Anti-Fungal Activity of Cathelicidins and their Potential Role in Candida albicans Skin Infection

Belén López-García, Phillip H. A. Lee, Kenshi Yamasaki, and Richard L. Gallo
Division of Dermatology, University of California San Diego, and VA San Diego Healthcare Center, San Diego, California, USA

Cathelicidins have broad anti-microbial capacity and are important for host defense against skin infections by some bacterial and viral pathogens. This study investigated the activity of cathelicidins against Candida albicans. The human cathelicidin LL-37, and mouse cathelicidin mCRAMP, killed C. albicans, but this fungicidal activity was dependent on culture conditions. Evaluation of the fungal membrane by fluorescent dye penetration after incubation with cathelicidins correlated membrane permeabilization and inhibition of fungal growth. Anti-fungal assays carried out in an ionic environment that mimicked human sweat and with the processed forms of cathelicidin such as are present in sweat found that the cleavage of LL-37 to forms such as RK-31 conferred additional activity against C. albicans. C. albicans also induced an increase in the expression of cathelicidin in mouse skin, but this induction did not confer systemic or subcutaneous resistance as mCRAMP-deficient mice were not more susceptible to C. albicans in blood-killing assays or in an intradermal infection model. Therefore, cathelicidins appear active against C. albicans, but may be most effective as a superficial barrier to infection.

Key words: anti-microbial peptides/defensins/infection/innate immunity/LL-37/mCRAMP/sweat


Cathelicidins have been shown to contribute to defense against invasive bacterial infection by Group A Streptococcus (GAS) (Dorschner et al., 2001; Nizet et al., 2001) and vaccinia virus (Howell et al., 2004). Whereas cathelicidins are typically expressed at low levels in normal keratinocytes, they are induced during inflammation and increase locally because of release from neutrophils and mast cells (Frohm et al., 1997). Recently, it has been demonstrated that cathelicidins are produced by the eccrine apparatus and secreted into human sweat (Schitteck et al., 2001; Murakami et al., 2002). After secretion onto the skin surface, LL-37 is processed by a serine protease-dependent mechanism into multiple distinct anti-microbial peptides, such as RK-31 or KS-30 (Murakami et al., 2004) that are more potent compared with the uncleaved mature peptide.

The function of cathelicidins as a natural barrier to fungal infections of the skin has not been well characterized. This study characterized the anti-fungal activity of the cathelicidins against Candida albicans and studied the possible role of this family of peptides in skin fungal infection.

Results

Anti-fungal activity of cathelicidins The anti-fungal activity of cathelicidins was measured against C. albicans in low salt 20% mDixon medium (pH 5.5). Two similar α-helical cathelicidin homologues, human (LL-37) and mouse (mCRAMP), showed similar activity with delay or complete inhibition of growth at an minimum inhibitory concentration (MIC) range of 15–20 μM, whereas the linear porcine cathelicidin PR-39 was inactive (Fig 1A). Activities of the human and mouse cathelicidins were pH dependent but not affected by change in temperature from 25°C to 37°C. Growth inhibition of C. albicans in the presence of 15 μM of LL-37 was observed at acidic and neutral pH values, with growth of 19%, 21%, and 60% at pH 4.5, 5.5, and 7.2, respectively (Fig 1B). Growth in the absence of peptide under identical conditions was considered 100% growth.

Processed forms of LL-37 have been shown to kill C. albicans in vitro (Murakami et al., 2004). To compare the activity of these against PR-39, LL-37, and mCRAMP,
fungicidal activity was measured in 20% mDixon, pH 5.5 (Fig 2). These results suggest that the shorter processed forms of LL-37 present at the skin surface (RK-31 and KS-30) have enhanced anti-fungal effect against the yeast compared with forms of cathelicidins released by neutrophils such as mCRAMP and LL-37 (Fig 2).

LL-37 and RK-31 permeabilize C. albicans To evaluate the kinetics and mechanism of killing of C. albicans by cathelicidins, the survival and permeability of the yeast were investigated in the presence of LL-37 or RK-31 (Fig 3). A higher initial concentration of yeast was used in these experiments compared with Fig 2. At this initial C. albicans load (10^6 colony-forming units per mL), 25 μM LL-37 had less fungicidal activity than at (2.5–5 × 10^4 CFU per mL), whereas 8 μM RK-31 continued to kill approximately 90% of the initial inoculum within 15 min (Fig 3A). To determine fungal membrane integrity simultaneously, an assay based on the uptake of the fluorescent dye Sytox green (SG) was used (Fig 3B). SG does not penetrate live eukaryotic or prokaryotic cell membranes or walls but binding to nucleic acids results in a 4–500-fold enhancement in its fluorescence emission, thus enabling sensitive detection of a disruption in the outer membrane (Roth et al., 1997; Thevissen et al., 1999). Incubation of yeast cells with 25 μM LL-37 or 8 μM RK-31 allowed rapid uptake of SG in both cases (Fig 3B). After 5 min of incubation, LL-37 and RK-31 permeabilized more than 50% of the cells versus 12% permeable cells observed in samples without peptide. These results indicate that the kinetics of permeabilization correlated with the fungicidal effect of RK-31 and LL-37.

Candida skin infection increases cathelicidin expression In humans and mice, cathelicidins are not easily
detectable in normal adult skin, but are usually abundantly expressed after inflammation or injury (Frohm et al., 1997; Dorschner et al., 2001). To determine the expression after fungal infection, mice were subcutaneously infected with C. albicans and tissues were sampled. Immunohistochemical analysis showed that mCRAMP expression was higher in infected tissue (Fig 4B and C) compared with expression in healthy tissue (Fig 4A). mCRAMP appeared to be induced specifically at areas of C. albicans infection. This induction of mCRAMP protein expression was confirmed by western blot (data not shown). Control sections stained with rabbit pre-immune serum used as a control. Brown staining in (B) shows increased expression of mCRAMP in C. albicans-infected skin compared with uninfected skin in (A). (C) High power (scale bar = 10 μm) of dermis from (B) shows evidence of multiple C. albicans organisms indicated by arrows and mCRAMP containing neutrophils (hematoxylin counterstain; scale bar = 40 μm in (A, B, D)).

Role of cathelicidins in C. albicans infection in vivo To explore whether cathelicidins are responsible for controlling C. albicans infection in mice, two complementary experiments were carried out. Using blood from mice deficient for mCRAMP (Cnlp−/−), an in vitro killing assay was carried out to determine fungicidal activity against C. albicans. No statistical differences were seen between the growth of the yeast in blood from wild-type versus knockout Cnlp−/− mice (Fig 5A). As reported previously, however, a difference was observed with GAS (Nizet et al., 2001). Next, subcutaneous skin infections of C. albicans were carried out in wild-type and Cnlp−/− mice. Three days after inoculation of yeast, lesions were biopsied and colony counts were performed. Colony-forming units per milliliter were similar from knockout and wild-type mice (Fig 5B).

Processing of LL-37 in sweat increases activity against C. albicans The apparent discordant results between killing in vitro and our inability to detect a contribution for cathelicidins in mouse blood or subcutaneous injections suggested that cathelicidins may only be active against C. albicans on the skin surface. Since the anti-microbial activity of cathelicidins is dependent on ionic and serum components, and can be neutralized in ionic environments containing high concentrations of NaCl (Bals et al., 1998; Turner et al., 1998; Zasloff, 2002), we next evaluated whether cathelicidins were active in the ionic environment of the human skin surface, that of sweat. First, the abundance of cathelicidin peptides in the sweat may be dependent on ionic and serum components, and can be neutralized in ionic environments containing high concentrations of NaCl (Bals et al., 1998; Turner et al., 1998; Zasloff, 2002). We next evaluated whether cathelicidins were active in the ionic environment of the human skin surface, that of sweat. First, the abundance of cathelicidin peptides in the sweat was collected, analyzed by high-pressure liquid chromatography (HPLC), and the mass of fractions eluting at the expected site for KS-30 and LL-37 was confirmed by mass spectrometry. This analysis yielded an approximate concentration of 1.165 μM for KS-30/RK-31 and 0.013 μM of LL-37 in normal human sweat (Fig 6A).

The human sweat was next analyzed for its anti-Candida activity before and after concentration by evaporation.
Following partial evaporation to reduce the volume 4-fold, C. albicans growth was inhibited by the sweat concentrate containing a complex mixture of cathelicidins and other potential anti-microbials (Murakami et al, 2002) (Fig 6B). This inhibitory effect of concentrated sweat was not because of concentrated salt content, as a salt buffer mimicking sweat (SwB) and concentrated 4-fold in salt solution that mimics the ionic composition of normal human sweat. In buffer containing 40 mM NaCl, 10 mM KCl, 1 mM CaCl2, and 1 mM MgCl2, the derived peptides KS-30 and RK-31 were fungicidal against C. albicans, whereas LL-37 in the absence of proteolytic processing was not active (Fig 6C).

Since the buffer conditions of C. albicans growth strongly influence the apparent activity of cathelicidin peptides, additional experiments were performed to mimic the effects of sweat evaporation at the skin surface. When added to the SwB solution, concentration by a factor of 5 × or greater decreased the relative fungicidal activity (Fig 7A), thus suggesting that by itself the cathelicidin peptides may be inactivated in high ionic strength solutions. When cathelicidin peptides were added to normal sweat concentrated by 2 × or 5 × (Fig 7B), the anti-fungal activity of the concentrated sweat was greater than any additional activity detectable by supplementing with synthetic peptides.

**Discussion**

Little is known about the contribution of naturally occurring anti-microbial peptides to defense against fungal infection.
In this study, we characterized the anti-fungal activity of human and mice cathelicidin peptides. The form of these peptides found in human neutrophils (LL-37) or mouse granulocytes (mCRAMP) was effective against *C. albicans* in 20% mDixon medium with an MIC of 15–20 μM and minimal fungicidal concentration (MFC) of 25–30 μM. This activity was pH and growth media dependent. As previously published (Marr et al, 1999), pH is a critical factor in anti-fungal susceptibility testing. Using an acidic pH of 4–5 rather than neutral pH decreased the MIC of LL-37 against *C. albicans*, an effect seen with other anti-fungal agents (Rogers and Galgiani, 1986; McIntyre and Galgiani, 1989). Also, comparison of Figs 2 and 6B shows that the MFC of cathelicidin peptides is greater in salt-containing solutions than in low-salt Dixon media. Thus, conclusions regarding cathelicidin activity against *C. albicans* must be qualified by recognition of the assay conditions, a variable that is also true for other anti-microbial peptides and other target organisms including Gram- and Gram + bacteria. Importantly, the activity of cathelicidin against *C. albicans* could be demonstrated by the direct addition of the peptides in natural human sweat, and human sweat itself becomes anti-fungal once concentrated such as would normally occur during evaporation. Thus, the most superficial topical environment of human skin contains anti-fungal activity that may be partly because of the contribution of cathelicidins.

Several studies have addressed the mode of action of anti-microbial peptides (Oren and Shai, 1998; Epand and Vogel, 1999; Shai et al, 2001; Zasloff, 2002; López-García et al, 2004). Membrane permeabilization drives the anti-microbial activity of most cationic and amphipathic peptides including LL-37 and mCRAMP. Our experiments using fluorescent SG indicate that the perturbation of the yeast membrane is a step in the anti-fungal activity of cathelicidins. Both eukaryotic and bacterial cells can be made transiently permeable with retention of viability; thus, permeability alone does not predict activity (Shapiro, 2001). Our data, however, support a good correlation between both permeability and anti-fungal activity for LL-37 and RK-31 on *C. albicans* (Fig 3). Other factors may also modulate the activity and specificity of peptide anti-microbial activity. For example, the cathelicidin PR-39 affects DNA and protein synthesis (Boman et al, 1993) and is a non-competitive and reversible inhibitor of the proteasome function (Gaczynska et al, 2003) but does not immediately disrupt bacterial membranes. Moreover, LL-37 can neutralize endotoxin, and has chemoattracting effects on leukocytes, which could provide additional mechanisms for protection independent of direct microbial killing (Larrick et al, 1995; Agerberth et al, 2000; De et al, 2000). Together, these observations support a role for membrane permeabilization in the activity of cathelicidins against *C. albicans*, but do not conclusively prove that this is the sole mechanism for cell killing.

Injection of *C. albicans* into mouse skin indicated that cathelicidin is induced in the skin in response to *C. albicans* infection, but this induction did not confer subcutaneous resistance, as mCRAMP-deficient mice were not more susceptible to *C. albicans* in a subcutaneous model. Unlike *Streptococcus pyogenes*, blastospores of *C. albicans* grew equally well in blood from wild-type and Cnlp−/− mice. This lack of response likely reflects several differences between *S. pyogenes* and *C. albicans* but may also several conditions in the subcutaneous environment that act to inhibit cathelicidin function. These include higher pH and concentrations of cations in vivo than in the fungal culture conditions and/or inactivation of peptide by interaction with high-molecular-weight serum proteins (Wang et al, 1998). Susceptibility to *C. albicans* infection may also vary with the strain of mouse used (Hay et al, 1983). Given the results observed with mice and in mouse blood, these findings suggested that cathelicidins may not be involved in protection against *C. albicans* infection once it has penetrated the skin surface.

An alternative role for cathelicidins in fungal infection may be to provide a superficial barrier against proliferation and potential funglal invasion. Our data show that cathelicidin peptides have activity against *C. albicans* when added to normal human sweat, or in ionic environments that mimic sweat. But LL-37 was poorly active in this environment without proteolytic processing. In this paper, we estimated that the concentration of these processed forms (KS-30 or RK-31) and LL-37 is 1.165 and 0.013 μM, respectively (Fig 6A). These concentrations are likely a large underestimate of the actual abundance of cathelicidins at the skin surface. Cathelicidin peptides such as the human cathelicidins will partition into lipid components and bind anionic molecules and will not partition completely into the soluble sweat fraction recovered. But although the actual concentrations may be an underestimate, it appears that the proportion of LL-37 related to forms such as RK-31 is low. LL-37 is a minor form of human cathelicidin on the skin surface.

The actual contribution of the human cathelicidins KS-30 and RK-31 to topical defense against *C. albicans* growth is difficult to determine. This data confirm that these peptides are present, and show activity against *C. albicans* under select growth conditions. Interestingly, RK-31 shows higher activity than KS-30 in higher ionic strength media (SwB, Fig 6C) but lesser activity in the low ionic strength medium (20% mDixon, Fig 2). This suggests that KS-30 activity may be more sensitive to inactivation by salt than RK-31. In SwB solutions alone, the amount of RK-31 required to completely kill *C. albicans* was greater than the amount of RK-31 estimated in normal sweat. Upon addition of 8 μM peptide to normal sweat, however, inhibitory activity was detectable. Activity at 8 μM in the complex mixture of normal sweat compared with 25 μM in SwB alone suggests that these peptides are most likely to be effective in combination with other factors, including other anti-microbial peptides.

In summary, our findings show that naturally produced cathelicidins are present at sites of fungal infection and may form a barrier to *C. albicans* infection. Superficial infection with *C. albicans* is a common dermatological condition that is exacerbated by several factors including host immune status, location, and medications. In humans, abnormal production or an inability to appropriately increase cathelicidin expression following skin inflammation has been associated with disease (Frohm et al, 1997; Dorschner et al, 2001; Conner et al, 2002; Marchini et al, 2002; Ong et al, 2002; Heilborn et al, 2003). The observations made in this study suggest that unlike bacterial infections with *S. pyogenes*, the relevance of cathelicidins to *C. albicans* infection is more likely to be important at the skin surface. Understanding such interactions with innate immune effector
molecules such as cathelicidins may explain the pathophysiology of common fungal skin diseases and offer alternative therapeutic approaches to conventional anti-fungal therapy.

Materials and Methods

Peptides All peptides were prepared by Synpep (Dublin, Oregon), purified by HPLC (95% purity), and identity confirmed by mass spectrometry. A 10 x stock solution was prepared in H2O for each peptide.

Media Primary Candida culture medium was a modified Dixon (mDixon) prepared in the laboratory from 4% malt extract (Fluka Biochemika, Steinheim, Germany), 0.6% Bacto Peptone (Becton, Dickinson and Company, Sparks, Maryland), 1% glucose (Sigma-Aldrich, St Louis, Missouri), and 1% Tween-80 (Sigma-Aldrich). The pH of this medium was 5.0 ± 0.1. For anti-microbial assays, 20% mDixon medium in 1 mM sodium phosphate buffer pH 7 containing 16 μg/mL chloramphenicol was used. The final pH of the mixture was 5.5. Our 20% mDixon medium contained 5.0 mM Na+ , 1.6 mM K+ , 4 mg per dL phosphate, and undetectable amounts of Ca2+ , Mg2+ , and Cl-.

Microorganism C. albicans American Type Cultures Collection 14053 was cultured on mDixon agar medium (mDixon broth + mDixon broth medium) and grown overnight at 37°C with shaking. The turbidity of each suspension was adjusted to OD600 of 0.01 (corresponding to 2.5–5 x 10^5 CFU per mL).

In vitro anti-fungal activity assays The susceptibility of C. albicans was tested by broth microdilution assay. Yeast was grown on 14053 was cultured on mDixon agar medium (mDixon broth + mDixon broth medium and grown overnight at 37°C with shaking. The pH of this medium was 5.0 ± 0.1. For anti-microbial assays, 20% mDixon medium in 1 mM sodium phosphate buffer pH 7 containing 16 μg/mL chloramphenicol was used. The final pH of the mixture was 5.5. Our 20% mDixon medium contained 5.0 mM Na+ , 1.6 mM K+ , 4 mg per dL phosphate, and undetectable amounts of Ca2+ , Mg2+ , and Cl-.

Mouse infection and tissue sampling Procedures were approved by the Veterenrs Affairs (VA) San Diego Healthcare System subcommittee on animal studies. The backs of BALB/c mice were shaved and hair removed by chemical depilation (Nair). Subcutaneous injections with 150 μL of 10^6 CFU per mL C. albicans complexed to Cytofect beads as a carrier were carried out. Three days after injection, the site was measured, then biopsied, and tissue weighted prior to homogenization in PBS for release of organisms and measurement of surviving C. albicans. Some skin specimens were immediately embedded in optimal cutting temperature compound (Sakura Finetechanical, Tokyo, Japan) frozen into liquid nitrogen, and kept at -80°C for immunohistochemistry. Also, skin tissues from areas of normal skin from the same mice were sampled. Sections from fresh-frozen tissues were cut at 10 μm. For protein extraction, skin from infected and non-infected areas was biopsied and homogenized immediately in 1% Triton X-100 in PBS buffer.

Immunohistochemistry Fresh-frozen sections were cut at 10 μm and sections fixed with 10% buffered formalin for 5 min at room temperature and immersed in PBS for 10 min. Endogenous peroxidase activity was blocked with 30 min in 0.3% H2O2. Sections were blocked with 2% goat serum in PBS for 30 min, and incubated with primary antibody, rabbit anti-CRAMP polyclonal antibody (1:500 in PBS) (Dorschner et al, 2001), and 3% bovine serum albumin (BSA) overnight at 4°C. As a negative control, the polyclonal antibody was replaced by normal rabbit pre-immune IgG diluted with PBS containing 3% BSA at the same protein concentration as the primary antibody. After washing the slides 3 x with PBS for 15 min, the signal was detected with goat biotinylated anti-rabbit IgG and a Vectastain ABC Rabbit IgG Elite kit (Vector Laboratories, Burlingame, California) according to the manufacturer’s instructions. Finally, sections were incubated in 0.02% 3,3′-di-aminobenzidine with 0.03% H2O2/urea in PBS for 3–5 min. Sections were counterstained with hematoxylin for 1 s.

Protein extraction and western blotting Skin was dissolved in 1 mL of 1% Triton X-100 in PBS buffer containing protease inhibitor (Complete Mini, EDTA-free, Roche Diagnostics, Mannheim, Germany) and then vortexed, incubated 1–2 h on ice, homogenized with a Dounce homogenizer (Kente Glassio, Vineland, New Jersey) and centrifuged for 10 min at 16,000 x g. Five microliters of 6 x SDS loading buffer containing 10% 2-mercaptoethanol was added to 25 μL of the supernatants of each sample. Proteins were separated using 16% Tris/tricine peptide gels (GeneMate Express Gels, ISC BioExpress, Kaysville, Utah) and transferred to a PVDF membrane (0.45 μm) Immobilon-P (Millipore, Bedford, Massachusetts). For positive control, 80 ng mCRAMP synthetic peptide was applied. The membrane was treated with blocking solution (3% non-fat dry milk, 0.5% BSA, 0.87% NaCl, and 0.3% Tween-20 in TSB) overnight at 4°C and then detected with rabbit anti-CRAMP polyclonal antibody (1:5000 in blocking solution) for 4 h at 4°C. After washing 3 x with 0.1% TTBS for 15 min, the signal was detected by using a goat anti-rabbit IgG conjugated with horseradish peroxidase (Dako AS, Glostrup, Denmark) and developed by using Western Lightning Chemiluminescence Regent Plus (Perkin Elmer, Boston, Massachusetts).

Whole-blood killing assay Three hundred to 500 μL of mouse blood was collected into a tube with 2 μL of heparin (1000 U per mL). Ten microliters of mDixon containing C. albicans at 1.3 x 10^5 CFU per mL (final concentration 3 x 10^5 CFU per mL) was added to 35 μL of fresh blood. The mixture was incubated for 4 h at 37°C and dilutions of the mixture plated on agar plates for enumeration of colony-forming units. As a control, the experiment was carried out with GAS as this organism has previously been shown to depend on mouse cathelicidin in the blood-killing assay. Ten microliters of TSB containing GAS 2.7 x 10^5 CFU per mL was added to 35 μL of blood and the enumeration of colony-forming units was carried out after 1 h of incubation at 37°C. The growth index was
calculated as the ratio of colony-forming units recovered to the initial inoculum.

**Sweat collection and analysis** Human sweat was collected on paper tissues (Kimwipes, Kimberly-Clark, Neenah, Wisconsin) from three healthy volunteers (two females and one male) after exercise as previously described (Murakami et al, 2002, 2004). The sweat was removed from paper tissue by centrifugation. For analysis of cathelicidin in human sweat, 1 mL was separated by reverse phase (RP) HPLC with a C18 column. The sweat was eluted at a flow rate of 2 mL per min using gradients of 10%–20% and 20%–70% ace-tonitrile containing 0.1% trifluoroacetic acid (solution B) for 2 and 32 min, respectively. Column effluent was monitored at 214, 230, and 280 nm. Identified peaks in sweat fractions were compared with the peaks of synthesized peptides (LL-37, KS-30, and RK-31) in an identical HPLC protocol, and the concentrations of peptides were estimated by the peak areas of chromatograms using UNICORN 3.00 (Amersham Pharmacia Biotech, Piscataway, New Jersey). The identity of samples was confirmed by Mass spectrometry (MALDI-MS) performed by Center for Mass Spectrometry, The Scripps Research Institute (La Jolla, California) as previously described (Murakami et al, 2004). Mass assignments were assigned with an accuracy of approximately ± 0.1% (± 1 Da/1000 Da).

**Anti-fungal activity in sweat** To test the anti-fungal activity of human sweat, 4 μL of a 20 × human sweat concentrate was added to 2.5–5 × 10^4 CFU per mL of C. albicans in 16 μL of 20% mDixon medium (final concentration). To study the importance of the proteolytic processing of LL-37 in more active peptides as KS-30 and RK-31 for the anti-fungal activity, C. albicans at 2.5–5 × 10^4 CFU per mL (final concentration) was incubated in 1 × sweat with the peptides LL-37 and RK-31 from 10 × stock solutions. In some samples, 2 μL of a 10 × protease inhibitor solution composed of a mixture of several protease inhibitors with broad inhibitory specificity against serine, cysteine, and metalloproteases, as well as calpains (Complete Mini, EDTA-free protease inhibitor, Roche Diagnostics) was added. To mimic the human sweat ionic environment, and evaluate individual synthetic cathelicidin peptides, an SwB composed of 40 mM NaCl, 10 mM KCl, 1 mM CaCl_2, 1 mM MgCl_2, and 1 mM NaHPO_4, pH 6.5 was also prepared (Murakami et al, 2002). This was sterile filtered using 0.2 μm filter and concentrated 20 × to replicate evaporated conditions in some experiments. Two microliters of 10 × synthetic cathelicidin peptides, LL-37, KS-30, and RK-31 were added to 2.5–5 × 10^4 CFU per mL of C. albicans in 18 μL of 1 × SwB. To test whether the peptides are active in sweat after the evaporation process, peptides RK-31, KS-30, and KR-20 were mixed in sweat or SwB, at a final concentration of 2 or 10 μM of each peptide. Each sample was lyophilized and dissolved in H_2O to final concentrations of 2 × , 4 × , and 10 × . Ten microliters of 9 × 10^4 CFU per mL of C. albicans was incubated with 10 μL of each sample of mixed peptides in SwB or sweat. In all cases, each sample contained 16 μg per mL chloramphenicol to inhibit bacterial growth. Assay mixtures were incubated in sterile 96-well microtiter plates at 37°C for 6 or 24 h and then aliquots of each sample were plated in mDixon agar plates to determine the colony-forming units per milliliter. These plates were then incubated at 37°C for 1 d and colonies were counted.

Address correspondence to: Dr Richard L. Gallo, Division of Dermatology, University of California, San Diego, and VA San Diego Healthcare System, San Diego, California 92161, USA Email: rgallo@vapop.ucsd.edu

**References**


Gallo RL, Ono M, Povsic T, Page C, Eriksson E, Klagsbrun M, Bernfield M: Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds. Proc Natl Acad Sci USA 91:11035–11039, 1994


Lee is supported by the Generalist Physician-Scientist Training Program NIH-NCI 1T32 CA81211. Kenshi Yamasaki is a recipient of a post-doctoral fellowship from the Association for Preventive Medicine of Japan.

In the moment...

Wu M, Maier E, Benz R, Hancock RE: Mechanism of interaction of different classes of cationic antimicrobial peptides with planar bilayers and with the cytoplasmic membrane of Escherichia coli. Biochemistry 38:7235–7242, 1999