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Synthetic modifications of the immunomodulating peptide thymopentin to confer anti-mycobacterial activity

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Effective global control of tuberculosis (TB) is increasingly threatened by the convergence of multidrug-resistant TB and the human immunodeficiency virus (HIV) infection. TB/HIV coinfections exert a tremendous burden on the host’s immune system, and this has prompted the clinical use of immunomodulators to enhance host defences as an alternative therapeutic strategy. In this study, we modified the clinically used synthetic immunomodulatory pentapeptide, thymopentin (TP-5, RKDVY), with six arginine residues (RR-6, RRRRRR) at the N- and C-termini to obtain the cationic peptides, RR-11 (RRKDVYRRRRR-NH2) and RY-11 (RRRRRRKDVY-NH2), respectively. The arginine residues conferred anti-mycobacterial activity to TP-5 in the peptides as shown by effective minimum inhibitory concentrations of 125 mg/L and killing efficiencies of >99.99% against both rifampicin-susceptible and -resistant Mycobacterium smegmatis. The immunomodulatory action of the peptides remained unaffected as shown by their ability to stimulate TNF-α production in RAW 264.7 mouse macrophage cells. A distinct change in surface morphology after peptide treatment was observed in scanning electron micrographs, while confocal microscopy and dye leakage studies suggested bacterial membrane disruption by the modified peptides. The modified peptides were non-toxic and did not cause hemolysis of rat red blood cells up to a concentration of 2000 mg/L. Moreover, RY-11 showed synergism with rifampicin and reduced the effective concentration of rifampicin, while preventing the induction of rifampicin resistance. The synthetic peptides may have a potential application in both immunocompetent and immunocompromised TB patients.

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1. Introduction

Tuberculosis (TB) is caused by the bacillus Mycobacterium tuberculosis and ranks as the second leading cause of death from a single infectious agent, after the human immunodeficiency virus (HIV) [1]. Although TB incidence and mortality rates have fallen globally, the rapid emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB continues to threaten decades of progress in global TB control. MDR-TB, with an estimated 450,000 incident cases in 2012, is defined as resistance to isoniazid and rifampicin, the two most powerful first-line anti-TB drugs. XDR-TB strains, which accounts for 9% of MDR-TB cases, are further generated when MDR-TB strains develop additional resistance to a fluoroquinolone and a second line injectable agent [2]. In addition, efforts to tackle MDR-TB are complicated by TB/HIV coinfections, which dramatically reduce host immunity and increase individuals’ susceptibility to TB infection and reinfection, including with drug-resistant strains [3]. Malabsorption of anti-TB drugs, particularly rifampicin, in TB/HIV patients may also predispose them to acquisition of drug resistance [4]. Progress towards early diagnosis and the development of shorter, less toxic and more efficacious treatment regimens in MDR-TB is clearly a priority in the global management of TB.

Cationic host defense peptides (HDPs) are a diverse group of molecules produced by the innate immune system in response to infectious agents. They have recently been identified as a potential new class of anti-infectives for drug development, given their broad-spectrum activity through both direct bactericidal and adjunctive immunomodulatory actions [5]. The direct anti-
microbial mechanism of HDPs is largely attributed to their ability to fold into amphiphilic structures with hydrophobic and cationic domains, facilitating physical interactions with the negatively charged bacterial cell membrane and in some cases, bacterial cell penetration to act on intracellular targets, leading to the disruption of membrane integrity and cellular processes, respectively [6]. Their aggregate actions on several components essential to bacterial cell survival thus make development of drug resistance much less likely [7,8]. Rational design of directly anti-microbial peptides based on structure-function relationships has thus been widely employed to produce candidates with greater bactericidal efficiency, although often at the expense of safety, which may explain why most peptides are used topically in clinical trials. The adjunctive immunomodulatory actions of HDPs, on the other hand, have been demonstrated in animal models to be important for pathogen clearance via the regulation of chemotactic activities of dendritic and T-cells, and induction of pro-inflammatory cytokines, leading to enhanced leukocyte recruitment to the site of infection [9]. Given their pleiotropic targets and effects and the lack of clear structural requirements for immunomodulation, rational design of immunomodulatory HDPs is relatively more challenging and lags behind the development of microbial HDPs. Nonetheless, immunomodulation is recognized as a highly effective strategy to combat MDR infections especially in immunocompromised patients, as the target of action is the immune system rather than the pathogen itself.

In the present study, we explored synthetic modifications of a clinically used immunomodulator, thymopeitin (TP-5), to confer cationicity to mimic the dual immunomodulatory and anti-microbial effects of HDP. TP-5 is a synthetic pentapeptide consisting of five amino acids, Arg-Lys-Asp-Val-Tyr, that correspond to the 32–36 amino acid sequence of the thymus hormone thymopoietin Refs. [10,11]. TP-5 reproduces the immunomodulatory activity of thymopeitin, which is responsible for thymocyte differentiation and maturation [10,11]. Besides inducing the phenotypic differentiation of T precursor cells in vitro, TP-5 can regulate the expression of CD4 and CD8 cell surface markers on human thymocytes [11,12]. TP-5 reproduces the immunomodulatory activity of thymopoietin, which is responsible for thymocyte differentiation and maturation [10,11]. Besides inducing the phenotypic differentiation of T precursor cells in vitro, TP-5 can regulate the expression of CD4 and CD8 cell surface markers on human thymocytes [11,12].

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For each treatment, bacterial cells exposed to a sub-MIC concentration (1/8 of MIC at 2.10). Simulation of drug resistance

Peptides Peptides sequence MIC (mg/L) HC 50 (mg/L)

| Peptides | Peptides sequence | MIC (mg/L) | HC 50 (mg/L) |
|----------|------------------|------------|-------------|
| TP-5     | RKDVY-NH2        | >1000      | ND          |
| RR-6     | RRRRRR-NH2       | 125        | 125         |
| RR-11    | RRRRRRRRRRR-NH2  | 125        | 125         |
| RY-11    | RRRRRRRDVY-NH2   | 125        | 125         |

Table 1

Minimum inhibitory concentration (MIC) of synthetic peptides, TP-5, RR-6, RR-11 and RY-11, against M. smegmatis and their 50% hemolysis concentration (HC50).

Bacterial cells were prepared as in MIC measurements but treated with the peptide RR-11 (250 mg/L) for a shorter incubation time (2 h). After treatment, the bacteria were centrifuged at 4000 rpm for 5 min, and washed 3 times with PBS. Sample fixation was performed with 2.5% glutaraldehyde for 30 min, followed by washing with PBS and finally deionized water. Sample dehydration was carried out using a series of graded ethanol solutions (35%, 50%, 75%, 90% and 100%), followed by drying for two days. The dried samples were mounted on carbon tape, sputtered with platinum coating and analyzed under a field emission scanning electron microscope equipped with a 63 × 1.4 (oil) Plan apochromate lens.

2.8. Hemolytic activity test

To assess the safety of the peptides against mammalian cells, their hemolytic activity was tested using freshly drawn rat red blood cells (rRBCs) obtained from Animal Holding Unit (AHU), Biomedical Research Center (BRC), Singapore. The rRBCs were diluted 25 times with PBS to give 4% blood content as reported previously. Sequential dilution of the peptides was prepared using PBS with concentrations ranging from 0 to 2000 mg/L. Equal volumes (250 μL) of the peptide solution and blood suspension were mixed. Subsequently, the mixtures were centrifuged at 37 °C for 2 h to facilitate the interactions between the rRBCs and peptides. Subsequently, the mixtures were centrifuged at 4000 g for 15 min and 100 μL of the supernatant was transferred into a 96-well microplate. Hemoglobin release was determined spectrophotometrically by measuring the absorbance of the samples at 576 nm via the microplate reader (TECAN, Switzerland). Untreated rRBCs served as a negative control while rRBCs treated with 1% Triton-X served as a positive control. Each test was carried out in 4 replicates and reproduced twice. The degree of hemolysis was calculated using the following formula: Hemolysis (%) = (|O.D.576 nm of the treated sample - O.D.576 nm of the negative control|/|O.D.576 nm of positive control - O.D.576 nm of negative control|) × 100.

2.9. Chequerboard assay

Chequerboard assays were performed to determine the effect of combining the peptide RY-11 and rifampicin treatments against M. smegmatis. A set of mixtures of 100 μL each were prepared by varying RY-11 and rifampicin concentrations according to Table 2 and added to 100 μL of M. smegmatis suspension (containing approximately 107 CFU/mL) in each well of a 96-well plate. The plates were then incubated at 37 °C and read after 72 h. Assessment of microbial growth was done visually or spectrophotometrically via OD readings at 600 nm (TECAN, Switzerland). The fractional inhibitory concentration index (FICI) was calculated for each combination using this equation: FICI = FIC A + FIC B, where FIC A = MIC of drug A in combination/MIC of drug A alone, and FIC B = MIC of drug B in combination/MIC of drug B alone. FICI of ≤ 0.5 was interpreted as synergy, 0.5 < FICI ≤ 1.0 as additive, 1.0 < FICI ≤ 4.0 as indifferent, and FICI > 4.0 as antagonism.

2.10. Simulation of drug resistance

Drug resistance was induced in M. smegmatis by repeated treatments with RR-11 or RY-11 for 10 passages and established via MIC measurement as abovementioned. For each treatment, bacterial cells exposed to a sub-MIC concentration (1/8 of MIC at that particular passage) were re-grown to the log phase and reused for the following passage’s MIC measurement. Changes in the MIC were depicted by normalizing the MIC at passage n to that of the first passage.

2.11. TNF-α stimulation assay

The ability of the peptides RR-11 and RY-11 as well as TP-5 to modulate immune response was evaluated by measuring the production of the proinflammatory cytokine tumor necrosis factor (TNF-α) in RAW 266.7 mouse macrophage cells. The cells were kindly provided by Dr Ho Han Kiat (Department of Pharmacy, National University of Singapore, Singapore) and cultured in DMEM supplemented with 10–15% FBS, 10 μL/mL penicillin G and 100 μg/mL streptomycin in a humidified atmosphere at 37 °C containing 5% CO2. The cells were seeded at density of 4 × 105 cells/mL in 24-well plates for 24 h, after which they were treated with TP-5, RR-11 and RY-11 at a final concentration of 5 μg in serum-free medium for 1 h. The media was then collected and centrifuged at 5000 g for 5 min to spin down any cells. This cell-free supernatant was used to conduct TNF-α ELISA assay as per manufacturer’s instructions. Lipopolysaccharide (LPS), a bacterial cell wall component, which is known to stimulate TNF-α production in RAW 266.7 cells was used at a concentration of 500 ng/mL as the positive control. Absorbance readings were taken at 450 nm with wavelength correction at 570 nm using the Infinite® 200 Pro spectrophotometer (TECAN, Switzerland). The amount of TNF-α released upon peptide treatment was quantified using the regression equation from the generated standard curve. All the experiments were performed in triplicates and reported as mean ± S.E.M.

Passage's MIC measurement. Changes in the MIC were depicted by normalizing the MIC at passage n to that of the first passage.

Fig. 1. Colony formation units (CFUs) of M. smegmatis after incubation with RR-11 for various periods of time. Experiments were performed in triplicate and the bacterial activities are expressed as mean log (CFU/ml ± standard deviations shown by the error bars. The bactericidal effect RR-11 at MIC and 2 × MIC was validated by the 3 log10 of CFU/ml comparing with initial inoculums.
2.12. Statistical analysis

Data were analyzed using one-way ANOVA followed by Dunnett’s post-hoc analysis (SPSS, Chicago, IL). The difference between values for the treatments was considered to be statistically significant at $p < 0.001$.

3. Results and discussion

3.1. In vitro anti-mycobacterial activity

It has been reported that anti-microbial activity of peptides could be enhanced by replacing the acidic residues by basic ones to increase the net positive charge [31,32]. Hence, in order to amplify the anti-microbial ability, it is reasonable to increase the cationicity by including positive charged amino acids in the peptide sequence. Furthermore, peptide consisting of six or more arginine entered cells far more effectively than peptides of equal length containing lysine, ornithine or histidine. However, peptides of fewer than six amino acids were ineffective [33,34]. Therefore, we engineered a short peptide composed of six arginine residues alone (RR-6) and this short peptide was capable of inducing pronounced growth inhibition of both rifampicin (RIF)-susceptible and RIF-resistant M. smegmatis with an MIC value of 125 mg/L (Table 1, Fig. S1). RIF-resistant M. smegmatis was developed by multiple treatments of drug-susceptible M. smegmatis with rifampicin at a sub-MIC concentration ($0.125 \times$ of MIC) and the MIC concentration of rifampicin increased from 7.81 mg/L to 500 mg/L (Fig. S2). As expected, TP-5 itself could not inhibit bacterial growth up to the highest concentration used (1000 mg/L). However, upon addition of six

Fig. 3. Confocal microscopic images of M. smegmatis incubated with FITC-conjugated dextran (250 mg/L) in the presence of PBS (A) or RR-11 (500 mg/L) for 10 min (B), 30 min (C) and 1 h (D). Notes: A1-D1: Green represents fluorescence of FITC-conjugated dextran. A2-D2: Bright field. A3-D3: Overlapping images. Scale bar: 20 μm. The entry of FITC-conjugated dextran indicates the microbial cell membrane damage after treatment with the peptide. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
arginine residues at either C- or N-terminus of TP-5, the modified peptides, RR-11 and RY-11, became active against *M. smegmatis* at MIC similar to RR-6. In addition, killing efficiencies greater than 99.99% were achieved at respective MICs, suggesting a bactericidal effect of the peptides against RIF-susceptible and RIF-resistant *M. smegmatis* (Fig. S3). This effect was further validated using *in vitro* time-kill assays to analyze the fractional cell survival upon RR-11 peptide treatment at none, ½× MIC, MIC and 2× MIC over 72 h. Fig. 1 shows that RR-11 was bactericidal (defined as ≥3 log10 decrease in the initial inoculum [35]) at 48 h at both MIC and 2× MIC. Our results support the successful conferment of anti-microbial activity on the immunomodulator TP-5 via increasing the cationic charge density of the peptide.

### 3.2. Anti-mycobacterial mechanism of action

LUVs were employed to contain the fluorescent calcein dye and to mimic the loss of cellular content upon membrane rupture in bacteria. A concentration- and time-dependent increase in dye leakage was observed with RR-11 peptide treatment, indicating that the peptide was highly efficient in disrupting the integrity of the simulated bacterial membrane (Fig. 2). To verify the potential of RR-11 in damaging bacterial cell membrane, *M. smegmatis* was exposed to RR-11 (500 mg/L) for 10 min, 30 min and 60 min in the presence of fluorescent FITC-labeled 100 kDa dextran molecules (250 mg/L) and observed under the confocal microscope for evidence of dextran uptake (Fig. 3). In contrast to the control group incubated with PBS alone, FITC-dextran gained entry into *M. smegmatis* readily after 10 min incubation with RR-11 to give a fluorescent signal, which becomes more intense with a longer incubation. The entry of the FITC dye indicated that the microbial cell membrane was damaged after the treatment with RR-11. These observations were supported by SEM visualization of cell lysis, which demonstrated the change of surface morphology in *M. smegmatis* from a rod-shaped structure with smooth surface to a flaccid and shrunken structure with distorted surfaces after 2 h incubation with RR-11 (250 mg/L) (Fig. 4).

### 3.3. Hemolytic activity

With its influence on the structural integrity of the bacterial membrane, we were interested to find out the relative specificity of the peptide on mammalian cells. This is evaluated by hemolysis tests, which measure the ability of the peptides to lyse rat red blood cells *in vitro*. Fig. 5 shows that the peptides displayed minimal hemolytic effects even up to a concentration as high as 2000 mg/L, a concentration which is 16 times that of the MIC of peptides (125 mg/L) (Table 1). This confirms that the peptides have high selectivity towards bacterial cells, which is desirable to expand the therapeutic window for clinical application.

### 3.4. Synergism with first-line anti-TB drug rifampicin

Cationic peptides are considered as promising antibiotic candidates based on the fact that these peptides are less likely to develop drug resistance and have broad spectrum of activity. However, in spite of the advantages, the clinical usage is limited due to drawbacks such as poor potency, specificity and in vivo stability [36]. To overcome the shortcomings and translate laboratory discovery to the clinic, synergistic effect between peptides and traditional antibiotics can be applied as synergy can reduce the dose of each drug in combination, prevent drug resistance and result in greater antibacterial effect than the sum of the effects due to single agent [37,38]. Rifampicin has been reported to act synergistically when administered in conjunction with anti-microbial peptides, possibly as a consequence of the peptide-mediated membrane disruption, which in turn enhances uptake of the drug [36,39,40]. To determine the interactions between RY-11 and rifampicin, synergy studies were performed using the chequerboard assays. Fig. 6 shows that the minimum effective concentrations for each agent to be used in combination to achieve synergy in inhibiting the growth of *M. smegmatis* was ¼× MIC of RY-11 and ¼× MIC of rifampicin. The calculated FICI value was 0.5. Time-kill studies were performed to

![Fig. 5.](image-url) Hemolytic activity of the anti-mycobacterial peptides. All peptides displayed minimal hemolytic effect up to the concentration of 2000 mg/L.

![Fig. 4.](image-url) FE-SEM images of *M. smegmatis* showing the difference on the morphology between untreated (A) and treated (B) bacteria. A flaccid and shrunken structure with distorted surfaces was seen upon treatment with RR-11 (250 mg/L) for 2 h, in comparison to the untreated control.
validate the synergistic effect. Fig. 7 shows that rifampicin and RY-11 used alone as a single agent at $\frac{1}{4}\times$ MIC could not inhibit the growth of *M. smegmatis*, while their combination successfully inhibited the bacterial growth. Synergy was defined as $>2\log_{10}$ decrease in CFU/ml by the drug combination when compared to its most active constituent [35]. Synergistic activity was observed for combination group at 48 h and 72 h, confirmed by the reduction in viable colony counts of $>2\log_{10}$ as compared to the single most active constituent.

3.5. Effect in drug-resistant *M. smegmatis*

The induction of drug resistance was used to evaluate the possibility of peptides to induce drug resistance in mycobacteria after multiple treatments with low sub-MIC concentrations. Fig. 8 shows that after repeated treatments of rifampicin at $\frac{1}{4}\times$ MIC concentration, *M. smegmatis* became resistant to rifampicin as early as passage 2 and the MIC against *M. smegmatis* rose dramatically by approximately 30 fold by passage 10. In contrast, RR-11 and RY-11 remained equally active from passage 1 to 10, as demonstrated by the maintenance of MIC value throughout. This result supports the hypothesis that the different mechanisms of anti-mycobacterial action may be exploited in combination therapy to evade drug resistance to a single agent.

3.6. TNF-α stimulation assay

TP-5 has been shown to induce the cytokine TNF-α production in unstimulated macrophages as part of its immunomodulatory functions [41]. Hence, to address the retention of biological activity of TP-5 in the modified peptides RR-11 and RY-11, ELISA assays were performed to measure and compare the level of TNF-α released after peptide treatment in RAW 264.7 macrophage-like cells. The amount of TNF-α released did not differ between the peptides although the levels were comparable to that released in response to LPS stimulation of the macrophages ($p > 0.05$) (Fig. 9). These levels were significantly higher than when the peptides were absent in the cell media ($p < 0.001$). Our results show that despite the structural modifications, the biological activity of TP-5 remained unperturbed in RR-11 and RY-11. Notably, it has been reported that TNF-α is essential in controlling TB infection [42]. In inflammatory bowel disease (IBD) patients who were also latently infected with *M. tuberculosis*, the approach to counteract the increased serum levels of TNF-α with TNF-α-neutralizing agents such as infliximab has led to TB reactivation [43,44]. The ability to induce TNF-α production of TP-5 was maintained in our modified peptides.
production specifically in our modified peptides may thus complement their anti-mycobacterial actions for a more effective therapeutic management of TB.

4. Conclusion

The present study demonstrates that cationic charges bestowed by repeated arginine residues have conferred anti-mycobacterial activity against both drug-susceptible and drug-resistant strains on the clinically used immunomodulator TP-5. Mechanistic investigations using dye leakage assays and microscopy reveal that the modified peptides function via membrane-lytic actions, which may account for their synergistic actions with the first-line anti-TB drug rifampicin. Despite the structural modifications, hemolysis hence toxicity is kept low and the biological activity of TP-5 is well retained as indicated by its ability to stimulate TNF-α production in macrophages. These results demonstrate that synthetic peptides, which possess dual anti-mycobacterial and immunomodulatory effects may have the potential to evade drug resistance in both immunocompetent and immunocompromised TB patients.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2013.12.049.

References

[1] WHO. Global tuberculosis report 2013. Geneva: World Health Organization; 2013.
[2] Walter ND, Strong M, Belknap R, Ordway DJ, Daley CL, Chan ED. Translating basic science insight into public health action for multibacillary and extensively drug-resistant tuberculosis. Respir Res 2012;17:772–91.
[3] Pawloski A, Jansson M, Skold M, Kallenius G. Tuberculosis and HIV co-infection. PLoS Pathog 2012;8:e1002464.
[4] Sundal E, Bertelletti D. Thymopentin treatment of rheumatoid arthritis. Arzneimittelforschung 1994;44:1145–9.
[5] Liu J, Xu K, Wang H, Tan PJ, Fan W, Venkattraman SS, et al. Self-assembled cationic peptide nanoparticles as an efficient antimicrobial agent. Nat Nanotech 2009;4:457–63.
[6] Wang H, Xu K, Liu L, Tan JP, Chen Y, Li Y, et al. The efficacy of self-assembled cationic antimicrobial peptide nanoparticles against Cryptococcus neoformans for the treatment of meningitis. Biomaterials 2010;31:2874–81.
[7] Leung DY, Hirsch RL, Schneider L, Li SH, et al. Thymopentin therapy reduces the clinical severity of atopic dermatitis. J Allergy Clin Immunol 1990;85:927.
[8] Malaise MG, Franchimont P, Bachandersen R, Gerber H, Stocker H. Hauwerta E, et al. Treatment of active rheumatoid-arthritis with slow intravenous injections of thymopentin – a double-blind placebo-controlled randomized study. Lancet 1985;1:823–8.
[9] Sundal E, Bertelletti D. Thymopentin treatment of rheumatoid-arthritis. Arzneimittelforschung 1994;44:1145–9.
[10] Alacan NJ, Yeuang AT, Pena OM, Hancock RE. Therapeutic potential of host defense peptides in antibiotic-resistant infections. Curr Pharm Des 2012;18:807–19.
[11] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002;415:389–95.
[12] Malik AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol 2006;6:468–75.
[13] Eliopoulos GM, Moellering RC. Antibiotic combinations. 3rd ed. Baltimore, MD: The Williams & Wilkins Co.; 1991.
[14] Tencza SB, Creighton DJ, Yuan T, Vogel HJ, Montelaro RC. Antimicrobial peptides prepared by ring-opening metathesis polymerization: manipulating antimicrobial properties by organic counterion and charge density variation. Chemotherapy 2009;15:11715–22.
[15] Mitchell DJ, Kim DT, Steinman L, Fathman CG, Rothbard JB. Polymicrobial enteric cells more efficiently than other polyacyclic homopolymers. J Pept Res 2000;56:318–25.
[16] NCCLS. Methods for determining bacterial activity of antimicrobial agents: approved guideline. Wayne, Pennsylvania, USA: NCCLS; 1999.
[17] Alacan NJ, Yeuang AT, Pena OM, Hancock RE. Therapeutic potential of host defense peptides in antibiotic-resistant infections. Curr Pharm Des 2012;18:807–19.
[18] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002;415:389–95.
[19] Malik AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol 2006;6:468–75.
[20] Alacan NJ, Yeuang AT, Pena OM, Hancock RE. Therapeutic potential of host defense peptides in antibiotic-resistant infections. Curr Pharm Des 2012;18:807–19.
[21] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002;415:389–95.
[22] Malik AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol 2006;6:468–75.
[23] Rand K, Houck H, Brown P, Bennett D. Reproducibility of the microdilution checkerboard method for antibiotic synergy. Antimicrob Agents Chemother 1993;37:613–5.
[24] Leung DY, Hirsch RL, Schneider L, Li SH, et al. Thymopentin therapy reduces the clinical severity of atopic dermatitis. J Allergy Clin Immunol 1990;85:927.
[25] Leung DY, Hirsch RL, Schneider L, Li SH, et al. Thymopentin therapy reduces the clinical severity of atopic dermatitis. J Allergy Clin Immunol 1990;85:927.
[26] Leung DY, Hirsch RL, Schneider L, Li SH, et al. Thymopentin therapy reduces the clinical severity of atopic dermatitis. J Allergy Clin Immunol 1990;85:927.
[27] Alacan NJ, Yeuang AT, Pena OM, Hancock RE. Therapeutic potential of host defense peptides in antibiotic-resistant infections. Curr Pharm Des 2012;18:807–19.
macrophage cells: inhibitory analysis and a role of signal cascades. Expert Opin Ther Targets 2011;15:1337–46.
Møller M, Hoal EG. Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. Tuberculosis (Edinb) 2010;90:71–83.

[43] Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001;345:1098–104.

[44] D’Haens G, Daperno M. Advances in biologic therapy for ulcerative colitis and Crohn’s disease. Curr Gastroenterol Rep 2006;8:506–12.