

De Novo Generation of Cationic Antimicrobial Peptides: Influence of Length and Tryptophan Substitution on Antimicrobial Activity

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Comparison of human immunodeficiency virus lentiviral lytic peptide 1 with other host-derived peptides indicates that antimicrobial properties of membrane-active peptides are markedly influenced by their cationic, hydrophobic, and amphipathic properties. Many common themes, such as Arg composition of the cationic face of an amphipathic helix and the importance of maintaining the hydrophobic face, have been deduced from these observations. These studies suggest that a peptide with these structural properties can be derived de novo by using only a few strategically positioned amino acids. However, the effects of length and helicity on antimicrobial activity and selectivity have not been objectively evaluated in the context of this motif. To address these structure-function issues, multimers of a 12-residue lytic base unit (LBU) peptide composed only of Arg and Val residues aligned to form idealized amphipathic helices were designed. Bacterial killing assays and circular dichroism analyses reveal a strong correlation between antibacterial activity, peptide length, and propensity to form a helix in solvent mimicking the environment of a membrane. Increasing peptide length beyond two LBUs (24-residue peptides) resulted in no appreciable increase in antimicrobial activity. Derivatives (WLBU) of the LBU series were further engineered by substituting Trp residues in the hydrophobic domains. The 24-residue WLBU2 peptide was active at physiologic NaCl concentrations against *Staphylococcus aureus* and mucoid and nonmucoid strains of *Pseudomonas aeruginosa*. Further, WLBU2 displayed the highest antibacterial selectivity of all peptides evaluated in the present study by using a coculture model of *P. aeruginosa* and primary human skin fibroblasts. These findings provide fundamental information toward the de novo design of an antimicrobial peptide useful for the management of infectious diseases.

Despite the establishment of safe and effective antibiotics, the management of infectious diseases remains a worldwide public health concern. One untapped resource of novel antimicrobial agents, evolutionarily proven as anti-infectives, are the host-derived antimicrobial peptides (12, 13). In vertebrates, insects, and even bacteria, these compounds represent the first line of defense against pathogens challenging the host (13). These peptides commonly adopt an amphipathic conformation in which positively charged and hydrophobic groups segregate onto opposing faces of an α -helix, a β -sheet, or some other tertiary structure (20). These general structural properties confer an ability to disrupt or traverse a phospholipid membrane. The spectrum of different amino acid side chain chemical properties affords a variety of peptide sequences to present a cationic amphipathic helical peptide. As a result, hundreds of different natural cationic antimicrobial peptides (CAPs) with widely varying sequences have been described. The diversity of antimicrobial peptides is likely a consequence of each peptide evolving to function in a particular environment against a specific subset of microbial pathogens.

We have previously reported the conservation of a peptide derived from the extreme C terminus of the human immunodeficiency virus type 1 (HIV-1) transmembrane protein that is referred to as lentivirus lytic peptide 1 (LLP1) (17). Like other

membrane interactive peptides, LLP1 is predicted to have an amphipathic α -helical structure and may be involved in the pathogenicity of HIV-1 because of its calmodulin binding (18, 28, 35) and natural endogenous reverse transcriptase activity of HIV-1 (36). Investigations of the antimicrobial potential of LLP1 demonstrate that the parent sequence is remarkably potent compared to other host-derived antimicrobial peptides (27). Based on these studies, it is reasonable to predict that CAP derivatives can be engineered for enhanced potency and selectivity by increasing cationicity by using Arg residues on the polar face and hydrophobicity by using Val residues on the nonpolar face (26). This potency and selectivity can be further enhanced by increasing peptide length and by including Trp residues on the nonpolar face as demonstrated by Vogel et al. (33).

Similar to studies by McLaughlin and coworkers (15) and DeGrado and coworkers (29), we reasoned that a de novo-designed peptide presenting an optimized amphipathic α -helix with exclusively Arg residues on the hydrophilic face and Val residues on the hydrophobic face would demonstrate antimicrobial activity. An important question requiring attention was the optimal length of the peptide and the influence of Trp residues on the hydrophobic face. We describe here the design and evaluation of de novo-engineered peptides of various lengths and Trp contents for their in vitro activity and selectivity in a bacterium-host cell coculture model. The results demonstrate that it is possible to engineer an effective antimicrobial compound by using a 24-residue peptide comprised only of Arg and Val residues. Notably, increasing peptide length beyond 24 residues did not enhance antimicrobial po-

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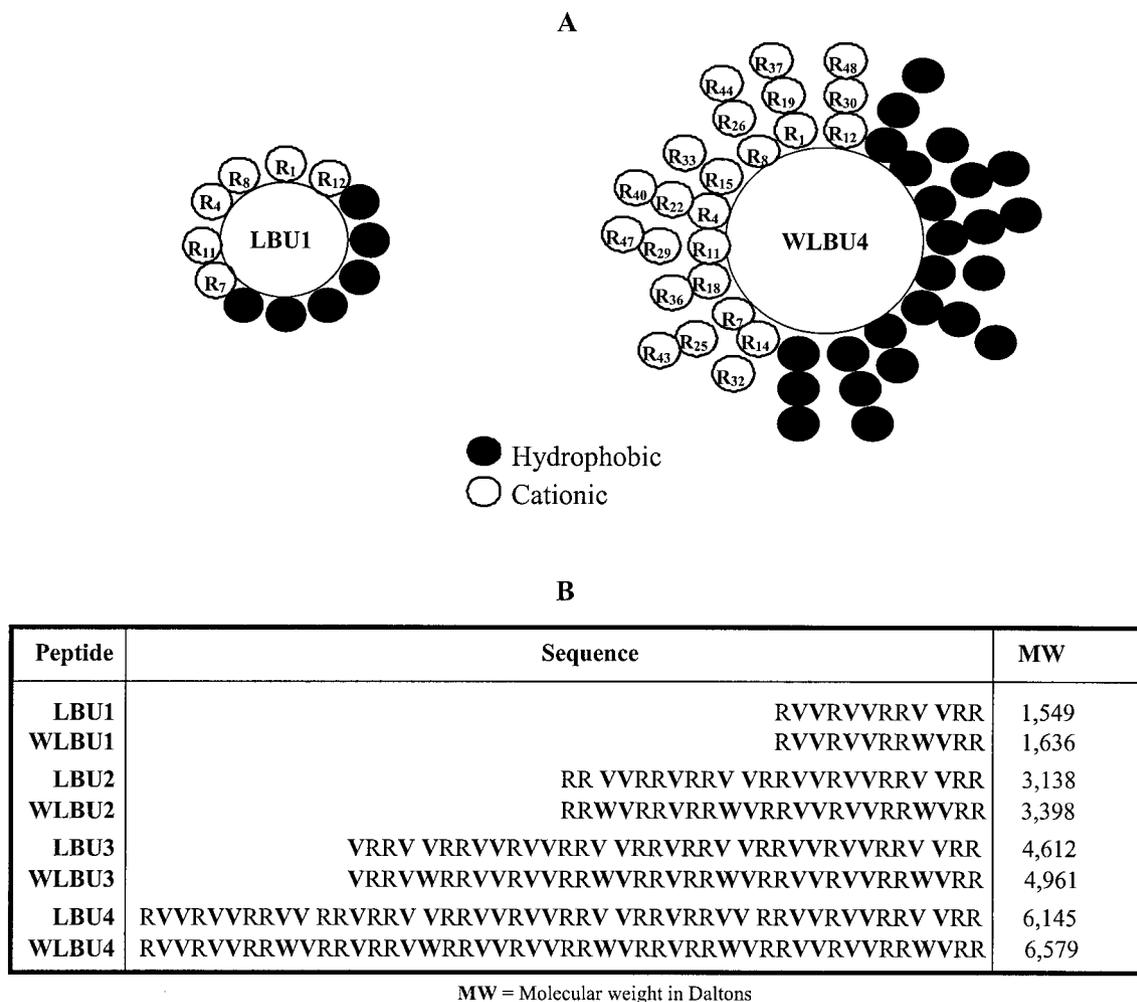


FIG. 1. Peptide design. (A) The cationic amphipathic peptides were designed as demonstrated in helical wheel diagrams. Arg, Val, and Trp residues were arranged to form idealized amphipathic α -helices, with the hydrophilic and hydrophobic faces indicated in clear and shaded circles, respectively. The 12- and 48-mers LBU1 and WLBU4 are shown as representatives of the LBU and WLBU series, respectively. (B) Primary sequences of the LBU and WLBU peptides used in the present study. The shortest peptide forms one lytic base unit (LBU1) of 12 amino acids, and the others were designed as multimers (2, 3, and 4) of LBU1. The WLBU peptides were derived from the LBU series by substituting Trp residues at the indicated positions. Not shown here are the peptide hydrophobic moments calculated according to the method of Eisenberg et al. (8).

tency in vitro. In addition, the inclusion of Trp residues on the hydrophobic face conferred increased selectivity in the bacterium-host cell coculture model.

MATERIALS AND METHODS

Peptide synthesis. The LBU and WLBU series (Fig. 1), as well as the host-derived antimicrobial peptide LL37 (10, 31), were synthesized by using standard Fmoc (9-fluorenylmethoxy carbonyl) synthesis protocols as previously described (9). Synthetic peptides were characterized and purified by reverse-phase high-pressure liquid chromatography on Vydac C_{18} or C_4 columns (The Separations Group, Hesperia, Calif.), and the identity of each was established by mass spectrometry (Electrospray Quatro II triple quadrupole mass spectrometer; Micromass, Inc., Manchester, United Kingdom). Peptide concentrations were determined by using a quantitative ninhydrin assay as previously described (9).

Bacterial killing assay. The objective of the present study was to compare a repeated sequence idealized to form an amphipathic helical structure with Arg predominating on the hydrophilic face and Val/Trp on the hydrophobic face for the effect of NaCl and for selective toxicity against killing bacteria over human skin fibroblasts (HSF). Thus, the following features of the standard NCCLS assay were modified: (i) the medium in which the experiment was performed, (ii) the

exposure time, (iii) the excipient addition (albumin), and (iv) the MIC definition. This modified NCCLS bacterial killing assay is identical to that previously described (27) and uses two prototypical organisms, a clinical isolate of *Pseudomonas aeruginosa* (PA1244) and a laboratory-passaged strain of *Staphylococcus aureus* (27). These isolates were confirmed by the clinical laboratory at Children's Hospital of Pittsburgh to be *P. aeruginosa* and *S. aureus*. Also used for the present study were two mucoid *P. aeruginosa* strains purchased from American Type Culture Collection and a series of clinical isolates identified by Children's Hospital of Pittsburgh as *P. aeruginosa* exhibiting either a mucoid or a nonmucoid phenotype.

To test for susceptibility of these index bacteria to the peptides described, bacterial suspensions ($\sim 10^6$ CFU/ml) in 10 mM potassium phosphate buffer (PB) or phosphate buffer containing 150 mM NaCl (PBS; pH 7.2) were incubated with twofold dilutions of peptides for 30 min at 37°C. Serial peptide dilutions were performed and plated on tryptic soy agar (Difco, Detroit, Mich.). Surviving colonies were counted the next day to determine the minimum bactericidal concentration (MBC), defined as the molar concentration of peptide reducing the viable bacteria within a suspension by 3 orders of magnitude. Values were expressed on a molar basis, with a lower MBC corresponding to increased peptide potency. The results were expressed as an average of MBCs obtained from three to five independent experiments. MBCs may be converted to micro-

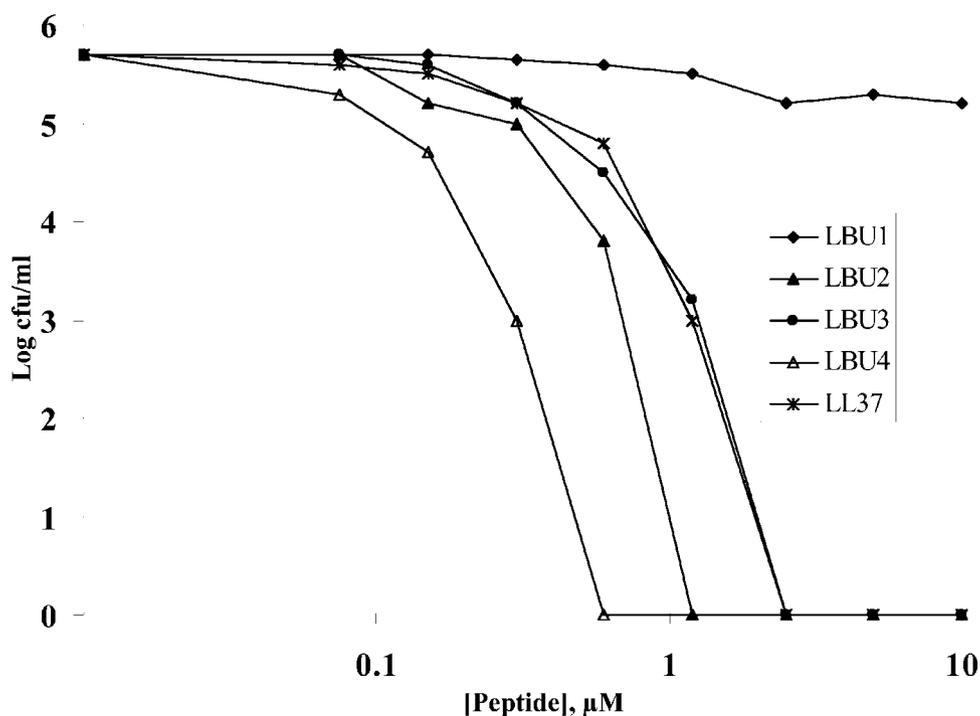


FIG. 2. Relationship between length and antibacterial activity. *P. aeruginosa* (PA1244) isolate (10^5 to 10^6 bacteria/ml) was incubated with twofold serially diluted peptides at 37°C for 30 min. Bacterial survival at corresponding peptide concentrations was evaluated by broth dilution assays. The activity of the LBU series against *P. aeruginosa* in 10 mM PB is both length and dose dependent, reaching an optimal level at 24 residues in length. Twofold differences in peptide activity are not statistically significant.

grams per milliliter by dividing the molecular mass (in grams/mole) by 1,000 and then multiplying that value by the value in micromolar units.

CD analysis. Circular dichroism (CD) was performed as previously described (9). Briefly, CD measurements were taken with an Aviv spectrometer (Aviv Instruments, Lakewood, N.J.) at room temperature, over the range of 190 to 300 nm, and in PB or 30% trifluoroethanol (TFE) in PB. The average mean residue ellipticities ($[\theta]/1,000 \times [\text{degree} \times \text{square centimeters/decimole}]$) for 8 to 10 scans per experimental trial were plotted against wavelength (in nanometers), and the program K2D (<http://www.embl-heidelberg.de/~andrade/k2d/>) was used to determine the percent helicity for each peptide in TFE (1). To confirm that the peptide structure could be induced by bacterial components, experiments were performed in the presence of smooth lipopolysaccharide (LPS; purified from *Salmonella enterica* serovar Typhimurium; Sigma) and phosphatidylglycerol bearing liposomes at increasing concentrations.

Selective cytotoxicity. Primary HSF cells at passage 20 were propagated at 37°C and in 5% CO_2 to over 80% confluence in Iscove modified Dulbecco medium (IMDM; Life Technologies, Grand Island, N.Y.) containing 10% fetal bovine serum and transferred (after treatment with trypsin) to each well of a 96-well plate to a final count of 0.5×10^5 cells/well. After a 24-h incubation at 37°C , the medium was aspirated and a $100\text{-}\mu\text{l}$ suspension of *P. aeruginosa* 1244 ($\sim 10^6$ cells/ml in 50% serum-free IMDM) was added to each well. Using peptide concentrations from 0 to $10 \mu\text{M}$ in $1 \times$ PBS, the bacterial-HSF media were further diluted to 25% IMDM. The coculture was then incubated at 37°C for 1 h. To determine bacterial survival, the coculture medium was serially diluted after gentle pipetting of $20\text{-}\mu\text{l}$ aliquots to another 96-well plate containing $180 \mu\text{l}$ of PBS per well, and threefold serial dilutions were made to tryptic soy agar, followed by incubation overnight at 37°C , and bacterial counts were determined and are expressed as CFU per milliliter.

Measurements of HSF cell viability were accomplished by using a tetrazolium-based colorimetric assay (5, 11, 19). After two washes with PBS, the coculture medium was replaced with $100 \mu\text{l}$ of IMDM containing 10% fetal bovine serum (vol/vol) and 0.5 mg of MTT Formazan (MTT; Sigma, Lakehood, N.J.)/ml. The reaction mixtures were incubated at 37°C in 5% CO_2 for 4 h, after which equal volumes of 0.1 N HCl-isopropanol were added. The percent viability was assessed by taking absorbances at 570 nm by using a Dynatech MR5000 (Germantown, Md.). As controls, cells were treated with 25% IMDM (the test medium)

in the presence or absence of bacteria, without the addition of peptide and with 100% lysis buffer (0.15 M NaCl, 25 mM Tris-HCl [pH 8.0], 1% [wt/vol] deoxycholate and Triton X-100). The experiments were performed in triplicate, and viability data were averaged. The final HSF toxicity values are expressed as mean percent toxicity for each test condition minus the percent toxicity in the presence of bacteria alone.

RESULTS

Studies of the conservation of Arg and Val residues in the LLP1 sequences among diverse HIV-1 isolates (27), coupled with engineering of the LLP1 sequence for greater potency (23, 26, 27, 32) and other studies related to host-derived antimicrobial peptides (15, 23, 32), led us to propose that effective de novo antimicrobial peptides could be synthesized solely from Arg and Val residues if positioned to optimize an amphipathic α -helix. What was not clear from previous studies was the minimum peptide length required for optimal antimicrobial activity. In addition, since the membrane perturbation properties of Trp-rich antimicrobials (21, 33) and fusogenic peptides (22) have been well described, we hypothesized that the inclusion of Trp residues on the hydrophobic face of an α -helix would increase antimicrobial potency. To address both of these issues, the series of peptides described in Fig. 1 was designed based on the concept of a repeating lytic base unit of 12 residues.

Antibacterial potency of LBU peptides of increasing length. Using a standard broth dilution assay (27), the potency (i.e., antimicrobial activity on a per-mole basis) of the LBU series and the host-derived human cathelicidin, LL37, was compared

TABLE 1. Influence of length and salinity on antibacterial activity^a

Peptide	Length (no. of residues)	MBC (μM) against:			
		<i>P. aeruginosa</i> PA1244 in:		<i>S. aureus</i> in:	
		PB	PBS	PB	PBS
LL37	37	1.2	1.2	2.5	>10
LBU1	12	>10	>10	>10	>10
LBU2	24	0.6	0.6	1.2	>10
LBU3	36	1.2	0.6	0.6	1.2
LBU4	48	0.3	0.3	0.3	0.3
WLBU1	12	0.6	>10	>10	>10
WLBU2	24	0.3	0.3	0.3	0.3
WLBU3	36	2.5	0.6	2.5	0.3
WLBU4	48	2.5	0.6	2.5	0.6
LBU1.3	15	1.2	>10	>10	>10
LBU1.6	18	1.2	>10	2.5	>10
LBU1.9	21	0.6	2.5	1.2	>10

^a The MBCs (peptide concentrations promoting 99.9% killing) of the LBU and WLBU series in PB and in PB plus 150 mM NaCl (PBS) were derived from the dose-dependent survival curves as previously described (26). The human cathelicidin LL37 was used as a comparative host-derived peptide in these experiments. There was no significant change in antibacterial activity in the peptides of 24 to 48 residues in length, but the peptide LBU2 was salt sensitive against *S. aureus*. The relationship between length and activity was further characterized by using peptides of 12, 15, 18, 21, and 24 residues. The results of these studies reveal that a minimum length of 15 residues is required for significant antibacterial activity. Average MBCs were obtained from three to five experimental trials.

for killing *P. aeruginosa* in PB alone. Figure 2 shows the dose-dependent bacterial survival in log CFU/ml after peptide treatment. There was a significant increase in activity from the 12-residue LBU1, which did not achieve an MBC at the concentrations tested, to the 24-residue LBU2, which demonstrated an MBC of 0.6 μM (Table 1). There was no appreciable increase in potency against *P. aeruginosa* for the 36-residue LBU3 and 48-residue LBU4 peptides. Interestingly, the LBU2 and LBU4 were more active than the host-derived human cathelicidin, demonstrating the increased potency of the de novo peptide component to a reference natural antimicrobial peptide. Based on electron microscopic experiments from our laboratory and others, this killing is the result of membrane dissolution and not an artifact of aggregation induced by the monovalent peptide.

To define the minimum length for bacterial killing, a series of LBU peptides ranging from 12 to 24 residues were tested for their antimicrobial potency in PB and PBS against two index organisms, *P. aeruginosa* and *S. aureus*. The results (Table 1) indicate that at a length of 24 residues, optimal peptide activity (MBC of 0.6 μM) was achieved for *P. aeruginosa* in both PB and PBS, although some antimicrobial activity was initially observed with the 15- and 18-residue derivatives.

Influence of Trp residues on antimicrobial potency of the LBU peptides. Trp is known to be a highly membrane active amino acid and is prevalent in many antimicrobial peptide sequences (33). The contribution of Trp residues to antimicrobial activity was investigated when included on the hydrophobic side of the LBU peptide series (Fig. 1). As summarized in Table 1, a single Trp substitution in WLBU1 significantly increased antipseudomonal potency compared to the parent peptide LBU1 in PB, with an MBC of 0.6 μM . The 24-residue WLBU2 derivative (three Trp residues in the hydrophobic

face) displayed a slight increase in activity against *P. aeruginosa* in comparison to the LBU2, but it showed a significantly higher potency against *S. aureus* with an average MBC of 0.3 μM in both PB and PBS. These findings suggest that the inclusion of Trp residues can render peptides less salt sensitive and significantly more potent against *S. aureus*. Like the LBU series, WLBU3 and WLBU4 did not demonstrate any increased potency, although both peptides were refractory to the presence of salt for both index organisms.

Relationship of helicity in membrane mimetic solvents to antimicrobial activity. The relationship between the propensity to form a helix and antibacterial activity was investigated using CD analysis. The LBU peptides described in Fig. 1 were subjected to structural analysis in PB (mimicking an aqueous environment) and PB containing 30% TFE (mimicking a membrane environment). Not surprisingly, no appreciable structure was observed for all peptides in PB (data not shown). In contrast, the helical propensity in membrane mimetic solvents did correlate with bacterial killing (Fig. 3). In general, a percent helical value of >80% correlated with the potency of bacterial killing (Table 2). A similar activity was shown upon the addition of mixed vesicles comprised of LPS and phosphatidylglycerol, although the helical-inducing properties were less efficient (data not shown). This finding is significant for the design of future de novo antimicrobial peptides, suggesting that helical content in a membrane environment should be preserved for maximal antimicrobial potency.

Activity of WLBU2 against a battery of *P. aeruginosa* isolates. Based on the experimental results described above, WLBU2 demonstrated the optimal peptide activity, i.e., the shortest, most potent peptide active against *P. aeruginosa* and *S. aureus* under physiological NaCl conditions. To investigate whether the potent activity displayed by WLBU2 was active against a panel of organisms in addition to the index strain, standard bacterial killing assays were performed in PBS with 16 clinical isolates of *P. aeruginosa*, six of which were of mucoid phenotype. The results are reported in Table 3 and demonstrate that WLBU2 maintains potent activity against all strains tested but also displayed a 2- to 16-fold higher (depending on the strain) bactericidal activity than that of LL37.

Selective toxicity. Initial characterization of CAP cytotoxicity for human cells in our lab and others have been based on the human erythrocyte lysis assay (7, 16, 27, 30). The information obtained by red blood cell lysis, while informative, does not reflect the challenges of antimicrobial peptides that must be selectively toxic for bacterial cells over eukaryotic cells presented at the identical time and under identical conditions. To more accurately assess the concept of selective toxicity, we developed a coculture assay in which primary HSF are infected with *P. aeruginosa* prior to peptide treatment. After 1 h of exposure to peptide, these cocultures were examined for bacterial killing and eukaryotic cell viability as described in Materials and Methods. LBU2 and WLBU2 were chosen for study in this assay because they were the minimal-length peptides that demonstrated potent antimicrobial activity (Table 1) and low hemolytic activity (data not shown). Figure 4 plots increasing concentrations of LBU2 (Fig. 4A) and WLBU2 (Fig. 4B) as a function of bacterial killing (left axis) and eukaryotic cell toxicity (right axis). The cytotoxic effects of LBU2 and WLBU2 on primary HSF cells were negligible at concentrations (<8

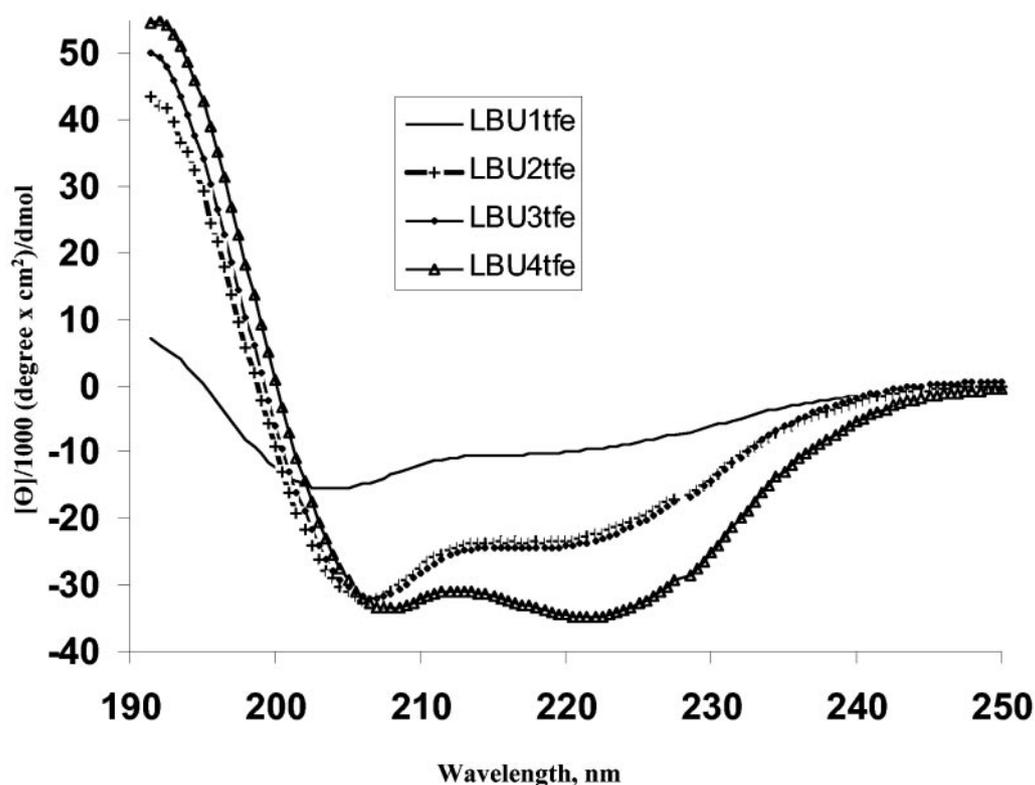


FIG. 3. Structure of de novo-engineered antimicrobial peptides. CD analysis was performed on the LBU and WLBUs peptides under two conditions: (i) in 10 mM PB (not shown) and (ii) in 30% TFE (as shown above) (9). Mean residue ellipticities ($[\Theta]/1,000 \times [\text{degree} \times \text{square centimeters/decimole}]$) were plotted against wavelength, and the CD spectra shown here are representative of three independent experimental trials.

and $< 2 \mu\text{M}$, respectively) that decrease bacterial load by 99.9%. These data suggest the inclusion of Trp residues can significantly increase antimicrobial potency in a selective toxicity setting. By comparison, the host-derived LL37 was moderately toxic to HSF cells (31% reduction at $10 \mu\text{M}$), and a 99.9% reduction in *P. aeruginosa* was not attained at the max-

imum concentration tested (Fig. 4C). The finding of increased potency of the de novo-designed antimicrobial peptides compared to LL37 underscores that peptides of improved potency and selectivity can be generated.

DISCUSSION

In this study we demonstrated that antimicrobial peptides can be designed based on a cationic amphipathic motif by using Arg residues on the cationic face and Val residues on the hydrophobic face. Furthermore, we demonstrated that increased potency can be achieved by the inclusion of Trp residues on the hydrophobic face, which could be predicted based upon the predominance of these residues in the indolicidins (25). The current study identifies LBU2 and WLBUs as lead compounds for de novo antimicrobials based upon the minimal length of 24 residues to deliver maximal antimicrobial potency. The observation that these lead compounds are broadly active against our prototype gram-positive and -negative organisms, including mucoid and nonmucoid clinical isolates of *P. aeruginosa*, suggests that these peptides may be a template on which to base further derivatives.

A de novo approach to peptide synthesis with natural amino acids is now predictable based on a comparison of the large number of host-derived peptides described over the past two decades that exhibit a cationic amphipathic motif (4, 6). A review of the antimicrobial peptide literature leads to the question of

TABLE 2. Relationship between length and helical content^a

Peptide	Length (no. of residues)	% Helicity
LBU1	12	27
LBU2	24	82
LBU3	36	80
LBU4	48	92
WLBUs	12	25
WLBUs	24	81
WLBUs	36	92
WLBUs	48	75
LBU1.3	15	42
LBU1.6	18	45
LBU1.9	21	82

^a To evaluate the relationship between length and helical content, the percent helicity was obtained from the CD data by using the program K2D over the internet (www.embl-heidelberg.de/~andrade/k2d/) (3). Although the length seems to correlate with helicity, there is little increase in helical content in peptides longer than 24 residues. These data, together with the results of the bacterial killing assays, reveal a similar correlation between helicity and antibacterial activity in PB. The data tabulated are representative of two to three experimental trials.

TABLE 3. Antibacterial activity of WLBU2 is not strain specific^a

<i>P. aeruginosa</i> strain	MBC (μ M)	
	WLBU2	LL37
PA1244	0.3	2
PACO1*	0.5	2
PACO3*	0.3	1
PACO4	0.5	2
PACO6	0.5	2
PACO7*	0.5	2
PACO8	0.3	1
PACO9	0.3	1
PACO10	0.3	2
PACO11	0.5	2
PACO12	0.5	2
PACO13*	0.5	2
PACO14	0.3	2
ATCC 19142*	0.3	5
ATCC 33468*	0.3	2
PAO1	0.5	1

^a To investigate whether the potent activity displayed by WLBU2 was dependent on the specificity of the clinical strains, we performed standard bacterial killing assays (in PBS) with a panel of 16 strains of *P. aeruginosa*, 6 of which were of mucoid phenotype. The peptide not only maintained its potent effects against all of these strains but also demonstrated a 4- to 13-fold-higher bactericidal activity than that of LL37. The data tabulated are representative of two experimental trials. *, Mucoid type.

why a single sequence has not evolved as a predominant antimicrobial peptide functioning in the innate immunity of all vertebrate species. It seems that this cationic amphipathic motif has evolved in its place. One possibility is that peptides with the same motif but with unique sequences behave differently against different bacterial pathogens in a variety of environments.

McLaughlin and coworkers initiated the concept of multimeric peptide design from a unique sequence (15). However, they limited their studies to peptides with a maximum length of 21 residues and thus did not allow for a complete appreciation of the effects of length on antimicrobial activity. In addition, they used Lys as their predominant cationic residue. In contrast, we exploited the membrane-active properties of Arg that have been documented in the literature (24, 33, 34). We utilized Val on the hydrophobic face because of its predominance in the LLP1 derivatives that we have described previously (26, 27). Finally, substitution of the membrane-seeking amino acid Trp (22, 33) in the hydrophobic faces in the WLBU series also allowed us to appreciate its influence on antimicrobial activity and selectivity. The possibility of using only three amino acids and synthesizing longer and more potent peptides in the form of a base unit is a novel aspect of these studies that affects the economic feasibility of peptide synthesis for large scale antibiotic production. For example, the anti-HIV-1 drug T20 is synthesized through the condensation of peptide fragments (2, 3, 14).

We have shown that the activity of the LBU peptides was generally improved with increased chain length, whereas the 24-residue peptides achieved optimal antibacterial selectivity. Although antibacterial activity correlated consistently with helicity for the LBU series, the length-potency correlation could be explained by better optimization of both the hydrophilic and the hydrophobic faces with increasing length. However, hydrophobicity is the prime candidate in the observed increase in mammalian cytotoxicity. LBU2, the most amphipathic (mean hydrophobic moment of 0.80) (8) and highly helical in this

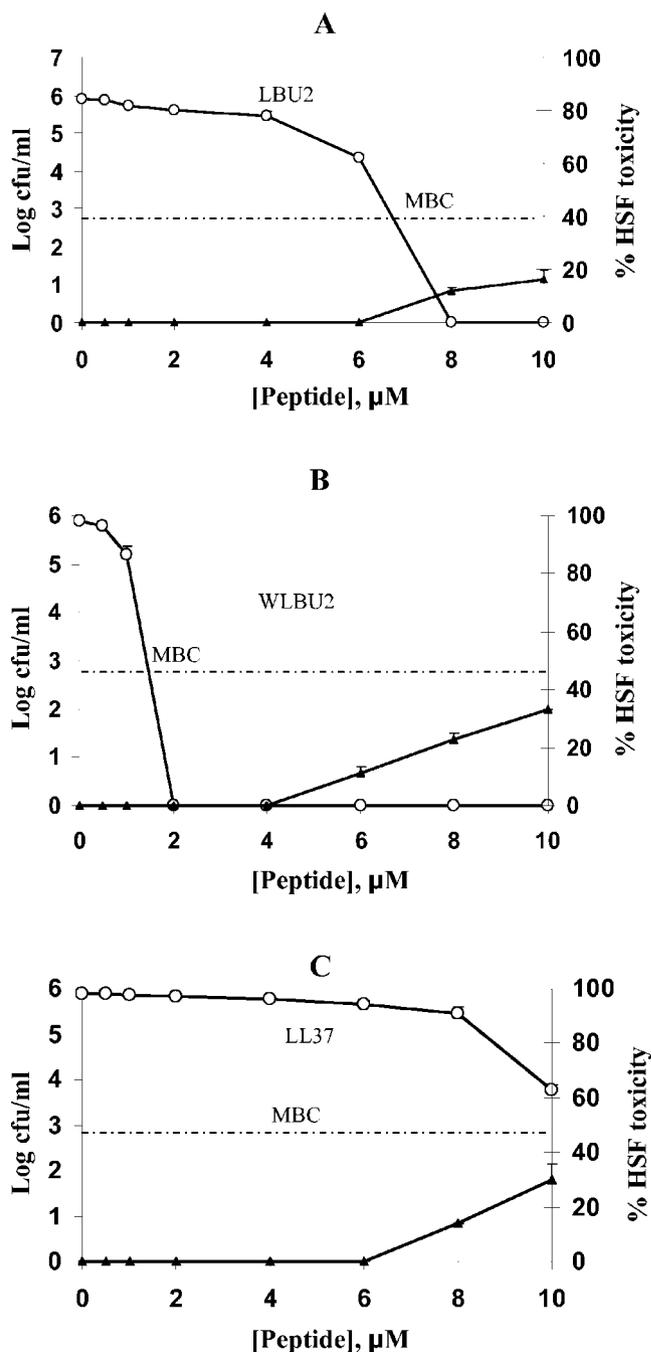


FIG. 4. Selective toxicity of antimicrobial peptides in a coculture system. Primary HSF (HSFCCD-986sk) at passage 20 and >80% confluence were infected with *P. aeruginosa* in 50% IMDM in PBS with no bovine fetal serum. The coculture was then treated for 1 h with twofold dilutions of peptides as described in Materials and Methods. Bacterial survival was determined as in a standard broth dilution assay, and HSF viability was evaluated by MTT staining (a tetrazolium-based assay that measures the activity of mitochondrial redox enzymes). Controls included were 25% IMDM in PBS (0% cytotoxicity), 25% IMDM plus bacteria (10^5 to 10^6 cells/ml), and $1\times$ standard lysis buffer (100% cytotoxicity). The selected peptides LBU2 (A) and WLBU2 (B) displayed high antibacterial potency and selectivity compared to the host-derived peptide LL37 (C). The data plotted are representative of three experimental trials.

series, has the highest Arg-to-Val ratio (13 to 11), thereby favoring strong initial interactions between the peptide and the highly electronegative LPS. This is supported by the observation that LBU3, with 36 residues and a lower hydrophobic moment (0.70), showed no net gain in activity in comparison to LBU2 against *P. aeruginosa*.

The peptide WLBU2, the most amphipathic of the WLBU series, was also the most potent in both PB and PBS and retained antipseudomonal selectivity, presumably because of its high hydrophobic moment ($\mu = 0.83$) (8). Moreover, the three Trp residues in the hydrophobic face rendered WLBU2 considerably active against *S. aureus* without significantly affecting its mammalian cytotoxicity. We are currently investigating the antibacterial activity of WLBU2 in human serum and its immunomodulatory effects on primary epithelial cells. This information will be relevant to the potential in vivo efficacy of de novo-engineered antimicrobial peptides.

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