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Review

Potential therapeutic applications of multifunctional host-defense peptides from frog skin as anti-cancer, anti-viral, immunomodulatory, and anti-diabetic agents

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ABSTRACT

Frog skin constitutes a rich source of peptides with a wide range of biological properties. These include host-defense peptides with cytotoxic activities against bacteria, fungi, protozoa, viruses, and mammalian cells. Several hundred such peptides from diverse species have been described. Although attention has been focused mainly on antimicrobial activity, the therapeutic potential of frog skin peptides as anti-infective agents remains to be realized and no compound based upon their structures has yet been adopted in clinical practice. Consequently, alternative applications are being explored. Certain naturally occurring frog skin peptides, and analogs with improved therapeutic properties, show selective cytotoxicity against tumor cells and viruses and so have potential for development into anti-cancer and anti-viral agents. Some peptides display complex cytokine-mediated immunomodulatory properties. Effects on the production of both pro-inflammatory and anti-inflammatory cytokines by peritoneal macrophages and peripheral blood mononuclear cells have been observed so that clinical applications as anti-inflammatory, immunosuppressive, and immunostimulatory agents are possible. Several frog skin peptides, first identified on the basis of antimicrobial activity, have been shown to stimulate insulin release both *in vitro* and *in vivo* and so show potential as incretin-based therapies for treatment of patients with Type 2 diabetes mellitus. This review assesses the therapeutic possibilities of peptides from frogs belonging to the Ascaphidae, Alytidae, Pipidae, Dicroglossidae, Leptodactylidae, Hylidae, and Ranidae families that complement their potential role as anti-infectives for use against multidrug-resistant microorganisms.

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1. Introduction

Skin secretions from many species of Anura (frogs and toads) contain a wide range of compounds with biological activity, often in very high concentrations, that have excited interest because of their potential for drug development. Among these substances are host-defense peptides with broad-spectrum antibacterial and antifungal activities, and the ability to permeabilize mammalian cells [16]. These peptides vary in size from as small as 8 up to 48 amino acid residues, and a comparison of their amino acid sequences reveals the lack of any conserved domains that are associated with cytotoxic activity. However, with few exceptions, these peptides are cationic (charge between +1 and +6 at pH 7) and contain between 40 and 70% hydrophobic amino acids [71]. Circular dichroism and nuclear magnetic resonance (NMR) studies have shown that they generally lack stable secondary structure in aqueous solutions but have the propensity to form an amphipathic α-helix in the environment of a phospholipid vesicle or in a membrane-mimetic solvent such as 50% trifluoroethanol–water. There is no single mechanism by which peptides produce cell death but their action generally does not involve binding to a receptor rather a non-specific interaction with the bacterial cell membrane or mammalian plasma membrane that results in loss of integrity and ultimate disintegration [7,13].

It is a common fallacy that all frog species produce peptides with antimicrobial activity in their skin secretions. Although considered to be a component of the animal's system of innate immunity, species distribution of these peptides is sporadic suggesting that their production in the skin may confer some evolutionary advantage to the organism but is not necessary for survival. It has been proposed that cutaneous symbiotic bacteria may provide the major system of defense against pathogenic microorganisms in the environment with antimicrobial peptides assuming a supplementary role in some species [17,74].

The emergence in all regions of the world of strains of pathogenic bacteria and fungi with resistance to commonly used antibiotics constitutes a serious threat to public health. This has necessitated a search for novel types of antimicrobial agent with appropriate pharmacokinetic and toxicological profiles that are active against these multidrug- or pandrug-resistant microorganisms. Over 26 years have passed since the discovery of the magainins in the skin of the African clawed frog, *Xenopus laevis* in the family Pipidae. These peptides, identified independently by Michael Zasloff at the National Institutes of Health, Bethesda, U.S.A. [78] and by the group of Dudley H. Williams at the University of Cambridge, U.K. [35], were the first amphibian peptides with antimicrobial activity to be fully characterized. Since that time several hundred such peptides have been isolated from the skin secretions of many other frog species belonging to different families [71]. However, despite showing potent activity against strains of antibiotic-resistant bacteria and against certain pathogenic fungi and protozoa, the potential of these peptides as therapeutic agents has not been realized. Enthusiasm for frog skin peptides within the pharmaceutical industry declined when the US Food and Drug Administration did not approve marketing of pexiganan, an analog of magainin-2 with potent, broad spectrum antimicrobial activity [34], for treatment of infected foot ulcers in diabetic patients on the grounds that efficacy had not been sufficiently demonstrated. No anti-infective compound based upon the structure of a frog skin peptide has yet been adopted in clinical practice. In consequence, alternative therapeutic applications are being explored.

It is now appreciated that cationic α-helical antimicrobial peptides are multi-functional displaying immunomodulatory, chemoattractant, and insulinotropic properties as well as cytotoxic activities [76]. Consequently, it is more informative to refer to them as host-defense peptides rather than as exclusively antimicrobial peptides. Analogs of naturally occurring amphibian peptides have been developed that show selective cytotoxicity against tumor cells and so have potential for development into anti-cancer agents [47]. As well as producing tumor cell death by disruption of the plasma membrane, certain cationic antimicrobial peptides can instigate apoptosis *via* the mitochondrial pathway and act as anti-angiogenic factors [48,58]. Similarly, certain peptides in frog skin secretions have demonstrated potent antiviral activity, either by directly inactivating the virus particles or by interfering with the initial steps of the viral reproductive cycle such as binding to specific cell surface receptors and subsequent entry into the cytoplasm [58]. These properties, combined with the short contact time required to induce killing, have led to their consideration as candidates for development into novel antiviral agents. The problems posed by the emergence of multidrug resistance in the treatment of bacterial infections are also encountered in cancer and viral chemotherapy [45]. Because of their non-specific and destructive mechanism of action, cell-penetrating peptides show therapeutic potential in cases where the tumor or virus is not responsive to conventional pharmaceutical therapy.

Frog skin host-defense peptides, in common with many other cationic antimicrobial peptides of diverse origins [15,76], display complex cytokine-mediated immunomodulatory properties. Effects on the production of both pro-inflammatory and anti-inflammatory cytokines by peritoneal macrophages and peripheral blood mononuclear cells have been observed so that

clinical applications as anti-inflammatory, immunosuppressive, and immunostimulatory agents are possible.

The current pandemic of Type 2 diabetes mellitus has necessitated a search for new types of therapeutic agents. Several peptide-based drugs that target the enteroinsular axis by stimulating the release of insulin are in clinical trials or have already been adopted in clinical practice [30,31]. A number of frog skin peptides that were first identified on the basis of their ability to inhibit the growth of microorganisms have subsequently been shown to possess the ability to release insulin from BRIN-BD11 cells at low concentrations that are not cytotoxic to the cells and to improve glucose tolerance in mice. The BRIN-BD11 rat clonal β-cell line is a well-established and convenient model to study insulin release in response to a range of nutrients, hormones, neurotransmitters and drugs [54]. Such peptides show therapeutic potential for the treatment of patients with Type 2 diabetes mellitus.

This review addresses the therapeutic possibilities of peptides isolated from skin secretions of frogs belonging to the families Ascaphidae, Alytidae, Pipidae, Dicroglossidae, Leptodactylidae, Hylidae, and Ranidae that are additional to their potential role as anti-infectives for use against multidrug-resistant microorganisms. The taxonomic recommendations of Frost [32] are adopted with regard to the naming of frog species.

2. Frog skin host-defense peptides with therapeutic potential as anti-cancer agents

The origins and primary structures of naturally occurring peptides and their analogs with anti-cancer properties described in this section are shown in Table 1.

2.1. Ascaphidae

The family Ascaphidae, comprising two species in the single genus *Ascaphus*, is the phylogenetically most ancient group of frogs and the taxon is sister-group to the clade containing all extant anurans [32]. Ascaphin-8 from skin secretions of *Ascaphus truei* shows relatively high cytotoxicity to human hepatocarcinoma-derived HepG2 cells (LC_{50} = 20 μM) but the peptide is appreciably hemolytic to human erythrocytes (LC_{50} = 55 μM) [20]. Structure–activity studies demonstrate that increasing cationicity from +4 in the native peptide to +5 in the [G8K] analog results in a 2.5-fold increase in potency against HepG2 cells (LC_{50} = 8 μM) but a 5-fold increase in hemolytic activity (LC_{50} = 11 μM). In contrast, the [G8k] analog (k = D-lysine) retains high potency against tumor cells (LC_{50} = 15 μM) but has relatively weak hemolytic activity (LC_{50} = 150 μM). Similarly, the [D4k] analog with a charge of +6 at pH 7 is active against HepG2 cells (LC_{50} = 22 μM) but is even less hemolytic (LC_{50} = 300 μM).

2.2. Alytidae

The family Alytidae currently comprises 12 species divided into three genera *Alytes* (5 species), *Discoglossus* (6 species), and *Latonia* (1 species) [32]. Alyteserin-2a from the midwife toad *Alytes obstetricans*, displays relatively weak antimicrobial and cytotoxic activities [21]. However, a structure–activity study has led to the design of analogs of the naturally occurring peptide with increased potencies and selectivities for tumor cells [23]. Incorporation of D-lysine residue at position 11 and an L-lysine residue at position 15 into alyteserin-2a generates a peptide that retains potency against human non-small cell lung adenocarcinoma A549 cells (LC_{50} = 15 μM) but whose therapeutic index (ratio of LC_{50} values for erythrocytes and tumor cells) is 13-fold elevated relative to the native peptide. [G11k,N15K]alyteserin-2a is also active against hepatocarcinoma HepG2 cells (LC_{50} = 26 μM), human breast adenocarcinoma MDA-MB-231 cells (LC_{50} = 20 μM), and human colorectal adenocarcinoma HT-29 cells (LC_{50} = 28 μM).

2.3. Pipidae

The African clawed frogs belonging to the family Pipidae are currently classified in the genera *Silurana* (2 species) and *Xenopus* (19 species), united in the sub-family Xenopodinae, together with the sister-taxa *Hymenochirus* (4 species) and *Pseudhymenochirus* (1 species) although new species are continuously being identified [32]. Magainin-2 from *X. laevis* and its C-terminally α-amidated, carboxypeptidase-resistant analog, magainin G show potential as anticancer agents displaying tumoricidal activity against human small cell lung cancer cell lines [59], the RT4, 647V, and 486P bladder cancer cell lines [42], and against suspension cultures of a wide range of hematopoietic cell lines [28]. Out of a range of antimicrobial peptides tested, the magainin-2 analog, pexiganan showed the greatest cytotoxic activity against the U937 human histiocytic lymphoma cell line [39]. The anti-tumor activity of protease-resistant all D-amino acid magainin-2 amide (MSI-238) is markedly superior to the parent compound displaying high potency *in vitro* against lung adenocarcinoma A549 cells and *in vivo* against P388 leukemia, S180 ascites, and a spontaneous ovarian tumor [10]. The cytotoxic mechanism of [F5W]magainin-2 against HeLa cells, has been investigated and involves initial interaction of the peptide with cell surface gangliosides [57].

Peptide XT-7, a caerulein precursor fragment (CPF) peptide from *Silurana tropicalis*, shows only moderate cytotoxic potency against hepatocarcinoma HepG2 cells (LC_{50} = 75 μM) but increasing the cationicity of the peptide by appropriate amino acid substitutions by L-lysine that preserve amphipathicity results in a progressive increase in activity ([S15K]XT-7, LC_{50} = 24 μM; [S15K,N16K]XT-7, LC_{50} = 10 μM; [P5K,S15K,N16K]XT-7, LC_{50} = 5 μM) [20]. However, this increase in potency against tumor cells is mirrored by a corresponding increase in hemolytic activity.

Hymenochirin-1B from *Hymenochirus boettgeri* shows high cytotoxic potency against lung adenocarcinoma A549 cells (LC_{50} = 2.5 μM), breast adenocarcinoma MDA-MB-231 cells (LC_{50} = 9.0 μM), colorectal adenocarcinoma HT-29 cells (LC_{50} = 9.7 μM), and hepatocarcinoma HepG2 cells (LC_{50} = 22.5 μM) with appreciably less hemolytic activity against human erythrocytes (LC_{50} = 213 μM) [8]. Structure–activity relationships were investigated by synthesizing analogs of hymenochirin-1B in which Pro^5, Glu^6 and Asp^9 on the hydrophilic face of the peptide helix are replaced by one or more L-lysine or D-lysine residues. The [D9K] analog displays the greatest increase in potency against all four cell lines (up to 6-fold) but hemolytic activity also increases (LC_{50} = 174 μM). The [D9k] and [E6k,D9k] analogs retain relatively high cytotoxic potency against the tumor cells (LC_{50} in the range 2.1–21 μM) but show reduced hemolytic activity (LC_{50} > 300 μM).

2.4. Leptodactylidae

The Leptodactylidae family of New World frogs is divided into three sub-families Leiuperinae (currently 90 species); Leptodactylinae (currently 98 species), and Paratelmatobiinae (currently 13 species) [32]. Pentadactylin from *Leptodactylus labyrinthicus* (Leptodactylinae) is cytotoxic to the B16F10 murine melanoma cell line (LC_{50} = 26 μM) but the specificity for tumor cells is not high. The LC_{50} value for non-neoplastic human fibroblast cells is 36 μM [44]. Incubation of the tumor cells with pentadactylin resulted in DNA fragmentation, cell cycle arrest at the S phase, and alteration in mitochondrial membrane potential suggesting an involvement of apoptosis in cell death.

Table 1
Origins and primary structures of frog skin peptides with therapeutic potential as anti-cancer agents.

Species	Family	Cytotoxic peptide	Primary structure
Tailed frog *Ascaphus truei*	Ascaphidae	Ascaphin-8	GFKDLLKGAAKALVKTVLF[a]
Midwife toad *Alytes obstetricans*	Alytidae	Alyteserin-2	ILGKLLSTAAGLLSNL[a]
South African clawed frog *Xenopus laevis*	Pipidae	Magainin-2	GIGKFLHSAKKFGKAFVGEIMNS
		Pexiganan	GIGKFLKKAKKFGKAFVKILKK[a]
Congo dwarf clawed frog *Hymenochirus boettgeri*	Pipidae	Hymenochirin-1B	KLSPETKDNLKKVLKGAIKGAIVAKMV[a]
Tropical clawed frog *Silurana tropicalis*	Pipidae	Peptide XT-7	GLLGPLLKIAAKVGSNL[a]
Pepper frog *Leptodactylus labyrinthicus*	Leptodactylidae	Pentadactylin	GLLDTLKGAAKNVVGSLASKVMEKL
Lemur leaf frog *Agalychnis lemur*	Hylidae	Dermaseptin L1	GLWSKIKEAAKAAGKAALNAVTGLVNQGDQPS
		Phylloseptin L1	LLGMIPLAISAISALSKL[a]
Giant monkey frog *Phyllomedusa bicolor*	Hylidae	Dermaseptin B2	GLWSKIKEVGKEAAKAAAKAAGKAALGAVSEAV
		Dermaseptin B3	ALWKNMLKGIGKLAGQAALGAVKTLVGAE
Green and golden bell frog *Litoria aureus*	Hylidae	Aurein 1.2	GLFDIIKKIAESF[a]
		Aurein 3.1	GLFDIVKKIAGHIAGSI[a]
Chiricahua leopard frog *Lithobates chiricahuensis*	Ranidae	Esculentin-2CHa	GFSSIFRGVAKFASKGLGK DLAKLGVDLVACKISKQC
Chinese brown frog *Rana chensinensis*	Ranidae	Temporin-CEa	FVDLKKIANIINSIF[a]
Foothill yellow-legged frog *Rana boylii*	Ranidae	Brevinin-1BYa	FLPILASLAAKFGPKLFCLVTKKC

[a] Denotes C-terminal α-amidation.

2.5. Hylidae

The extensive family Hylidae is divided into the sub-families Phyllomedusinae (currently 59 species in 5 genera), Pelodryadinae (currently 203 species in the single genus *Litoria*), and Hylinae (currently 674 species in 43 genera) [32]. Both dermaseptin L1 and phylloseptin L1, isolated from norepinephrine-stimulated skin secretions of the lemur leaf frog *Agalychnis lemur* (Phyllomedusinae), are cytotoxic to hepatocarcinoma HepG2 cells [19]. Dermaseptin L1 showed selective cytolytic activity against HepG2 cells (LC_{50} = 45 μM compared with an LC_{50} of 200 μM for human erythrocytes) whereas phylloseptin L1 was approximately equipotent against both HepG2 cells (LC_{50} = 35 μM) and erythrocytes (LC_{50} = 40 μM). Dermaseptin B2 and B3 from the South American tree frog *Phyllomedusa bicolor* (Phyllomedusinae) show both antitumor and angiostatic properties, inhibiting proliferation of prostatic adenocarcinoma PC-3 cells as well as proliferation and differentiation of bovine aortic endothelial cells [70]. Several aurein peptides from the green and golden bell frog *Litoria aureus* and the southern bell frog *Litoria raniformis* (Pelodryadinae) possess anti-cancer activity [65]. Aureins 1.2 and 3.1 show the most potent activity in the National Cancer Institute test regime with LC_{50} values in the 10–100 μM range.

2.6. Ranidae

According to the taxonomic recommendations of Frost [32], the widely distributed family Ranidae currently comprises 357 species divided into 16 genera but it must be pointed out that this classification is not accepted by all herpetologists. Esculentin-2CHa from the Chiricahua leopard frog *Lithobates chiricahuensis* shows relatively high cytotoxic potency against lung adenocarcinoma A549 cells (LC_{50} = 10 μM) with appreciably lower hemolytic activity against human erythrocytes (LC_{50} = 150 μM) [9]. The more cationic [D20K,D27K] analog displays a 3-fold increase against A549 cells (LC_{50} = 3 μM) but this is offset by a 14-fold increase in cytotoxicity against erythrocytes (LC_{50} = 11 μM). Kinetic studies showed that the analog at a concentration of 10 μM produces > 90% death of A549 cells after 30 min and 100% cell death after 6 h. Replacement of the Cys^{31} and Cys^{37} residues in the cyclic domain by L-serine generates an analog that retains cytotoxic activity against A549 cells (LC_{50} = 26 μM) but shows very low hemolytic activity (LC_{50} greater than 200 μM) [9].

Temporin-1CEa from the Chinese brown frog *Rana chensinensis* produces rapid cell death in MDA-MB-231 and MCF-7 human breast cancer cell lines by a mechanism that involves induction of cell-surface exposure of phosphatidylserine, elevation of plasma membrane permeability and rapid depolarization of transmembrane potential. Additionally, temporin-1CEa produces elevations in intracellular Ca^{2+} and reactive oxygen species as well as collapse of mitochondrial membrane potential [73].

An analog of brevinin-1BYa from *Rana boylii* in which the intramolecular disulfide bridge in the peptide is replaced by a metabolically stable, non-reducible dicarba bond shows increased anti-tumor activity compared with the naturally occurring peptide [36]. The dicarba derivative is associated with increased cytotoxicity against breast adenocarcinoma MDA-MB-231 cells (1.3-fold; LC_{50} = 7 μM), and hepatocarcinoma HepG2 cells (1.5-fold; 4 μM) but cytotoxicity against human erythrocytes also increases (2.5-fold; 4 μM). The acyclic [C18S,C24S] analog retains high anti-tumor activity (LC_{50} = 13 μM against MDA-MB-231cells and LC_{50} = 10 μM against HepG2 cells) but is appreciably less hemolytic (LC_{50} = 75 μM).

3. Frog skin host-defense peptides with therapeutic potential as anti-viral agents

The origins and primary structures of naturally occurring frog skin peptides with antiviral properties described in this section are shown in Table 2.

Table 2
Origins and primary structures of frog skin peptides with therapeutic potential as anti-viral agents.

Species	Family	Peptide	Primary structure
South African clawed frog *Xenopus laevis*	Pipidae	Magainin-2B	GIGKFLHAAKKFAKAFVAEIMNS[a]
Volcano clawed frog *Xenopus amieti*	Pipidae	PGLa-AM1	GMASKAGSVLGKVAKVALKAAL[a]
		CPF-AM1	GLGSVLGKALKIGANLL[a]
Painted-belly leaf frog *Phyllomedusa sauvagei*	Hylidae	Dermaseptin S4	ALWMTLLKKVLKAAAKAALNAVLVGANA
		Dermaseptin S9	GLRSKIWLWVLLMIWQESNKFKKM
White's tree frog *Litoria caerulea*	Hylidae	Caerin 1.1	GLLSVLGSVAKHVLPHVVPVIAEHL[a]
Red-eyed tree frog *Litoria chloris*	Hylidae	Caerin 1.9	GLFGVLGSIAKHVLPHVVPVIAEKL[a]
Green-eyed tree frog *Litoria genimaculata*	Hylidae	Maculatin 1.1	GLFGVLAKVAAHVVPAIAEHF[a]
Kanagawa frog *Pelophylax porosus*	Ranidae	Brevinin-1	FLPVLAGIAAKVVPALFCKITKKC
Green paddy frog *Hylarana erythraea*	Ranidae	B2RP-ERa	GVIKSVLKGVAKTVALGML[a]

[a] Denotes C-terminal α-amidation.

3.1. Pipidae

Magainin-1 and -2 show antiviral properties against herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) but were inactive against the arenavirus, Junin virus [6]. The peptides do not appear to inactivate the HSV particles directly but rather target important steps in the viral reproductive cycle. Magainin-2 and PGLa from *X. laevis* reduced markedly the infectivity of channel catfish virus but were less potent against frog virus 3 [14]. Magainin-1 was ineffective against both viruses. Mechanistic studies have shown that the Ala-substituted magainin-2 amide analog, magainin-2B directly inactivates vaccinia virus by disrupting and removing the outer membrane envelope [29].

Both CPF-AM1 and PGLa-AM1 from *Xenopus amieti*, are capable of destroying more than 90% of extracellular HSV-1 virions within the first 5 min of direct contact. In addition, these two peptides inhibit the viral penetration and replication in Madin-Darby Bovine Kidney (MDBK) cells when applied at non-toxic concentrations (≤200 μM). Similarly, peptide XT-7 from *S. tropicalis* can destabilize HSV-1 particles and block virus entry and/or replication with effective concentration inhibiting 50% viral replication, EC_{50} = 87 μM (S. Shishkov and M. Mechkarska, unpublished data).

3.2. Hylidae

Dermaseptins S1–S5 from *Phyllomedusa sauvagei* are effective inhibitors of HSV-1 [11] and HSV-2 [12] infectivity. Dermaseptin S4, the most effective peptide against HSV-1, demonstrates its inhibitory effect only when applied to the virus before, or during virus adsorption to the target cells, suggesting that the activity of the peptide is exerted at a very early stage of the viral multiplication cycle, most likely at the virus–cell interface. Dermaseptin S4 shows potent effects against acyclovir-sensitive and acyclovir-resistant strains of HSV-2 ($EC_{50} \leq 6.0$ μM) accompanied by high cytotoxicity [LC_{50} = 7.5 μM for African Green Monkey Kidney (Vero) cells]. However, the [M4K,N20K] analog of dermaseptin S4 retains the high potency of the native peptide against both strains ($EC_{50} \leq 5.4$ μM) but is appreciably less cytotoxic (specificity index SI, representing the ratio of LC_{50} to EC_{50} against the HSV-2 acyclovir-sensitive strain = 12.1) [12]. Dermaseptin S4 and its less cytotoxic [M4K] analog inhibit cell-free and cell-associated HIV-1 infection of P4-CCR5 indicator cells and human primary T lymphocytes [46]. The peptides act directly on the viral particles or during the initial phase of virus–cell interactions. [M4K] dermaseptin S4 also inhibits HIV-1 capture by dendritic cells and subsequent transmission to $CD4^+$ T cells. Dermaseptin S9, which does not resemble the other dermaseptins from *P. sauvagei* [43] showed very weak activity against HIV-1 but the analog in which three lysines are replaced by arginines possesses strong virus-inhibitory activity with a therapeutic index of 26 (ratio of concentration causing 50% inhibition of virus replication to concentration causing 50% reduction in viability of CEM-SS human T4 lymphoblastoid cells) [72].

Caerin 1.1, caerin 1.9, and maculatin 1.1, from skin secretions of three different species of Australian tree frogs, inhibit HIV infection in concentrations (EC_{50} = 7.8, 1.2 and 11.3 μM respectively) that are non-toxic to the target T-cells [69]. The effect is mediated through inactivation of the HIV virions, and caerin 1.9 can potently inhibit infection of cells by HIV when pseudotyped with envelopes from different viruses. Moreover, caerin 1.1 and caerin 1.9 can inhibit (with similar EC_{50} values) infection of T-cells indirectly (*trans* infection) by killing HIV captured by dendritic cells before it is transmitted to T-cells. The *trans* infection is inhibited with greater than 95% efficiency even when dendritic cells are exposed to the peptides as late as 8 hours following HIV capture. The effect of the caerin peptides appears to be virus-specific, as they do not inhibit infection caused by a non-enveloped reovirus.

3.3. Ranidae

Brevinin-1, from skin secretions of the Asian frog *Pelophylax porosus*, is a potent inhibitor of HSV-1 and HSV-2 infection of Vero cells [75]. Its antiviral activity is not affected by reduction and carboxamidomethylation of the cysteine residues, a procedure that reduces its otherwise prominent hemolytic and cytotoxic effects. Brevinin-1BYa from *R. boylii* and B2RP-ERa, a brevinin-2-related peptide from *Hylarana erythraea*, inhibit HSV-1 infection of MDBK cells (S. Shishkov and M. Mechkarska, unpublished data). Brevinin-1BYa affects multiple steps of the infection cycle including inactivation of free virions and/or intracellular virus replication with EC_{50} of 7.3 μM. However, brevinin-1BYa suffers from the disadvantage of high cytotoxicity to the MDBK cells. The less cytotoxic B2RP-ERa has a weaker effect on the virus particles and on virus penetration but does not affect virus replication.

4. Frog skin host-defense peptides with therapeutic potential as immunomodulatory agents

The origins and primary structures of naturally occurring frog skin peptides with immunomodulatory properties described in this section are shown in Table 3.

Table 3
Origins and primary structures of frog skin peptides with therapeutic potential as immunomodulatory agents.

Species	Family	Peptide	Primary structure
Tyrrhenian painted frog *Discoglossus sardus*	Alytidae	Frenatin 2D	DLLGTLGNLPLPFI[a]
Volcano clawed frog *Xenopus amieti*	Pipidae	Magainin-AM1	GIKEFAHSLGKFGKAFVGGILNQ
Mueller's clawed frog *Xenopus muelleri*	Pipidae	Tigerinin-1M	WCPPMIPLCSRF[a]
Vietnamese lowland frog *Hoplobatrachus rugulosus*	Dicroglossidae	Tigerinin-1R	RVCSAIPLPICH[a]
Santa Fe frog *Leptodactylus laticeps*	Leptodactylidae	Plasticin-L1	GLVNGLLSSVLGGGQGGGGLLGGIL
Orinoco lime frog *Sphaenorhynchus lacteus*	Hylidae	Frenatin 2.1S	GLVGTLLGHIGKAILG[a]
		Frenatin 2.2S	GLVGTLLGHIGKAILS[a]
Vaillant's frog *Lithobates vaillanti*	Ranidae	Tigerinin-1V	RICYAMWIPYPC
Guenther's frog *Hylarana guentheri*	Ranidae	Brevinin-2GUb	GVIIDTLKGAAKTVAAELLRKAHCKLTNSC
California red-legged frog *Rana draytonii*	Ranidae	Temporin-DRa	HFLGTLVNLAKKIL[a]

[a] Denotes C-terminal α-amidation.

4.1. Ascaphidae

Production of the pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α) from concanavalin A (ConA)-stimulated human peripheral blood mononuclear (PBM) cells is significantly reduced by incubation with [D4k] analog of ascaphin-8 [64]. Production of interferon-γ (IFN-γ) from unstimulated PBM cells was also significantly reduced by [D4k]ascaphin-8. The peptide was without effect on IL-17 production by unstimulated and ConA-stimulated cells.

4.2. Alytidae

The [G11k,N15K] analog of alyteserin-2a from *A. obstetricans* inhibits the production of the anti-inflammatory cytokine IL-10 and transforming growth factor-β (TGF-β) from unstimulated and ConA-stimulated PBM cells [23]. Antimicrobial activity is not an essential pre-requisite for immunomodulatory activity. Frenatin 2D from *Discoglossus sardus* lacks both antimicrobial and hemolytic activities but stimulates production of the pro-inflammatory cytokines TNF-α and IL-1β by mouse peritoneal macrophages, but the peptide did not potentiate the stimulation produced by lipopolysaccharide (LPS) [22]. The peptide increased IL-12 production in both unstimulated and LPS-stimulated cells but stimulatory effects on IL-6 production were not significant.

4.3. Pipidae

Magainin-AM1 from *X. amieti*, as well as showing potent growth-inhibitory activity against a panel of oral and respiratory pathogens, increases production of the pro-inflammatory cytokine IL-8 by oral fibroblasts at concentrations of 1 and 10 μM (F. Lundy, unpublished data). In contrast, production of TNF-α from ConA-stimulated human PBM cells is significantly reduced by incubation with [G4K]XT-7 [64]. The [E6k,D9k] analog of hymenochirin-1B (10 μg/ml) increases the production of anti-inflammatory IL-10 from both unstimulated and Con A-stimulated PBM cells without increasing the rate of production of pro-inflammatory TNF-α and IL-17 [56]. In addition, the peptide enhances production of anti-inflammatory IL-4 in unstimulated cells and IFN-γ in Con A-stimulated cells.

The tigerinins are a family of cationic, cyclic peptides of unknown biological function produced in the skins of diverse frog species that also lack antimicrobial and hemolytic activities. The tigerinins display complex effects on the production of pro- and anti-inflammatory cytokines in mice that vary with genetic backgrounds of the strains studied. Tigerinin-1M from *Xenopus muelleri* (20 μg/ml) increases production of pro-inflammatory IL-12 and IL-23 in unstimulated peritoneal macrophages from C57BL/6 mice but had no effect on IL-12 and IL-23 production in BALBc mice [63]. The peptide increases IL-6 production in both unstimulated and LPS-stimulated cells from both mouse strains. Incubation with peritoneal macrophages from both BALB/c and C57BL/6 mice increases production of anti-inflammatory IL-10 and potentiates the stimulation produced by LPS. The peptide (5 μg/ml) downregulates production of IFN-γ in splenocytes from both C57BL/6 and BALBc mice and, in a concentration as low as 1 μg/ml, increases production of IL-10 in unstimulated and LPS-stimulated human PBM cells.

4.4. Dicroglossidae

At this time, the Afro-Asian frogs of the family Dicroglossidae are divided into the sub-families Dicroglossinae containing 162 species in 13 genera and Occidozyginae containing 20 species in 2 genera [32]. Incubation of tigerinin-1R (20 μg/ml) from *Hoplobatrachus rugulosus* (Dicroglossinae) with peritoneal macrophages from both BALB/c and C57BL/6 mice increases production of anti-inflammatory IL-10 and potentiates the stimulation produced by LPS [63]. Tigerinin-1R increases production of IL-6 in LPS-stimulated macrophages from C57BL/6 mice, but not from BALBc mice, but was without effect on the production of proinflammatory IL-12 and IL-23 by macrophages from either mouse strain. The peptide in a concentration as low as 1 μg/ml increases production of IL-10 in unstimulated and LPS-stimulated PBM cells from a healthy human subject. In a population of mononuclear cells derived from the spleens of both C57BL/6 and BALBc mice, tigerinin-1R stimulates production of anti-inflammatory Th2/Treg-derived IL-10 but was without effect on the production of pro-inflammatory Th1-derived IFN-γ and Th17-derived IL-17 [63].

4.5. Leptodactylidae

Plasticin-L1 from *Leptodactylus laticeps* lacks both antimicrobial and hemolytic activities at concentrations up to 500 μg/ml but incubation of the peptide (20 μg/ml) with peritoneal macrophages from both C57BL/6 and BALB/C mice stimulates production of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-12 [66]. Plasticin-L1 also increases IL-6 production by unstimulated and LPS-stimulated macrophages from BALB/C mice while in the case

of macrophages from C57BL/6 mice, the effect is significant only for unstimulated cells. Plasticin-L1 has no effect on production of anti-inflammatory IL-10 from either unstimulated or LPS-stimulated cells.

4.6. Hylidae

Frenatin 2.1S and 2.2S from *Sphaenorhynchus lacteus* in the sub-family Hylinae significantly increases production of proinflammatory cytokines IL-1β and IL-23 by LPS-stimulated mouse peritoneal macrophages and frenatin 2.1S also enhances production of TNF-α. Effects on IL-6 production were not significant [25]. Frenatin 2.2S significantly downregulates production of IL-10 by LPS-stimulated cells.

4.7. Ranidae

In terms of production of pro-inflammatory cytokines, esculentin-2CHa from *Lithobates chiricahuensis* increases synthesis of TNF-α by peritoneal macrophages from BALBc mice but effects on IL-6 and IL-1β production are not significant [9]. Tigerinin-1V from *L. vaillanti* increases production of IL-23 in unstimulated macrophages from C57BL/6 mice and potentiates IL-6 production in LPS-stimulated macrophages from both C57BL/6 and BALB/c mice [63]. Tigerinin-1V (5 μg/ml) also downregulates the production of IFN-γ in spleen cells from both C57BL/6 and BALBc mice. In contrast, production of TNF-α from ConA-stimulated human PBM cells is reduced by incubation with brevinin-2GUb from *Hylarana guentheri*, the brevinin-2-related peptide B2RP-ERa from *Hylarana erythraea*, and the [T5k]analog of temporin-DRa from *Rana draytonii* [64]. Release of IFN-γ from unstimulated PBM cells is also significantly reduced by brevinin-2GUb [64].

In terms of production of anti-inflammatory cytokines, [T5k]temporin-DRa and B2RP-ERa increase synthesis of TGF-β, IL-4, and IL-10 by both unstimulated and ConA-treated PBM cells [64] and esculentin-2CHa potentiates IL-10 production by unstimulated and Con A-stimulated mouse lymphoid cells [9]. Incubation of tigerinin-1V from *Lithobates vaillanti* with peritoneal macrophages from both BALB/c and C57BL/6 mice increases production of IL-10 and potentiates the stimulation produced by LPS [63]. The peptide, in a concentration as low as 1 μg/ml, also significantly increases production of IL-10 in unstimulated and LPS-stimulated human PBM cells.

The effects of those frog skin peptides tested to-date on the production of pro-inflammatory and anti-inflammatory cytokines are summarized in Table 4.

Table 4
An overview of the effects of frog skin host-defense peptides on the production of pro-inflammatory and anti-inflammatory cytokines.

Cytokine	Peptides that stimulate production	Peptides that inhibit production
Pro-inflammatory		
TNF-α	Frenatin 2D	[D4k]ascaphin-8
	Frenatin 2.1S	[G4K]XT-7
	Plasticin-L1	Brevinin-2GUb
	Esculentin-2CHa	B2RP-ERa
		[T5k]temporin-DRa
IFN-γ	[E6k,D9k]hymenochirin-1B	[D4k]ascaphin-8
		Tigerinin-1M
		Tigerinin-1V
		Brevinin-2GUb
IL-1β	Frenatin 2D	
	Frenatin 2.1S	
	Frenatin 2.2S	
	Plasticin-L1	
IL-6	Tigerinin-1M	
	Tigerinin-1R	
	Tigerinin-1V	
	Plasticin-L1	
IL-8	Magainin-AM1	
IL-12	Frenatin-2D	
	Tigerinin-1M	
	Plasticin-L1	
IL-23	Tigerinin-1M	
	Tigerinin-1V	
	Plasticin-L1	
Anti-inflammatory		
TGF-β	[T5k]temporin-DRa	[G11k,N15K]alyteserin-2a
	B2RP-ERa	
IL-4	[E6k,D9k]hymenochirin-1B	
	[T5k]temporin-DRa	
	B2RP-ERa	
IL-10	[E6k,D9k]hymenochirin-1B	[G11k,N15K]alyteserin-2a
	Tigerinin-1M	Frenatin 2.2S
	Tigerinin-1R	
	Tigerinin-1V	
	[T5k]temporin-DRa	
	B2RP-ERa	
	Esculentin-2CHa	

k denotes D-lysine.

5. Frog skin host-defense peptides with therapeutic potential as anti-diabetic agents

The origins and primary structures of naturally occurring frog skin peptides with the ability to stimulate the release of insulin *in vitro* and *in vivo* described in this section are shown in Table 5.

5.1. Alytidae

Alyteserin-2a from *A. obstetricans* produces a significant stimulation of the rate of insulin release from BRIN-BD11 clonal β cells at a concentration of 30 nM with a maximum response of 296 ± 26% of basal release at 3 μM [62]. The peptide does not stimulate release of the cytosolic enzyme lactate dehydrogenase (LDH) at concentrations up to 3 μM indicating that the integrity of the plasma membrane had been preserved. Membrane depolarization and an increase in intracellular Ca^{2+} concentration are involved in the mechanism of action of the peptide. Structure–activity studies led to the design of the [S7k,G11k] analog which showed appreciably increased potency (a significant increase in the rate of insulin release at a concentration of 0.01 nM) and a comparable maximum response (308 ± 14% of basal release at 3 μM) compared with the naturally occurring peptide [62]. However, both alyteserin-2a (LC_{50} = 140 μM) and [S7k,G11k]alyteserin-2a (LC_{50} = 185 μM) display moderate hemolytic activity against human erythrocytes limiting their clinical utility [23].

5.2. Pipidae

Ten peptides belonging to the magainin, peptide glycine-leucine-amide (PGLa), xenopsin precursor fragment (XPF), and caerulein precursor fragment (CPF) families with the ability to stimulate the release of insulin from BRIN-BD11 cells were identified in skin secretions of *X. laevis* [67]. CPF-1, CPF-3, CPF-5 and CPF-6 are the most potent peptides producing a significant increase in the rate of insulin release at concentration of 0.03 nM with CPF-7 producing the maximum stimulation of insulin release (571 ± 30% of basal rate at 3 μM). CPF-SE1 from skin secretions of the tetraploid frog *Silurana epitropicalis* also produces a significant increase in the rate of insulin release at 0.03 nM with a 514 ± 13% increase over basal rate at 3 μM [67]. No CPF peptide stimulated release of LDH from BRIN-BD11 cells at concentrations up to 3 μM. However, CPF peptides generally show hemolytic activity at higher concentration *e.g.* LC_{50} = 50 μM for CPF-SE1 [24] which severely limits their clinical utility. The mechanism of action of the CPF peptides involves,

Table 5
Origins and primary structures of frog skin peptides with therapeutic potential as anti-diabetic agents.

Species	Family	Peptide	Primary structure
South African clawed frog *Xenopus laevis*	Pipidae	CPF-1	GLASFLGKALKAGLKIGAHLLGGAPQQ
		CPF-3	GFGSFLGKALKAALKIGANALGGSPQQ
		CPF-5	GFGSFLGKALKTALKIGANALGGSPQQ
		CPF-6	GFASFLGKALKAALKIGANMLGGAPQQ
		CPF-7	GFGSFLGKALKAALKIGANALGGAPQQ
Cameroon clawed frog *Silurana epitropicalis*	Pipidae	CPF-SE1	GFLGPLLKLGLKGVAKVIPHLIPSRQQ
Marsabit clawed frog *Xenopus borealis*	Pipidae	Caerulein-B1	<EQDY(SO_3)GTGWMDF[a]
Volcano clawed frog *Xenopus amieti*	Pipidae	Xenopsin	<EGKRPWIL
		Xenopsin-AM2	<EGRRPWIL
Santa Fe frog *Leptodactylus laticeps*	Leptodactylidae	Ocellatin-L2	GVVDILKGAAKDLAGHLATKVMDKL[a]
Vietnamese lowland frog *Hoplobatrachus rugulosus*	Dicroglossidae	Tigerinin-1R	RVCSAIPLPICH[a]
Splendid leaf frog *Cruziohyla calcarifer*	Hylidae	RK-13	RRKPLFPLIPRPK
Lemur leaf frog *Agalychnis lemur*	Hylidae	Phylloseptin L2	FLSLIPHVISALSSL[a]
Paradoxical frog *Pseudis paradoxa*	Hylidae	Pseudin-2	GLNALKKVFQGIHEAIKLINNHVQ
Rough-skinned frog *Glandirana emeljanovi*	Ranidae	Gaegurin-6	FLPLLAGLAANFLPTIICKISYKC
Montane brown frog *Rana ornativentris*	Ranidae	Temporin-Oe	ILPLLGNLLNGLL[a]
American bullfrog *Lithobates catesbeianus*	Ranidae	Ranatuerin-2CBd	GFLDIIKNLGKTFAGHMLDKIRCTIGTCPPSP
		Brevinin-1CBb	FLPFIARLAAKVFPSIICSVTKKC
Mink frog *Lithobates septentrionalis*	Ranidae	Brevinin-2-related peptide	GIWDTIKSMGKVFAGKILQNL[a]

[a] Denotes C-terminal α-amidation, <denotes pyroglutamate.

at least in part, membrane depolarization and an increase in intracellular Ca^{2+} concentration.

Caerulein-B1 from *Xenopus borealis* at concentrations ≥30 nM stimulates the rate of insulin secretion from BRIN-BD11 cells with a maximum response (360% of basal rate) at 3 μM. The peptide (1 μM) also produces a significant (128%, $P < 0.05$) stimulation of the rate of insulin release in the presence of 16.7 mM glucose [77]. Unfortunately, the therapeutic potential of caerulein-B1 may be limited by the observation that repeated intraperitoneal injections of caerulein into rats and mice produce acute edematous pancreatitis with cellular necrosis. Xenopsin and xenopsin-AM2 from the octoploid frog *Xenopus amieti* also stimulate the rate of insulin secretion from BRIN-BD11 cells at concentrations ≥30 nM but the peptides produce a significantly lower maximum response than caerulein-B1 (198% of basal rate for xenopsin and 207% of basal rate for xenopsin-AM2 at a concentration of 3 μM) [77].

5.3. Dicroglossidae

The immunomodulatory peptide tigerinin-1R stimulates the rate of release of insulin from BRIN-BD11 cells at concentrations ≥0.1 nM [60]. The maximum response is 405% of the basal rate at 5.6 mM ambient glucose concentration and 290% of basal rate at 16.7 mM glucose. The magnitude of this effect is greater than that produced under the same experimental conditions by the antidiabetic drug, tolbutamide and the well-characterized insulinotropic peptides, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). C-terminal α-amidation is necessary for high potency and the mechanism of action of the peptide involves, at least in part, membrane depolarization and an increase in intracellular Ca^{2+} concentration. Administration of tigerinin-1R (75 nmol/kg body weight) to mice fed a high fat diet to induce insulin resistance and obesity enhances insulin release and improves glucose tolerance during the 60 min period after an intraperitoneal glucose load (18 mmol/kg body weight).

Structure–activity studies have shown that the [A5W], [L8W] and [I10W] analogs of tigerinin-1R are more potent than the native peptide producing a significant increase in the rate of insulin release from BRIN-BD11 rat clonal β cells at concentration of 0.01 nM [68]. The increase in the rate of insulin release produced by a 3 μM concentration of the [S4R], [H12K], and [I10W] analogs from both BRIN-BD11 cells and isolated mouse islets was significantly greater than that produced by tigerinin-1R. In contrast to alyteserin-2a analogs and CPF peptides, the tigerinin peptides did not produce detectable hemolysis of human erythrocytes at concentrations up to 500 μM [68]. In addition to its direct effects on insulin release, tigerinin-1R at concentrations ≥0.1 nM stimulates the release of the incretin peptide, GLP-1 from the enteroendocrine GLUTag cell line [61].

5.4. Leptodactylidae

Plasticin-L1 from *L. laticeps* at relatively high concentrations (≥1 μM) produces a modest (139% of basal rate at 3 μM), increase in the rate of glucose-induced release of insulin from BRIN-BD11 cells without increasing the rate of release of LDH [18]. A peptide, termed ocellatin-L2 that is identical to the previously described ocellatin-L1 except for the substitution $Asn^{23} \rightarrow Asp$, is also present in *L. laticeps* skin secretions. Ocellatin-L2 is devoid of antimicrobial and hemolytic actions but shows modest activity in stimulating insulin release from BRIN-BD11cells (181% of basal rate at 3 μM) [18].

5.5. Hylidae

Preliminary studies led to the identification of peptides with significant insulinotropic effects from three species in the subfamily Phyllomedusinae, notably *Phyllomedusa trinitatis* [52], *Agalychnis litodryas* [53] and *Cruziohyla calcarifer* [2]. The peptides from *P. trinitatis* and *A. litodryas* were shown to be dermaseptins but their potencies and mechanisms of action were not determined. A 13-amino-acid residue peptide, termed RK-13, with structural similarity to the N-terminal region of the proline–arginine-rich antimicrobial peptide PR-39 is present in skin secretions of *C. calcarifer* [2]. This peptide lacks antimicrobial activity but stimulates insulin release from BRIN-BD11 cells by a mechanism that may involve protein kinase A but is independent of pertussis toxin-sensitive G proteins.

Phylloseptin L2 from *A. lemur* (Phyllomedusinae) produces a stimulation of insulin release from BRIN-BD11 cells (134% of basal rate at a concentration of 30 nM, with a maximum response of 301% of basal rate at a concentration of 3 μM) without increasing the rate of release of LDH [4]. Administration of phylloseptin L2 (50 nmol/kg body weight) into healthy mice increases total release of insulin and improves glucose tolerance during the 60 min period following after an intraperitoneal injection of glucose (18 mmol/kg body weight) [4].

Pseudin-2 from the skin of the South American paradoxical frog *Pseudis paradoxa* (Hylinae) at the concentrations of 0.1 and 1 μM stimulates insulin release from BRIN-BD11 and is without β-cell cytotoxicity at concentrations up to 3 μM [5]. Increasing the cationicity of pseudin-2 from +2 to +3 by the substitution $Asn^{18} \rightarrow Lys$ increases insulin-releasing potency and maximum response compared with the naturally occurring peptide. The [N18K] analog produces a 46% increase in the rate of insulin release from BRIN-BD11 cells at 1 nM and a 215% increase in insulin release at 1 μM. However, increasing the molecular charge on the peptide to +6 in [N3K,Q10K,E14K]pseudin-2 and to +7 in [N3K,Q10K,E14K,N21K]pseudin-2 produces peptides that had no significant stimulatory effect on insulin release. Increasing the hydrophobicity of the peptide by the substitution $Ile^{16} \rightarrow Phe$ generates an analog that retains insulinotropic action but has increased hemolytic activity [5].

5.6. Ranidae

Preliminary studies led to the identification of brevinin-1 Pa from *Lithobates pipiens* [50], palustrin-1c from *Lithobates palustris* [49], and brevinin-1E, brevinin-2Ec, esculentin-1, and esculentin-1b from *Pelophylax saharicus* [51] with the ability to stimulate insulin release from BRIN-BD11 cells but their potencies were not determined. A peptide of the brevinin-1 family (termed "gaegurin-6" in the publication) from the Korean frog *Glandirana emeljanovi* stimulates insulin release from rat RINm5F insulinoma-derived cells and increases intracellular Ca^{2+} concentrations [38].

Out of seven temporins studied (temporin-Va, -Vb, and -Vc from *Lithobates virgatipes*, temporin-DRa and -DRb from *R. draytonii*, temporin-Oe from *Rana ornativentris*, and temporin-TGb from *Rana tagoi*), temporin-Oe shows the greatest ability to stimulate insulin release from BRIN-BD11 cells (2.6-fold greater than the rate of release produced by 5.6 mM glucose only) [1]. The peptide is active in the concentration range 0.01–1 μM and was not cytotoxic.

Peptidomic analysis of norepinephrine-stimulated skin secretions of the American bullfrog *Lithobates catesbeianus* led to the identification and characterization of seven peptides with *in vitro* insulin-releasing activity (brevinin-1CBb, ranatuerin-1CBa, ranatuerin-2CBc, ranatuerin-2CBd, palustrin-2CBa, temporin-CBa, and temporin-CBf) [55]. Ranatuerin-2CBd is the most potent peptide producing a significant stimulation of insulin release (119% of basal rate) from BRIN-BD11 cells at a concentration of 30 nM, with a maximum response (236% of basal rate) at a concentration of 3 μM. Ranatuerin-2CBd does not stimulate release of LDH at concentrations up to 3 μM indicating low cytotoxicity. Brevinin-1CBb produces the maximum stimulation of insulin release (285% of basal rate at 3 μM) but the peptide is cytotoxic at this concentration.

Brevinin-2GUb is the most potent insulin-releasing peptide identified in an extract of the skin of *H. guentheri* [26]. The peptide significantly stimulates insulin release (139% of basal rate) from BRIN-BD11 cells at a concentration of 0.1 μM with a maximum response (373% of basal rate) at a concentration of 3 μM. Administration of brevinin-2GUb (75 nmol/kg body weight) into healthy mice improves glucose tolerance following an intraperitoneal injection of glucose (18 mmol/kg body weight). Brevinin-2-related peptide (B2RP) from *Lithobates septentrionalis* produces a stimulation of insulin release from BRIN-BD11 cells (148% of basal rate at a concentration of 1 μM with a maximum response of 222% of basal rate at a concentration of 3 μM) without increasing the rate of release of LDH [3]. Increasing cationicity of B2RP while maintaining amphipathicity by the substitution $Asp^{4} \rightarrow Lys$ enhances the insulin-releasing potency (137% of basal rate at a concentration of 0.3 μM) with no stimulation of LDH release at 3 μM. In contrast, the [L18K] and [D4K,L18K] analogs are toxic to the cells and the [K16A] analog, with increased amphipathicity and hydrophobicity, shows reduced potency. Administration of [D4K]B2RP (100 nmol/kg body weight) to mice fed a high fat diet to induce obesity and insulin-resistance enhances insulin release and improves glucose tolerance during the 60 min period following an intraperitoneal glucose load (18 mmol/kg body weight) [3].

6. Conclusion and future directions

It is fair to say that the therapeutic potential of the host-defense peptides present in frog skin secretions has not yet been realized. Although this review has focused upon potential therapeutic applications that do not involve antimicrobial activity, it is not the authors' intention to discount the role of such peptides as anti-infective agents in antimicrobial therapy against multidrug-resistant microorganisms. Examples of peptides that show therapeutic potential include [E4K]alyteserin-1c, active against colistin-resistant strains of multidrug-resistant *Acinetobacter baumannii* (MIC = 1.25–5 μM) [27], CPF-SE3, active against a range of clinical isolates of methicillin-resistant *Staphylococcus aureus* (MIC = 5 μM) [24], and [E6k,D9k]hymenochirin-1B, active against a range of New Delhi metallo-β-lactamase-1 (NDM-1) carbapenemase-producing clinical isolates of Gram-negative bacteria (MIC = 3–6 μM) [56]. The relatively rapid rate of clearance of the peptides from the circulation probably precludes systemic administration unless long-acting, non-toxic analogs can be developed. Peptides applied to infected skin or skin lesions in the form of sprays or ointments can penetrate into the *stratum corneum* to kill microorganisms so that future therapeutic applications are more likely to involve topical administration such as for treatment of infected diabetic foot ulcers, skin conditions such as impetigo, and to promote wound healing.

The study of frog skin host-defense peptides as anti-viral agents is still in its infancy. However, preliminary data suggest that peptides, such as dermasptin-S4, may represent templates for the design of analogs for topical use in the treatment of *Herpes labialis* (cold sores) produced by acyclovir-resistant HSV-1 and genital herpes produced by resistant HSV-2. Similarly, non-cytotoxic analogs of brevinin-1BYa may also find application in topical treatment of HSV-1 infections. Although the half-lives in the circulation of frog skin host-defense peptides such as caerin 1.1 and caerin 1.9 are

too short for systemic administration, long-acting, non-cytotoxic analogs may find applications in the treatment of HIV infection.

Endotoxemic complications, such as severe sepsis and septic shock following infection by Gram-negative bacteria, are caused by release of LPS from bacterial membrane into the bloodstream and are a major cause of death particularly in intensive care units. Cytokine production by cells of the innate immune system plays a critical role in the maintenance of homeostasis and regulation of immune responses during sepsis. The importance of agents that modulate the immune function of the host in the treatment of sepsis is well recognized [40,41]. The ability of peptides such as the tigerinins [63] and [E6k,D9k]hymenochirinin-1B [56] to stimulate production of IL-10 in both macrophages and PBM cells without increasing the rate of production of pro-inflammatory cytokines suggests that these peptides may represent templates for the design of potent anti-inflammatory agents for use in the treatment of sepsis.

The pathogenesis of *acne vulgaris* is multifactorial involving infection of the pilosebaceous unit with the anaerobe *Propionibacterium acnes* and a cytokine-mediated inflammatory response. [D4k]ascaphin-8 and [T5k]temporin-DRa are potent inhibitors of the growth of *P. acnes*, inhibit the release of pro-inflammatory cytokines, and stimulate the release of anti-inflammatory cytokines [64]. A possible therapeutic role in the treatment of *acne vulgaris* is clearly indicated. Although generation of high levels of pro-inflammatory cytokines may lead to toxicity, enhancing their selective release may represent a possible therapeutic application of non-cytotoxic, neutral frog skin peptides such as plasticin-L1 [66], frenatin-2D [22], and frenatin 2.1S [25]. Pro-inflammatory cytokines function as immunostimulatory agents and several compounds that stimulate cytokine release are in clinical practice [41]. The peptides may have an enhancing effect on the innate immune response to microbial infection and tumorigenesis. This scenario could be beneficial in a clinical setting in which the first line of defense should be enhanced without possible complications due to induction of autoimmunity. Such peptides may provide a template to develop a new immunomodulatory class of molecules to be used in concert with other antimicrobial and/or anti-cancer drugs.

Incretin-based therapies are becoming increasingly important in the treatment of patients with Type 2 diabetes [30,31]. An incretin is a factor released by the gut in response to nutrients that facilitates uptake of glucose by peripheral tissues by stimulating secretion of insulin. Long-acting analogs of the endogenous incretins, GLP-1 [33] and GIP [37] promise effective treatments to improve glycemic control in patients with Type 2 diabetes. The ease of synthesis, lack of toxicity, potent insulin-releasing activity *in vitro*, and efficacy *in vivo* indicate that peptides based upon the structure of tigerinin-1R may have potential in Type 2 diabetes therapy. On the negative side, the peptide is rapidly cleared from the circulation so that work is needed to develop long-acting analogs.

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References

[1] Abdel-Wahab Y, Marenah L, Flatt P, Conlon JM. Insulin releasing properties of the temporin family of antimicrobial peptides. Protein Pept Lett 2007;14:702–7.
[2] Abdel-Wahab Y, Marenah L, Orr DF, Shaw C, Flatt PR. Isolation and structural characterisation of a novel 13 amino acid insulin-releasing peptide from the skin secretion of *Agalychnis calcarifer*. Biol Chem 2005;386:581–7.
[3] Abdel-Wahab YH, Patterson S, Flatt PR, Conlon JM. Brevinin-2-related peptide and its [D4K] analogue stimulate insulin release in vitro and improve glucose tolerance in mice fed a high fat diet. Horm Metab Res 2010;42:652–6.
[4] Abdel-Wahab YHA, Power GJ, Flatt PR, Woodhams DC, Rollins-Smith LA, Conlon JM. A peptide of the phylloseptin family from the skin of the frog *Hylomantis lemur* (Phyllomedusinae) with potent *in vitro* and *in vivo* insulin-releasing activity. Peptides 2008;29:2136–43.
[5] Abdel-Wahab YHA, Power GJ, Ng MT, Flatt PR, Conlon JM. Insulin-releasing properties of the frog skin peptide pseudin-2 and its [Lys18]-substituted analogue. Biol Chem 2008;389:143–8.
[6] Albiol Matanic VC, Castilla V. Antiviral activity of antimicrobial cationic peptides against Junin virus and herpes simplex virus. Int J Antimicrob Agents 2004;23:382–9.
[7] Almeida PF, Pokorny A. Mechanisms of antimicrobial, cytolytic, and cell-penetrating peptides: from kinetics to thermodynamics. Biochemistry 2009;48:8083–93.
[8] Attoub S, Arafat H, Mechkarska M, Conlon JM. Anti-tumour activities of the host-defense peptide hymenochirin-1B. Regul Pept 2013;187:51–6.
[9] Attoub S, Mechkarska M, Sonnevend A, Radosavljevic G, Jovanovic I, Lukic ML, et al. Esculentin-2CHa: a host-defense peptide with differential cytotoxicity against bacteria, erythrocytes and tumor cells. Peptides 2013;39:95–102.
[10] Baker MA, Maloy WL, Zasloff M, Jacob LS. Anticancer efficacy of magainin 2 and analogue peptides. Anticancer efficacy of magainin 2 and analogue peptides. Cancer Res 1993;53:3052–7.
[11] Belaid A, Aouni M, Khelifa R, Trabelsi A, Jemmali M, Hani K. In vitro antiviral activity of dermaseptins against herpes simplex virus type 1. J Med Virol 2002;66:229–34.
[12] Bergaoui I, Zairi A, Tangy F, Aouni M, Selmi B, Hani K. In vitro antiviral activity of dermaseptin S4 and derivatives from amphibian skin against herpes simplex virus type 2. J Med Virol 2013;85:272–81.
[13] Bocchinfuso G, Palleschi A, Orioni B, Grande G, Formaggio F, Toniolo C, et al. Different mechanisms of action of antimicrobial peptides: insights from fluorescence spectroscopy experiments and molecular dynamics simulations. J Pept Sci 2009;15:550–8.
[14] Chinchar VG, Bryan L, Silphadaung U, Noga E, Wade D, Rollins-Smith L. Inactivation of viruses infecting ectothermic animals by amphibian and piscine antimicrobial peptides. Virology 2004;323:268–75.
[15] Choi KY, Chow LN, Mookherjee N. Cationic host defence peptides: multifaceted role in immune modulation and inflammation. J Innate Immun 2012;4:361–70.
[16] Conlon JM. Structural diversity and species distribution of host-defense peptides in frog skin secretions. Cell Mol Life Sci 2011;68:2303–15.
[17] Conlon JM. The contribution of skin antimicrobial peptides to the system of innate immunity in anurans. Cell Tissue Res 2011;343:201–12.
[18] Conlon JM, Abdel-Wahab Y, Flatt P, Leprince J, Vaudry H, Jouenne T, et al. A glycine-leucine-rich peptide structurally related to the plasticins from skin secretions of the frog *Leptodactylus laticeps* (Leptodactylidae). Peptides 2009;30:888–92.
[19] Conlon JM, Woodhams DC, Raza H, Coquet L, Leprince J, Jouenne T, et al. Peptides with differential cytolytic activity from skin secretions of the lemur leaf frog *Hylomantis lemur* (Hylidae: Phyllomedusinae). Toxicon 2007;50:498–506.
[20] Conlon JM, Galadari S, Raza H, Condamine E. Design of potent, non-toxic antimicrobial agents based upon the naturally occurring frog skin peptides, ascaphin-8 and peptide XT-7. Chem Biol Drug Des 2008;72:58–64.
[21] Conlon JM, Demandt A, Nielsen PF, Leprince J, Vaudry H, Woodhams DC. The alyteserins: two families of antimicrobial peptides from the skin secretions of the midwife toad *Alytes obstetricans* (Alytidae). Peptides 2009;30:1069–73.
[22] Conlon JM, Mechkarska M, Pantic JM, Lukic ML, Coquet L, Leprince J, et al. An immunomodulatory peptide related to frenatin 2 from skin secretions of the Tyrrhenian painted frog *Discoglossus sardus* (Alytidae). Peptides 2013;40:65–71.
[23] Conlon JM, Mechkarska M, Prajeep M, Arafat K, Zaric M, Lukic ML, et al. Transformation of the naturally occurring frog skin peptide, alyteserin-2a into a potent, non-toxic anti-cancer agent. Amino Acids 2013;44:715–22.
[24] Conlon JM, Mechkarska M, Prajeep M, Sonnevend A, Coquet L, Leprince J, et al. Host-defense peptides in skin secretions of the tetraploid frog *Silurana epitropicalis* with potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Peptides 2012;37:113–9.
[25] Conlon JM, Mechkarska M, Radosavljevic G, Attoub S, King JD, Lukic ML, et al. A family of antimicrobial and immunomodulatory peptides related to the frenatins from skin secretions of the Orinoco lime frog *Sphaenorhynchus lacteus* (Hylidae). Peptides 2014;56:132–40.
[26] Conlon JM, Power GJ, Abdel-Wahab Y, Flatt P, Jiansheng H, Coquet L, et al. A potent, non-toxic insulin-releasing peptide isolated from an extract of the skin of the Asian frog, *Hylarana guentheri* (Anura:Ranidae). Regul Pept 2008;151:153–9.
[27] Conlon JM, Sonnevend A, Pál T, Vila-Farrés X. Efficacy of six frog skin-derived antimicrobial peptides against colistin-resistant strains of the *Acinetobacter baumannii* group. Int J Antimicrob Agents 2012;39:317–20.
[28] Cruciani RA, Barker JL, Zasloff M, Chen HC, Colamonici O. Antibiotic magainins exert cytolytic activity against transformed cell lines through channel formation. Proc Natl Acad Sci USA 1991;88:3792–6.
[29] Dean RE, O'Brien LM, Thwaite JE, Fox MA, Atkins H, Ulaeto DO. A carpet-based mechanism for direct antimicrobial peptide activity against vaccinia virus membranes. Peptides 2010;31:1966–72.
[30] Flatt PR, Bailey CJ, Green BD. Recent advances in antidiabetic drug therapies targeting the enteroinsular axis. Curr Drug Metab 2009;10:125–37.

[31] Fonseca VA, Zinman B, Nauck MA, Goldfine AB, Plutzky J. Confronting the type 2 diabetes epidemic: the emerging role of incretin-based therapies. Am J Med 2010;123:S2–10.
[32] Frost DR. Amphibian species of the World: an online reference, version 6.0. Electronic database. New York, USA: American Museum of Natural History; 2014 http://research.amnh.org/herpetology/amphibia/index.php
[33] Gallwitz B. Glucagon-like peptide-1 analogues for Type 2 diabetes mellitus: current and emerging agents. Drugs 2011;71:1675–88.
[34] Ge Y, MacDonald D, Henry MM, Hait HI, Nelson KA, Lipsky BA, et al. In vitro susceptibility to pexiganan of bacteria isolated from infected diabetic foot ulcers. Diagn Microbiol Infect Dis 1999;35:45–53.
[35] Giovannini MG, Poulter L, Gibson BW, Williams DH. Biosynthesis and degradation of peptides derived from *Xenopus laevis* prohormones. Biochem J 1987;243:113–20.
[36] Hossain MA, Guilhaudis L, Sonnevend A, Attoub S, van Lierop BJ, Robinson AJ, et al. Synthesis, conformational analysis and biological properties of a dicarba derivative of the antimicrobial peptide, brevinin-1BYa. Eur Biophys J 2011;40:555–64.
[37] Irwin N, Gault V, Flatt PR. Therapeutic potential of the original incretin hormone glucose-dependent insulinotropic polypeptide: diabetes, obesity, osteoporosis and Alzheimer's disease. Expert Opin Investig Drugs 2010;19:1039–48.
[38] Kim JH, Lee JO, Jung JH, Lee SK, You GY, Park SH, et al. Gaegurin-6 stimulates insulin secretion through calcium influx in pancreatic beta Rin5mf cells. Regul Pept 2010;159:123–8.
[39] Koszałka P, Kamysz E, Wejda M, Kamysz W, Bigda J. Antitumor activity of antimicrobial peptides against U937 histiocytic cell line. Acta Biochim Pol 2011;58:111–7.
[40] Kotsaki A, Giamarellos-Bourboulis EJ. Emerging drugs for the treatment of sepsis. Expert Opin Emerg Drugs 2012;17:379–91.
[41] Landry Y, Gies JP. Drugs and their molecular targets: an updated overview. Fundam Clin Pharmacol 2008;22:1–18.
[42] Lehmann J, Retz M, Sidhu SS, Suttmann H, Sell M, Paulsen F, et al. Antitumor activity of the antimicrobial peptide magainin II against bladder cancer cell lines. Eur Urol 2006;50:141–7.
[43] Lequin O, Ladram A, Chabbert L, Bruston F, Convert O, Vanhoye D, et al. Dermaseptin S9, an alpha-helical antimicrobial peptide with a hydrophobic core and cationic termini. Biochemistry 2006;45:468–80.
[44] Libério MS, Joanitti GA, Azevedo RB, Cilli EM, Zanotta LC, Nascimento AC, et al. Anti-proliferative and cytotoxic activity of pentadactylin isolated from *Leptodactylus labyrinthicus* on melanoma cells. Amino Acids 2011;40:51–9.
[45] Lord CJ, Ashworth A. Mechanisms of resistance to therapies targeting BRCA-mutant cancers. Nat Med 2013;9:1381–8.
[46] Lorin C, Saidi H, Belaid A, Zairi A, Baleux F, Hocini H, et al. The antimicrobial peptide dermaseptin S4 inhibits HIV-1 infectivity *in vitro*. Virology 2005;334:264–75.
[47] Lu CX, Nan KJ, Lei Y. Agents from amphibians with anticancer properties. Anticancer Drugs 2008;19:931–9.
[48] Mader JS, Hoskin DW. Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. Expert Opin Investig Drugs 2006;15:933–46.
[49] Marenah L, Flatt P, Orr DF, McClean S, Shaw C, Abdel-Wahab YHA. Brevinin-1 and multiple insulin-releasing peptides in the skin of the frog *Rana palustris*. J Endocrinol 2004;181:347–54.
[50] Marenah L, Flatt P, Orr DF, Shaw C, Abdel-Wahab YHA. Characterization of naturally occurring peptides in the skin secretion of *Rana pipiens* frog reveal pipinin-1 as the novel insulin-releasing agent. J Pept Res 2005;66:204–10.
[51] Marenah L, Flatt P, Orr DF, Shaw C, Abdel-Wahab YH. Skin secretions of *Rana saharica* frogs reveal antimicrobial peptides esculentins-1 and-1B and brevinins-1E and-2EC with novel insulin releasing activity. J Endocrinol 2006;188:1–9.
[52] Marenah L, McClean S, Flatt P, Orr DF, Shaw C, Abdel-Wahab Y. Novel insulin-releasing peptides in the skin of *Phyllomedusa trinitatis* frog include 28 amino acid peptide from dermaseptin BIV precursor. Pancreas 2004;29:110–5.
[53] Marenah L, Shaw C, Orr DF, McClean S, Flatt PR, Abdel-Wahab YHA. Isolation and characterisation of an unexpected class of insulinotropic peptides in the skin of the frog *Agalychnis litodryas*. Regul Pept 2004;120:33–8.
[54] McClenaghan NH, Barnett CR, Ah-Sing E, Abdel-Wahab YHA, O'Harte FP, Yoon TW, et al. Characterization of a novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. Diabetes 1996;45:1132–40.
[55] Mechkarska M, Ojo OO, Meetani MA, Coquet L, Jouenne T, Abdel-Wahab YHA, et al. Peptidomic analysis of skin secretions from the bullfrog *Lithobates catesbeianus* (Ranidae) identifies multiple peptides with potent insulin-releasing activity. Peptides 2011;32:203–8.
[56] Mechkarska M, Prajeep M, Radosavljevic GD, Jovanovic IP, Al Baloushi A, Sonnevend A, et al. An analog of the host-defense peptide hymenochirin-1B with potent broad-spectrum activity against multidrug-resistant bacteria and immunomodulatory properties. Peptides 2013;50:153–9.
[57] Miyazaki Y, Aoki M, Yano Y, Matsuzaki K. Interaction of antimicrobial peptide magainin 2 with gangliosides as a target for human cell binding. Biochemistry 2012;51:10229–35.
[58] Mulder KC, Lima LA, Miranda VJ, Dias SC, Franco OL. Current scenario of peptide-based drugs: the key roles of cationic antitumor and antiviral peptides. Front Microbiol 2013;4:321.
[59] Ohsaki Y, Gazdar AF, Chen HC, Johnson BE. Antitumor activity of magainin analogues against human lung cancer cell lines. Cancer Res 1992;52:3534–8.
[60] Ojo OO, Abdel-Wahab YH, Flatt PR, Mechkarska M, Conlon JM. Tigerinin-1R: a potent, non-toxic insulin-releasing peptide isolated from the skin of the Asian frog, *Hoplobatrachus rugulosus*. Diabetes Obes Metab 2011;13:1114–22.
[61] Ojo OO, Conlon JM, Flatt PR, Abdel-Wahab YH. Frog skin peptides (tigerinin-1R, magainin-AM1, -AM2, CPF-AM1, and PGla-AM1) stimulate secretion of glucagon-like peptide 1 (GLP-1) by GLUTag cells. Biochem Biophys Res Commun 2013;431:14–8.
[62] Ojo OO, Abdel-Wahab YH, Flatt PR, Conlon JM. Insulinotropic actions of the frog skin host-defense peptide alyteserin-2a: a structure–activity study. Chem Biol Drug Des 2013;82:196–204.
[63] Pantic JM, Mechkarska M, Lukic ML, Conlon JM. Effects of tigerinin peptides on cytokine production by mouse peritoneal macrophages and spleen cells and by human peripheral blood mononuclear cells. Biochimie 2014;101:83–92.
[64] Popovic S, Urbán E, Lukic M, Conlon JM. Peptides with antimicrobial and anti-inflammatory activities that have therapeutic potential for treatment of *acne vulgaris*. Peptides 2012;34:275–82.
[65] Rozek T, Wegener KL, Bowie JH, Olver IN, Carver JA, Wallace JC, et al. The antibiotic and anticancer active aurein peptides from the Australian Bell Frogs *Litoria aurea* and *Litoria raniformis* the solution structure of aurein 1.2. Eur J Biochem 2000;267:5330–41.
[66] Scorciapino MA, Manzo G, Rinaldi AC, Sanna R, Casu M, Pantic JM, et al. Conformational analysis of the frog skin peptide, plasticin-L1 and its effects on the production of proinflammatory cytokines by macrophages. Biochemistry 2013;52:7231–41.
[67] Srinivasan D, Mechkarska M, Abdel-Wahab YH, Flatt PR, Conlon JM. Caerulein precursor fragment (CPF) peptides from the skin secretions of *Xenopus laevis* and *Silurana epitropicalis* are potent insulin-releasing agents. Biochimie 2013;95:429–35.
[68] Srinivasan D, Ojo OO, Abdel-Wahab YH, Flatt PR, Guilhaudis L, Conlon JM. Insulin-releasing and cytotoxic properties of the frog skin peptide, tigerinin-1R: a structure–activity study. Peptides 2014;55:23–31.
[69] VanCompernolle SE, Taylor RJ, Oswald-Richter K, Jiang J, Youree BE, Bowie JH, et al. Antimicrobial peptides from amphibian skin potently inhibit human immunodeficiency virus infection and transfer of virus from dendritic cells to T cells. J Virol 2005;79:11598–606.
[70] Van Zoggel H, Hamma-Kourbali Y, Galanth C, Ladram A, Nicolas P, Courty J, et al. Antitumor and angiostatic peptides from frog skin secretions. Amino Acids 2012;42:385–95.
[71] Wang G, Li X, Wang Z. APD2: the updated antimicrobial peptide database and its application in peptide design. Nucleic Acids Res 2009;37:D933–7.
[72] Wang G, Watson KM, Peterkofsky A, Buckheit Jr RW. Identification of novel human immunodeficiency virus type 1-inhibitory peptides based on the antimicrobial peptide database. Antimicrob Agents Chemother 2010;54:1343–6.
[73] Wang C, Tian LL, Li S, Li HB, Zhou Y, Wang H, et al. Rapid cytotoxicity of antimicrobial peptide temporin-1CEa in breast cancer cells through membrane destruction and intracellular calcium mechanism. PLoS One 2013;8:e60462.
[74] Woodhams DC, Vredenburg VT, Simon M-A, Billheimer D, Shakhtour B, Shyr Y, et al. Symbiotic bacteria contribute to innate immune defences of the threatened mountain yellow-legged frog, *Rana muscosa*. Biol Conserv 2007;138:390–8.
[75] Yasin B, Pang M, Turner JS, Cho Y, Dinh N-N, Waring AJ, et al. Evaluation of the inactivation of infectious herpes simplex virus by host-defense peptides. Eur J Clin Microbiol Infect Dis 2000;19:187–94.
[76] Yeung ATY, Gellatly SL, Hancock RE. Multifunctional cationic host defence peptides and their clinical applications. Cell Mol Life Sci 2011;68:2161–76.
[77] Zahid OK, Mechkarska M, Ojo OO, Abdel-Wahab YH, Flatt PR, Meetani MA, et al. Caerulein-and xenopsin-related peptides with insulin-releasing activities from skin secretions of the clawed frogs, *Xenopus borealis* and *Xenopus amieti* (Pipidae). Gen Comp Endocrinol 2011;172:314–20.
[78] Zasloff M. Magainins, a class of antimicrobial peptides from *Xenopus* skin: isolation, characterization of two active forms and partial cDNA sequence of a precursor. Proc Natl Acad Sci USA 1987;84:5449–53.