonstrated therapeutic efficacy in the LPS-hypoxia-ischemia neonatal brain injury model, which is considered a model for difficulties accompanying pre-term birth. *Ex vivo* studies showed that IDR-1018 significantly reduces LPS-induced inflammatory cytokine production (GM-CSF, IL-4, IL-10, TNF-α, IL-17, and KC) in microglia, the resident macrophages of the brain [28]. *In vivo*, a single therapeutic dose of IDR-1018 conferred impressive neuroprotection in neonatal mice suffering from LPS-induced brain damage. When given after LPS and hypoxia-ischemia insult, IDR-1018 reduced the amount of white and grey matter tissue loss as well as protected other areas of the brain such as the cerebral cortex, hippocampus, thalamus, and striatum [28]. Consistent with *ex vivo* results, the peptide-mediated neuroprotection was associated with a suppression of inflammatory signaling injury as well as cell death-associated pathways [28]. Intriguingly, pharmacokinetic studies accompanying this paper demonstrated that the peptide freely distributed throughout the body of neonatal mice including entry across the blood brain barrier, indicating that the peptide worked locally.

Anti-Biofilm Activity

Bacterial biofilms are between tenfold and 1000-fold more resistant to conventional antibiotics creating a significant clinical problem because they are associated with around 65% of all infections [29–31]. Recently, the human cathelicidin peptide LL-37, which

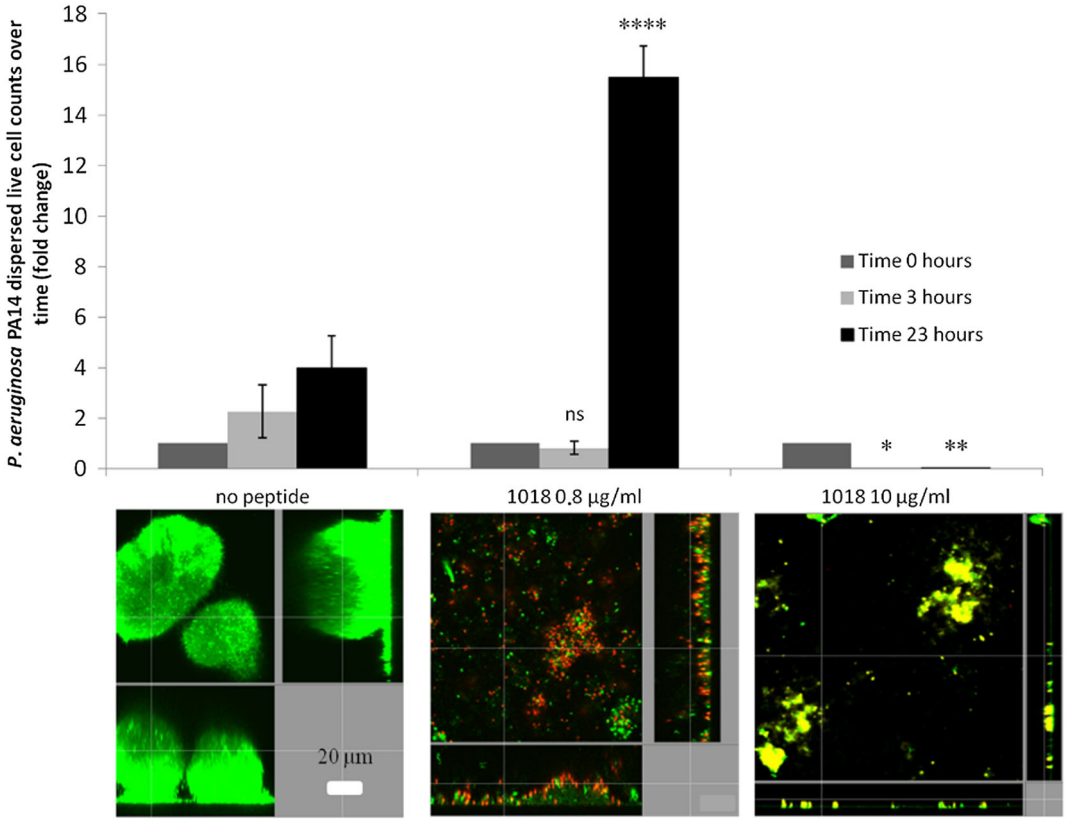


Figure 2. Effect of 1018 on biofilms. Two-day old *Pseudomonas aeruginosa* biofilms were treated with either 0.8 or 10 μg/ml of the peptide, and viable dispersed cells were collected from the effluent of the flow cell, and viable counts were determined after 0, 3, and 23 h of treatment. Lower images correspond to confocal micrographs of remaining cells of 2-day old *P. aeruginosa* biofilms treated with 0.8 and 10 μg/ml of 1018. Flow cell chambers were stained with SYTO-9, which permeates both live and dead cells. To differentiate between dead and live cells, flow cell chambers were costained with propidium iodide, which stains dead cells red. The overlap of stains appears as yellow. Reprinted from [5].

Journal of PeptideScience

has no useful antimicrobial activity versus planktonic cells [8], was shown to block biofilm growth and trigger disintegration of preformed biofilms in *Pseudomonas aeruginosa* at one sixteenth its MIC [6]. These findings prompted subsequent screening of synthetic peptide libraries aimed to select for anti-biofilm activity [4]. Resulting from these efforts was peptide 1037, which lacked direct antimicrobial activity (MIC 304 μg/ml) but showed moderate anti-biofilm activity against gram-negative bacteria and the gram-positive organism *Listeria monocytogenes*. Subsequently, it was demonstrated that IDR-1018 (named 1018 in the original anti-biofilm papers) exhibited potent broad-spectrum anti-biofilm activity versus *P. aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, methicillin resistant *S. aureus*, *Salmonella typhimurium*, and *Burkholderia cenocepacia* at concentrations well below its MIC [5]. The biofilm inhibitory activity was shown to be due to the dispersal of cells from biofilms at very low peptide concentrations peptide-induced biofilm cell death at higher concentrations (Figure 2) [5]. The mechanism of peptide 1018 was shown to involve binding to and subsequent stimulation of the degradation of second messenger stress-induced nucleotides (p)ppGpp, which are involved in biofilm formation and maintenance [5].

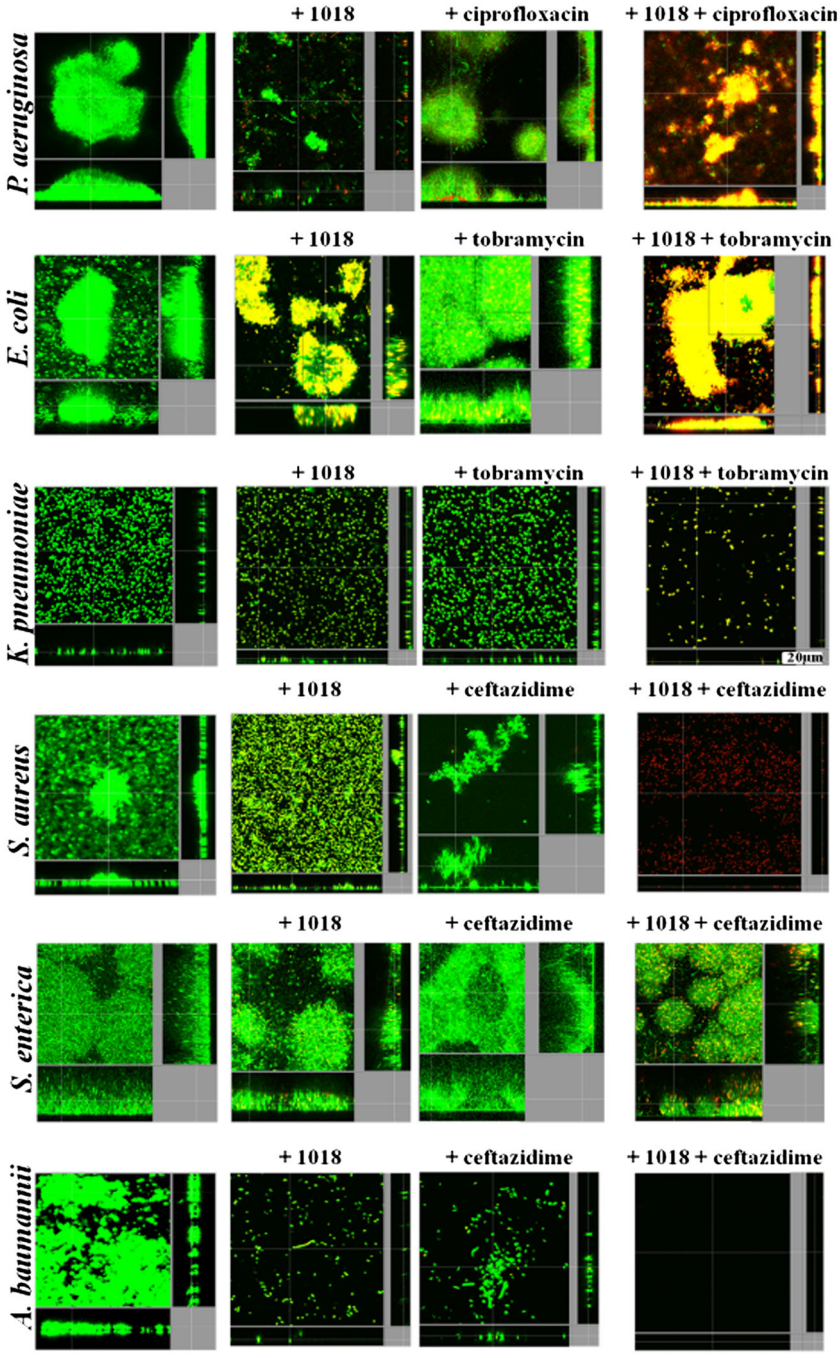


Figure 3. Anti-biofilm peptide 1018 is synergistic with antibiotics. Mature biofilms from *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Salmonella enterica*, and methicillin resistant *Staphylococcus aureus* treated with 1018 and various different antibiotics show synergistic interactions. Taken from [32] with permission. Copyright © 2014, American Society for Microbiology.

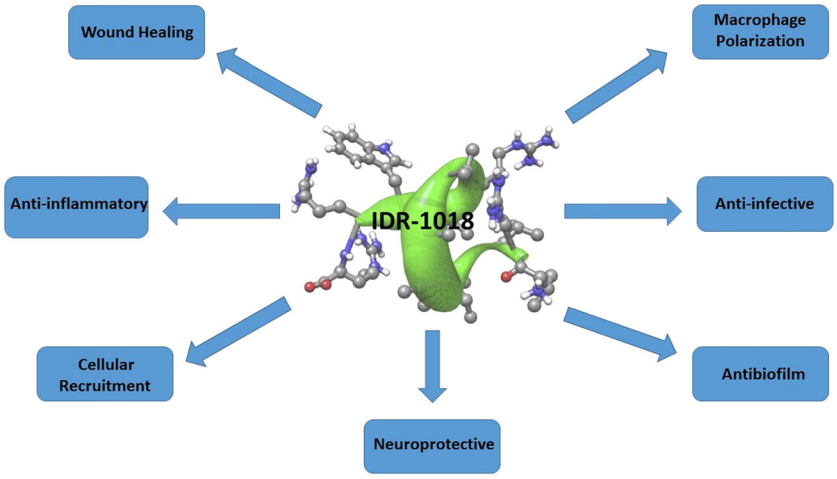


Figure 4. Overview of innate defense regulator (IDR)-1018 activities. The central peptide structure is from [17].

Interestingly, these studies served to separate the anti-biofilm and antimicrobial activities of peptides. For example, peptide 1037 was virtually inactive against planktonic bacteria but inhibited biofilm growth at sub-MIC levels [4], 1018 had weak direct antimicrobial activity (MIC 19 μg/ml [17]) but potent anti-biofilm activity (leading to dispersal at 0.8 μg/ml [6]), while HHC-10 (Table 1) showed the opposite pattern, lacking anti-biofilm activity [4]. Similarly, although planktonic *Burholderia* was completely resistant to the action of peptides, it was susceptible when grown as biofilms [4,5].

Peptide 1018 shows strong synergy with conventional antibiotics in both preventing biofilm formation and eradicate pre-existing biofilms [32]. Indeed, when the peptide was added in the presence of antibiotics ceftazidime, ciprofloxacin, imipenem, or tobramycin, the concentrations of antibiotic required to treat biofilms formed by *P. aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Salmonella enterica,* and methicillin resistant *S. aureus* are reduced by up to 64-fold (Figure 3) [32].

Therapeutic Potential of Dual Anti-Biofilm-Immunomodulatory Peptides

Although peptide-based immunotherapies are still in their infancy, given the numerous biological activities of IDR-1018 summarized in Figure 4, and the therapeutic efficacy validated in animal models, IDR-1018 has the potential to serve as a new host-directed therapy for a number of diseases.

The potential clinical applicability of IDR-1018 depends on a number of factors, namely, toxicity, biological stability, cost of production, and therapeutic administration. Undoubtedly, peptide IDR-1018 confers a low toxicity profile and is well tolerated in several different animal models. Moreover, radiolabeling studies revealed that although peaking in the blood within 2 min of delivery, IDR-1018 distributes rapidly in the blood, liver, brain, and spleen, reaching steady state concentrations [28].

The combined immunomodulatory and anti-biofilm activities make peptide IDR-1018 an ideal candidate for catheter-related and cutaneous biofilm infections. For example, as biofilm infections impede wound healing, the combined anti-biofilm and wound healing properties of IDR-1018 may help to alleviate wound infections faster than conventional therapies [33]. Moreover, cutaneous wounds have been associated with a prolonged inflammatory state, which alters the progression of skin wound healing by stimulating the release of harmful cytokines that ultimately destroy existing host tissue and provide a nidus for more biofilm growth [34]. Nevertheless, the ability of IDR-1018 to skew host responses to favor cellular recruitment while controlling excessive harmful inflammation makes this peptide an ideal candidate for treating these complex infections. Moreover, the ability to control inflammation while not completely suppressing host-directed responses would make IDR-1018 ideal for other conditions such as sepsis. Early stage sepsis is characterized by a hyper-inflammatory state, which results in impaired immune function and eventual immunosuppression [35]. Peptide IDR-1018 could help to balance these delicate host responses to reduce the prolonged production of pro-inflammatory mediators but also enhance the activity of leukocytes, which are important for baseline immune function.

Conclusions and Future Directions

There is currently a severe problem with treatment of bacterial infections, given the explosion of multiple antibiotic resistance, whereby our entire arsenal of antibiotics is gradually losing effectiveness, combined with the paucity of truly novel compounds under development or entering the clinic. Thus, the even greater resistance of biofilms adds to the major concerns being expressed by physicians and medical authorities. Consequently, there is an urgent need for new strategies to treat biofilm infections, and, along these lines, we have outlined here the therapeutic potential of newly identified anti-biofilm peptide IDR-1018. Indeed, this peptide eradicates biofilms formed by four of the so-called ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, and Enterobacter species), identified by the Infectious Diseases Society of America as the most recalcitrant and resistant organisms in our society. In addition, despite the marginal antimicrobial activity, IDR-1018 has displayed several anti-infective properties against Staphylococcus, *Plasmodium berghei,* and *M. tuberculosis*. This activity is highly associated with enhanced cellular recruitment by selectively upregulating chemokines associated with monocyte, macrophage, and neutrophil recruitment [17]. Moreover, the ability of IDR-1018 to attenuate excessive pro-inflammatory responses has led to increased survival against malaria and reduced lung pathology in tuberculosis infected mice. Furthermore,

Journal of PeptideScience

IDR-1018 possesses wound healing capabilities, which promote keratinocyte proliferation and increased epidermal healing in *S. aureus*-infected and non-infected animal models. Most importantly, the peptide was non-toxic in these models. In conclusion, peptide IDR-1018 may be used alone or as an adjuvant in combination with conventional antibiotics to treat drug-resistant bacterial infections, as well as other inflammatory conditions.

Future research will focus on rationally designing peptides with improved activity compared to that of IDR-1018 and establishing animal models of infection (e.g., mouse, *Caenorhabditis elegans*, *Galleria mellonella*, etc.) for both gram-negative and gram-positive bacteria. These models will provide an ideal experimental framework to assess the *in vivo* immunomodulatory and anti-biofilm activities of the new generation of peptides.

Acknowledgements

Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under the award number R21AI098701 and by a grant from the Canadian Institutes for Health Research MOP-74493. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. R. E. W. H. holds a Canada Research Chair in Health and Genomics. C. D. L. F.-N. received a scholarship from the Fundación 'la Caixa' and Fundación Canadá (Spain).

Additionally, C. D. L. F.-N and R. E. W. H. are co-inventors of a provisional patent application on the use of cationic anti-biofilm and innate defense regulator peptides (US Patent Application No. 61/870,655).

References

1 Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002; **415**(6870): 389–395.
2 Fjell CD, Hiss JA, Hancock REW, Schneider G. Designing antimicrobial peptides: form follows function. *Nat. Rev. Drug Discov.* 2011; **11**(1): 37–51.
3 Hancock REW Lehrer R Cationic peptides: a new source of antibiotics. *Trends Biotechnol.* 1998; **16**(2): 82–88.
4 de la Fuente-Núñez C, Korolik V, Bains M, Nguyen U, Breidenstein EB, Horsman S, Lewenza S, Burrows L, Hancock REW. Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. *Antimicrob. Agents Chemother.* 2012; **56**(5): 2696–2704.
5 de la Fuente-Núñez C, Reffuveille F, Haney EF, Straus SK, Hancock REW. Broad-spectrum anti-biofilm peptide that targets a cellular stress response. *PLoS Pathog.* 2014; **10**(5): e1004152.
6 Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, Hancock REW. Human host defense peptide LL-37 prevents bacterial biofilm formation. *Infect. Immun.* 2008; **76**(9): 4176–4182.
7 Mansour SC, Pena O, Hancock REW. Host defense peptides: front-line immunomodulators. *Trends Immunol.* 2014; **35**(9): 443–450.
8 Bowdish DM, Davidson DJ, Lau YE, Lee K, Scott MG, Hancock REW. Impact of LL-37 on anti-infective immunity. *J. Leukoc. Biol.* 2005; **77**(4): 451–459.
9 Tjabringa GS, Ninaber DK, Drijfhout JW, Rabe KF, Hiemstra PS. Human cathelicidin LL-37 is a chemoattractant for eosinophils and neutrophils that acts via formyl-peptide receptors. *Int. Arch. Allergy Immunol.* 2006; **140**(2): 103–112.
10 Mookherjee N, Brown KL, Bowdish DM, Doria S, Falsafi R, Hokamp K, Roche FM, Mu R, Doho GH, Pistolic J, Powers JP, Bryan J, Brinkman FS, Hancock REW. Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. *J. Immunol.* 2006; **176**(4): 2455–2464.
11 Davidson DJ, Currie AJ, Reid GS, Bowdish DM, MacDonald KL, Ma RC, Hancock REW, Speert DP. The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. *J. Immunol.* 2004; **172**(2): 1146–1156.
12 Hall-Stoodley L, Costerton JW. Stoodley bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.* 2004; **2**(2): 95–108.
13 Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; **284**(5418): 1318–1322.
14 O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. *Annu. Rev. Microbiol.* 2000; **54**: 49–79.
15 Kolter R. Biofilms in lab and nature: a molecular geneticist's voyage to microbial ecology. *Int. Microbiol.* 2010; **13**(1): 1–7.
16 de la Fuente-Núñez C, Reffuveille F, Fernández L, Hancock REW. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. *Curr. Opin. Microbiol.* 2013; **16**(5): 580–589.
17 Wieczorek M, Jenssen H, Kindrachuk J, Scott WR, Elliott M, Hilpert K, Cheng JT, Hancock REW, Straus SK. Structural studies of a peptide with immune modulating and direct antimicrobial activity. *Chem. Biol.* 2010; **17**(9): 970–980.
18 Scott MG, Dullaghan E, Mookherjee N, Glavas N, Waldbrook M, Thompson A, Wang A, Lee K, Doria S, Hamill P, Yu JJ, Li Y, Donini O, Guarna MM, Finlay BB, North JR, Hancock REW. An anti-infective peptide that selectively modulates the innate immune response. *Nat. Biotechnol.* 2007; **25**(4): 465–472.
19 Mookherjee N, Hamill P, Gardy J, Blimkie D, Falsafi R, Chikatamarla A, Arenillas DJ, Doria S, Kollmann TR, Hancock REW. Systems biology evaluation of immune responses induced by human host defence peptide LL-37 in mononuclear cells. *Mol. Biosyst.* 2009; **5**(5): 483–496.
20 Nijnik A, Madera L, Ma S, Waldbrook M, Elliott MR, Easton DM, Mayer ML, Mullaly SC, Kindrachuk J, Jenssen H, Hancock REW. Synthetic cationic peptide IDR-1002 provides protection against bacterial infections through chemokine induction and enhanced leukocyte recruitment. *J. Immunol.* 2010; **184**(5): 2539–2550.
21 Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH, Engebretsen IL, Bayles KW, Horswill AR, Kielian T. Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. *J. Immunol.* 2011; **186**(11): 6585–6596.
22 Pena OM, Afacan N, Pistolic J, Chen C, Madera L, Falsafi R, Fjell CD, Hancock REW. Synthetic cationic peptide IDR-1018 modulates human macrophage differentiation. *PLoS One* 2013; **8**(1): e52449.
23 Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol.* 2011; **11**(11): 723–737.
24 Cherkasov A, Hilpert K, Jenssen H, Fjell CD, Waldbrook M, Mullaly SC, Volkmer R, Hancock REW. Use of artificial intelligence in the design of small peptide antibiotics effective against a broad spectrum of highly antibiotic-resistant superbugs. *ACS Chem. Biol.* 2009; **4**(1): 65–74.
25 Steinstraesser L, Hirsch T, Schulte M, Kueckelhaus M, Jacobsen F, Mersch EA, Stricker I, Afacan N, Jenssen H, Hancock REW, Kindrachuk J. Innate defense regulator peptide 1018 in wound healing and wound infection. *PLoS One* 2012; **7**(8): e39373.
26 Achtman AH, Pilat S, Law CW, Lynn DJ, Janot L, Mayer ML, Ma S, Kindrachuk J, Finlay BB, Brinkman FS, Smyth GK, Hancock REW, Schofield L. Effective adjunctive therapy by an innate defense regulatory peptide in a preclinical model of severe malaria. *Sci. Transl. Med.* 2012; **4**(135): 135ra64.
27 Rivas-Santiago B, Castañeda-Delgado JE, Rivas Santiago CE, Waldbrook M, González-Curiel I, León-Contreras JC, Enciso-Moreno JA, del Villar V, Mendez-Ramos J, Hancock REW, Hernandez-Pando R. Ability of innate defence regulator peptides IDR-1002, IDR-HH2 and IDR-1018 to protect against Mycobacterium tuberculosis infections in animal models. *PLoS One* 2013; **8**(3): e59119.
28 Bolouri H, Sävman K, Wang W, Thomas A, Maurer N, Dullaghan E, Fjell CD, Ek CJ, Hagberg H, Hancock REW, Brown KL, Mallard C. Innate defense regulator peptide 1018 protects against perinatal brain injury. *Ann. Neurol.* 2014; **75**(3): 395–410.
29 Hoffman LR, D'Argenio DA, MacCoss MJ, Zhang Z, Jones RA, Miller SI. Aminoglycoside antibiotics induce bacterial biofilm formation. *Nature* 2005; **436**(7054): 1171–1175.
30 Loutet SA, Valvano MA. Extreme antimicrobial peptide and polymyxin B resistance in the genus burkholderia. *Front. Microbiol.* 2011; **2**: 159.
31 Otto M Bacterial evasion of antimicrobial peptides by biofilm formation. *Curr. Top. Microbiol. Immunol.* 2006; **306**: 251–258.
32 Reffuveille F, de la Fuente-Núñez C, Mansour S, Hancock REW. A broad-spectrum anti-biofilm peptide enhances antibiotic action against bacterial biofilms. *Antimicrob. Agents Chemother.* 2014.
33 Schierle CF, De la Garza M, Mustoe TA, Galiano RD. Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. *Wound Repair Regen.* 2009; **17**(3): 354–359.
34 Zhao G, Usui ML, Lippman SI, James GA, Stewart PS, Fleckman P, Olerud JE. Biofilms and inflammation in chronic wounds. *Adv. Wound Care (New Rochelle)* 2013; **2**(7): 389–399.
35 Bosmann M, Ward PA. The inflammatory response in sepsis. *Trends Immunol.* 2013; **34**(3): 129–136.

