Postprint: Antimicrobial Peptide Sublancin Enhances Acquired Immunity in Mice

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Abstract

This study aimed to investigate the effects of oral administration of antimicrobial peptide Sublancin on the acquired immune response in ovalbumin (OVA)-immunized mice. Sixty 6-week-old female BALB/c mice were randomly divided into 5 groups with 12 mice per group. Mice in the blank control group were orally administered normal saline, those in the positive control group received 2.5 mg/kg body weight of levamisole solution, and mice in the antimicrobial peptide Sublancin groups were orally administered Sublancin solutions at 0.5, 1.0, and 2.0 mg/kg body weight. All groups received continuous administration for 14 days. Twenty-four hours after the administration period, all mice were immunized subcutaneously with the model antigen OVA, followed by a booster immunization 14 days later. Seven days after the second immunization, blood and spleens were collected. Serum OVA-specific immunoglobulin G (IgG) and its subclasses IgG1 and IgG2a, as well as cytokine levels, were measured using indirect enzyme-linked immunosorbent assay (ELISA). The results showed: 1) Compared with the blank control group, serum OVA-specific IgG levels were significantly or extremely significantly increased in the 0.5, 1.0, and 2.0 mg/kg Sublancin groups and the positive control group ($P<0.05$ or $P<0.01$). 2) Compared with the blank control group, serum OVA-specific IgG1 levels were significantly or extremely significantly increased in the 1.0 and 2.0 mg/kg Sublancin groups and the positive control group ($P<0.05$ or $P<0.01$), serum OVA-specific IgG2a levels were significantly increased in the 1.0 mg/kg Sublancin group and the positive control group ($P<0.05$), and the stimulation index of splenic lymphocytes was significantly increased in the 1.0 mg/kg Sublancin group and the positive control group ($P<0.05$). 3) Compared with the blank control group, the levels of T helper type 1 (Th1) cytokines interferon- (IFN-) and interleukin-2 (IL-2) in the supernatant of splenic lymphocytes were significantly or extremely significantly increased in the 1.0 mg/kg Sublancin group and the positive control group ($P<0.05$ or $P<0.01$). 4) Compared with the blank control group, the levels of

T helper type 2 (Th2) cytokines interleukin-4 (IL-4) and interleukin-10 (IL-10) in the supernatant of splenic lymphocytes were significantly or extremely significantly increased in the 0.5 and 1.0 mg/kg Sublancin groups and the positive control group ($P<0.05$ or $P<0.01$). 5) No significant differences were observed in any of the above parameters between the 0.5, 1.0, and 2.0 mg/kg Sublancin groups and the positive control group ($P>0.05$). These results indicate that antimicrobial peptide Sublancin can induce a Th1/Th2 mixed-type immune response in OVA-immunized mice, enhancing both humoral and cellular immune functions.

Full Text

Study of Antimicrobial Peptide Sublancin Enhancing Acquired Immunity in Mice

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Abstract

This study investigated the effects of intragastric administration of antimicrobial peptide Sublancin on the acquired immune response in mice immunized with ovalbumin (OVA). Sixty 6-week-old female BALB/c mice were randomly divided into 5 groups (n=12 per group). The blank control group received normal saline, the positive control group received levamisole at 2.5 mg/kg body weight, and the Sublancin groups received Sublancin at 0.5, 1.0, and 2.0 mg/kg body weight, respectively. All treatments were administered daily for 14 consecutive days. Twenty-four hours after the final administration, mice were immunized subcutaneously with OVA as a model antigen. A booster immunization was given 14 days later, and blood and spleen samples were collected 7 days after the second immunization. Serum OVA-specific immunoglobulin G (IgG) and its subclasses IgG1 and IgG2a, as well as cytokine levels, were measured by indirect enzyme-linked immunosorbent assay (ELISA).

The results showed: (1) Serum OVA-specific IgG levels in the 0.5, 1.0, and 2.0 mg/kg Sublancin groups and the positive control group were significantly or extremely significantly higher than in the blank control group ($P<0.05$ or $P<0.01$). (2) Compared with the blank control group, serum OVA-specific IgG1 levels in the 1.0 and 2.0 mg/kg Sublancin groups and the positive control group were significantly or extremely significantly increased ($P<0.05$ or $P<0.01$), while serum OVA-specific IgG2a levels in the 1.0 mg/kg Sublancin group and the positive control group were significantly increased ($P<0.05$). The spleen lymphocyte stimulation index was also significantly increased in the 1.0 mg/kg Sublancin group and the positive control group ($P<0.05$). (3) The contents of T helper type 1 (Th1) cytokines interferon- (IFN-) and interleukin-2 (IL-2) in spleen

lymphocyte supernatant were significantly or extremely significantly increased in the 1.0 mg/kg Sublancin group and the positive control group ($P<0.05$ or $P<0.01$). (4) The contents of T helper type 2 (Th2) cytokines interleukin-4 (IL-4) and interleukin-10 (IL-10) in spleen lymphocyte supernatant were significantly or extremely significantly increased in the 0.5 and 1.0 mg/kg Sublancin groups and the positive control group ($P<0.05$ or $P<0.01$). (5) No significant differences were observed among the Sublancin groups and the positive control group for any of the measured parameters ($P>0.05$). These findings demonstrate that Sublancin can induce mixed Th1 and Th2 immune responses in OVA-immunized mice, thereby enhancing both humoral and cellular immune functions.

Keywords: antimicrobial peptide; Sublancin; mice; humoral immunity; cellular immunity

Introduction

Antimicrobial peptides (AMPs) are biologically active small polypeptides produced by living organisms and widely distributed across the biological kingdom. As essential components of the innate immune defense system, they constitute the first line of host defense against pathogenic microorganisms [1-2]. AMPs exhibit broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, mycobacteria, and fungi, while also demonstrating efficacy against parasites, viruses, and even tumor cells [1,3-4]. Most AMPs exert their antibacterial effects by disrupting bacterial cytoplasmic membrane integrity, though some can penetrate cell membranes and bind to intracellular targets, making them less prone to inducing bacterial resistance and thus a promising alternative to antibiotics [3,5]. As research on AMP structure and function has progressed, their immunomodulatory functions have gradually been discovered. Beyond direct antimicrobial activity, AMPs possess multiple immunomodulatory properties, including regulation of inflammatory responses, chemotaxis of immune cells, promotion of cell differentiation, and activation of innate and adaptive immunity [2,6]. Studies have shown that fish-derived -helical antimicrobial peptides and chicken -defensins (AvBD) 1-14 can enhance antigen-specific humoral and cellular immune responses in animals, thereby improving vaccine efficacy [7-9].

Sublancin is a cationic peptide consisting of 37 amino acids with two disulfide bonds, originally isolated from the fermentation broth of *Bacillus subtilis* 168 strain by Hansen' s research team at the University of Maryland [10-11]. Its amino acid sequence is GLGKAQCAALWLQCASGGTIGCGGGAVACQNYRQFCR, with a molecular weight of approximately 3,875.74 Da [10-11]. Sublancin is extremely stable, tolerating pH ranges from 1.5 to 9.5 and high-temperature environments [12]. It exhibits activity against Gram-positive bacteria including *Bacillus megaterium*, *Staphylococcus aureus*, *Streptococcus pyo-*

genes, and *Clostridium perfringens*, but shows no significant antibacterial effect against Gram-negative bacteria [13-15]. Paik et al. [13] proposed that Sublancin exerts its antibacterial activity by targeting specific molecules in bacterial membrane synthesis, leading to pore formation. Kouwen et al. [16] found that Sublancin' s antibacterial activity is associated with bacterial mechanosensitive ion channels, suggesting it may cause bacterial lysis by preventing channel closure and inducing rapid efflux of intracellular contents. Wang et al. [14] reported that Sublancin may inhibit bacterial division by disrupting energy metabolism. Current research on Sublancin has primarily focused on its structure, expression regulation, bactericidal activity, and mechanisms of action, while its role as an immunomodulatory molecule remains unexplored. This study utilized BALB/c mice as a model and ovalbumin (OVA) as a model antigen to investigate the effects of intragastric Sublancin administration on acquired immune responses, providing a theoretical basis for its application as an immunoenhancer in animal production.

Materials and Methods

1.1 Experimental Materials OVA and levamisole were purchased from Sigma-Aldrich (USA). Sublancin was produced by the National Feed Engineering Technology Research Center through construction of a novel recombinant plasmid transformed into *Bacillus subtilis* W800 for high-level expression, followed by purification using an AKTA system to obtain 99.6% pure peptide. Sublancin was dissolved in normal saline at concentrations of 0.04, 0.08, and 0.16 mg/mL for mouse administration. RPMI-1640 medium, erythrocyte lysing solution, and phosphate-buffered saline (PBS) were obtained from Gibco (USA). Cell Counting Kit-8 (CCK-8) was from Dojindo (Japan). Concanavalin A (ConA) was from Sigma-Aldrich (USA). Fetal bovine serum (FBS) was from Hyclone (USA). Penicillin (100 U/mL) and streptomycin (100 g/mL) were from Gibco (USA). Bovine serum albumin (BSA) was from Sigma-Aldrich (USA). Horseradish peroxidase (HRP)-labeled goat anti-mouse IgG was from Arigo (Taiwan, China). HRP-labeled goat anti-mouse IgG1 and IgG2a were from Abcam (UK). ELISA kits for IL-2, IFN- , IL-4, and IL-10 were from R&D Systems (USA).

Coating buffer (0.05 mol/L, pH 9.6) was prepared by dissolving 1.59 g Na CO and 2.93 g NaHCO in distilled water to a final volume of 1,000 mL, with pH adjusted to 9.6. PBST wash buffer (0.01 mol/L, pH 7.4) contained 8.00 g NaCl, 2.90 g Na HPO · 12H O, 0.20 g NaH PO , 0.20 g KCl, and 0.5 mL Tween-20 in 1,000 mL distilled water, pH adjusted to 7.2. Antibody diluent (1% BSA) was prepared by dissolving 1 g BSA in 100 mL wash buffer. Blocking solution (3% BSA) contained 3 g BSA in 100 mL wash buffer. Substrate buffer (pH 5.0) consisted of 3.68 g Na HPO · 12H O and 0.933 g citric acid in 100 mL distilled water. TMB substrate solution was prepared fresh before use by mixing 0.1 mL TMB stock solution (10 mg dissolved in 100 L DMSO and 900 L distilled water)

with 10 mL substrate buffer and 10 L 30% H O . Stop solution (2 mol/L H SO) was prepared by adding 22.2 mL concentrated sulfuric acid (98%) to 177.8 mL distilled water.

1.2 Experimental Design and Animal Management Sixty 6-week-old specific pathogen-free (SPF) female BALB/c mice were purchased from Beijing Huafukang Bioscience Co. and randomly divided into 5 groups (n=12 per group). The blank control group received normal saline, the positive control group received levamisole at 2.5 mg/kg body weight, and the Sublancin groups received Sublancin at 0.5, 1.0, and 2.0 mg/kg body weight, respectively. All treatments were administered intragastrically as 0.2 mL solutions daily for 14 consecutive days. Twenty-four hours after the final administration, mice were immunized subcutaneously with OVA (100 g/mouse in 0.2 mL) via multiple dorsal injections. A booster immunization with the same dose was administered 14 days later.

The experiment was conducted at the Mouse Nutrition and Metabolism Laboratory of the Ministry of Agriculture Feed Efficacy and Safety Evaluation Supervision and Testing Center (Beijing). Mice were housed in temperature- and humidity-controlled rooms (22±2°C, 45%±10% relative humidity) with a 12:12 h light-dark cycle, provided ad libitum access to feed and water, and maintained on regular bedding changes to ensure hygienic conditions.

1.3 Sample Collection Seven days after the second OVA immunization, blood samples were collected via retro-orbital bleeding using sterile capillary tubes (approximately 1 mm diameter) inserted into the orbital sinus to collect 200-300 L into sterile 1.5 mL centrifuge tubes. After standing at room temperature for 2 hours, samples were centrifuged at 3,000 rpm for 10 minutes at 4°C. The supernatant serum was carefully aspirated, aliquoted, and stored at -80°C until analysis. Following blood collection, mice were euthanized by cervical dislocation, and spleens were aseptically removed for preparation of spleen lymphocyte suspensions.

1.4 Detection Methods 1.4.1 Body Weight
Body weight was measured before intragastric administration, before the first immunization, before the second immunization, and before necropsy.

1.4.2 Serum OVA-Specific IgG and Subclass Detection
Serum OVA-specific IgG, IgG1, and IgG2a levels were measured by indirect ELISA. Microtiter plates were coated with 5 g/mL OVA in coating buffer (100 L/well) and incubated overnight at 4°C. After washing five times with PBST (300 L/well for 3 minutes each), plates were blocked with 3% BSA (150 L/well) at 37°C for 60 minutes. Following another five washes, diluted serum samples (1:1,000) were added (100 L/well, in duplicate) and incubated at 37°C for 60 minutes. After washing, HRP-labeled goat anti-mouse IgG (1:5,000), IgG1 (1:10,000), or IgG2a (1:5,000) was added (100 L/well) and incubated at 37°C

for 60 minutes. Following final washes, TMB substrate solution (100 L/well) was added and incubated at 37°C for 5 minutes in the dark. The reaction was stopped with 50 L/well of 2 mol/L H SO , and absorbance at 450 nm was measured immediately using a microplate reader.

1.4.3 Spleen Lymphocyte Proliferation Assay
Spleen lymphocyte activity was assessed using the CCK-8 assay. Seven days after the second OVA immunization, mice were euthanized and spleens were aseptically removed after 75% ethanol immersion for 3-5 minutes. Spleens were placed in 10 mL cold Hanks' solution and gently minced through a 200-mesh stainless steel screen. The screen was washed with 3 mL cold Hanks' solution, and cells were collected by centrifugation at 1,000 rpm for 5 minutes. Erythrocytes were lysed using 2 mL erythrocyte lysing solution for 5 minutes, followed by two washes with 3 mL Hanks' solution. Cells were resuspended in 1 mL RPMI-1640 complete medium containing 10% FBS. Viability was assessed by trypan blue staining (1:9 dilution), ensuring >95% viability before adjusting cell concentration to 2.5×10 cells/mL. Cell suspension (100 L/well) was added to 96-well plates, with ConA (5 g/mL final concentration) added to positive control wells, OVA (5 g/mL final concentration) to test wells, and medium alone to negative control and blank wells, bringing total volume to 200 L/well. After 68 hours incubation at 37°C with 5% CO , CCK-8 reagent (10 L/well) was added and incubated for an additional 4 hours. Absorbance at 450 nm was measured, and stimulation index was calculated as: (OD of stimulated wells - OD of medium) / (OD of unstimulated wells - OD of medium).

1.4.4 Cytokine Detection in Spleen Lymphocyte Supernatant
Spleen lymphocytes were prepared as described in section 1.4.3, with cell concentration adjusted to 5×10 cells/mL. Cell suspension (100 L/well) was added to 96-well plates, followed by 100 L OVA (5 g/mL final concentration) and incubated at 37°C with 5% CO for 48 hours. After incubation, plates were centrifuged at 1,000 rpm for 10 minutes, and supernatants were carefully collected. Th1 cytokines (IFN- , IL-2) and Th2 cytokines (IL-4, IL-10) were quantified using ELISA kits according to manufacturer protocols.

1.5 Statistical Analysis Data were analyzed using two-tailed independent samples t-test in GraphPad Prism 6.0. Results are expressed as means. $P<0.05$ was considered statistically significant, and $P<0.01$ was considered extremely significant.

Results

2.1 Effects of Sublancin on Mouse Body Weight As shown in Table 1 , no significant differences in body weight were observed among groups after 2 weeks of intragastric administration with normal saline, levamisole, or Sublancin ($P>0.05$). Similarly, no significant differences were found 14 days after the first

immunization or 7 days after the second immunization (P>0.05). These results indicate that Sublancin administration did not affect mouse body weight.

Table 1 Effects of antimicrobial peptide Sublancin on body weight of mice

Items	Before intragastric administration	After intragastric administration	14 days after first immunization	7 days after second immunization
Blank-control group	-	-	-	-
Positive control group	-	-	-	-
Antimicrobial peptide Sublancin level (mg/kg)	0.5	1.0	2.0	-
P-value	-	-	-	-

In the same row, values with no letter superscripts mean no significant difference (P>0.05).

2.2 Effects of Sublancin on Serum OVA-Specific IgG Levels The effects of Sublancin on serum OVA-specific IgG content are presented in Figure 1 [Figure 1: see original paper]. Compared with the blank control group, serum OVA-specific IgG levels were significantly or extremely significantly increased in the 0.5, 1.0, and 2.0 mg/kg Sublancin groups (P<0.05 or P<0.01) and significantly increased in the positive control group (P<0.05). No significant differences were observed between any Sublancin group and the positive control group (P>0.05).

Figure 1 Effects of antimicrobial peptide Sublancin on serum OVA-specific IgG content in mice (n=12)

NC: blank control group; PC: positive control group. Value columns with indicate significant difference compared with blank control group (P<0.05), ** indicate extremely significant difference (P<0.01). Value columns of antimicrobial peptide Sublancin groups with no letter indicate no significant difference compared with positive control group (P>0.05). The same applies below.*

2.3 Effects of Sublancin on Serum OVA-Specific IgG1 and IgG2a Levels The effects of Sublancin on serum OVA-specific IgG subclass levels are shown in Figure 2 [Figure 2: see original paper]. As shown in Figure 2-a, serum OVA-specific IgG1 levels were significantly increased in the 2.0 mg/kg Sublancin group and positive control group ($P<0.05$) and extremely significantly increased in the 1.0 mg/kg Sublancin group ($P<0.01$) compared with the blank control group. No significant differences were observed between any Sublancin group and the positive control group ($P>0.05$).

As shown in Figure 2-b, serum OVA-specific IgG2a levels were significantly increased in the 1.0 mg/kg Sublancin group and positive control group ($P<0.05$) compared with the blank control group. The 0.5 and 2.0 mg/kg Sublancin groups showed no significant difference from the blank control group ($P>0.05$). No significant differences were observed between any Sublancin group and the positive control group ($P>0.05$).

These results demonstrate that 2-week intragastric administration of Sublancin at 1.0 and 2.0 mg/kg differentially increased serum OVA-specific IgG1 and IgG2a levels in OVA-immunized mice, with effects comparable to the levamisole positive control group.

Figure 2 Effects of antimicrobial peptide Sublancin on serum OVA-specific IgG1 (a) and IgG2a (b) contents in mice (n=12)

2.4 Effects of Sublancin on Spleen Lymphocyte Proliferation The effects of Sublancin on spleen lymphocyte proliferation capacity are presented in Figure 3 [Figure 3: see original paper]. The spleen lymphocyte stimulation index was significantly increased in the 1.0 mg/kg Sublancin group and positive control group compared with the blank control group ($P<0.05$). No significant differences were observed in the 0.5 and 2.0 mg/kg Sublancin groups ($P>0.05$), and no significant differences were found between any Sublancin group and the positive control group ($P>0.05$).

Figure 3 Effects of antimicrobial peptide Sublancin on spleen lymphocyte proliferation capacity in mice (n=12)

2.5 Effects of Sublancin on Th1 Cytokine Levels in Spleen Lymphocyte Supernatant The effects of Sublancin on Th1 cytokine levels are shown in Figure 4 [Figure 4: see original paper]. As shown in Figure 4-a, IFN- levels were significantly increased in the 1.0 mg/kg Sublancin group and positive control group compared with the blank control group ($P<0.05$). No significant differences were observed in the 0.5 and 2.0 mg/kg Sublancin groups ($P>0.05$), and no significant differences were found between any Sublancin group and the positive control group ($P>0.05$).

As shown in Figure 4-b, IL-2 levels were extremely significantly increased in the 1.0 and 2.0 mg/kg Sublancin groups ($P<0.01$) and significantly increased in the positive control group ($P<0.05$) compared with the blank control group.

No significant differences were observed between any Sublancin group and the positive control group (P>0.05).

These results indicate that 2-week intragastric administration of Sublancin at 1.0 and 2.0 mg/kg promoted spleen lymphocyte secretion of Th1 cytokines IFN- and IL-2.

Figure 4 Effects of antimicrobial peptide Sublancin on IFN- (a) and IL-2 (b) levels in spleen lymphocyte supernatant of mice (n=12)

2.6 Effects of Sublancin on Th2 Cytokine Levels in Spleen Lymphocyte Supernatant The effects of Sublancin on Th2 cytokine levels are presented in Figure 5 [Figure 5: see original paper]. As shown in Figure 5-a, IL-4 levels were significantly increased in the 0.5 mg/kg Sublancin group and positive control group (P<0.05) and extremely significantly increased in the 1.0 mg/kg Sublancin group (P<0.01) compared with the blank control group. No significant differences were observed between any Sublancin group and the positive control group (P>0.05).

As shown in Figure 5-b, IL-10 levels were extremely significantly increased in the 1.0 mg/kg Sublancin group and positive control group (P<0.01) and significantly increased in the 0.5 mg/kg Sublancin group (P<0.05) compared with the blank control group. No significant differences were observed between any Sublancin group and the positive control group (P>0.05).

These results demonstrate that 2-week intragastric administration of Sublancin at 0.5 and 1.0 mg/kg promoted spleen lymphocyte secretion of Th2 cytokines IL-4 and IL-10.

Figure 5 Effects of antimicrobial peptide Sublancin on IL-4 (a) and IL-10 (b) levels in spleen lymphocyte supernatant of mice (n=12)

Discussion

In addition to antimicrobial activity, antimicrobial peptides such as -defensins and -defensins can bridge innate and adaptive immunity by inducing differentiation of macrophages and dendritic cells and chemoattracting dendritic cells, monocytes, and T cells [2,6]. Furthermore, antimicrobial peptides like -defensins can directly regulate adaptive immunity by promoting CD4+ T lymphocyte proliferation and cytokine secretion [17]. Consequently, antimicrobial peptides can enhance antigen-specific adaptive immune responses to model antigens or pathogenic microorganisms [18]. While Sublancin has demonstrated in vitro and in vivo antibacterial activity [13-15], its effects on adaptive immunity remain unclear. This study used a mouse model and OVA as a model antigen [7,19] to investigate Sublancin' s impact on adaptive immune responses.

Adaptive immunity comprises humoral immunity mediated by B lymphocytes

and cellular immunity mediated by T lymphocytes. Humoral immunity involves antigen-induced B cell activation, proliferation, differentiation, and antibody production that exert effects in body fluids [20]. Serum antigen-specific antibody levels are commonly used to reflect humoral immune function. Cellular immune responses involve three stages: (1) naive T cell recognition of specific antigens, (2) activation, proliferation, and differentiation into effector T cells, and (3) effector T cell-mediated antigen clearance [20]. T helper (Th) cells are the primary regulators of immune responses, producing numerous cytokines that control immune responses through complex regulatory networks. CD4+ Th cells can be classified into Th1 and Th2 subsets based on their cytokine profiles [21]. Th1 cells induce cellular immune responses through macrophage activation and cytokine release to clear intracellular antigens, while Th2 cells promote B cell proliferation, differentiation, and antibody production through IL-4, IL-5, IL-6, and IL-10 secretion, thereby supporting humoral immunity [20-21].

Our findings demonstrate that Sublancin administration increased serum OVA-specific IgG levels, indicating enhanced OVA-specific humoral immune responses. Beyond increasing total IgG, Sublancin promoted production of IgG1 and IgG2a subclasses, which are used to evaluate Th2- and Th1-type humoral immune responses, respectively [22]. These results suggest that Sublancin enhances both Th1- and Th2-type humoral immune responses.

Sublancin increased secretion of Th1 cytokines IL-2 and IFN- as well as Th2 cytokines IL-4 and IL-10 from spleen lymphocytes stimulated with OVA, indicating enhancement of both Th1 and Th2 immune responses. This finding aligns with the observed effects on IgG subclass production. Collectively, the results for OVA-specific antibody subclasses and cytokine profiles demonstrate that Sublancin can induce mixed Th1/Th2 immune responses.

Additionally, Sublancin increased the spleen lymphocyte stimulation index, an important indicator of cellular immune function [23]. These results suggest that Sublancin can facilitate robust cellular immune responses to OVA, consistent with its promotion of Th1 cytokine production.

Levamisole, the levo-isomer of synthetic tetramisole, has been extensively studied as an immunomodulatory agent that enhances both humoral and cellular immune responses, serving as a reference standard in many immunoenhancer studies [24-27]. Our results showed that Sublancin administration at 0.5, 1.0, and 2.0 mg/kg produced comparable immunoenhancing effects to 2.5 mg/kg levamisole in OVA-immunized mice. Importantly, Sublancin administration did not significantly affect mouse body weight, demonstrating excellent safety at doses of 0.5-2.0 mg/kg for 2 weeks.

Antimicrobial peptides influence both innate and adaptive immune responses [28]. Previous studies have shown that human neutrophil-derived -defensins increase serum antigen-specific IgG levels and promote IFN- , IL-5, IL-6, and IL-10 release from CD4+ T cells [29-30]. The synthetic antimicrobial peptide KLKL5KLK is an effective Th2-type immunity inducer [7], while synthetic adju-

vants combining KLKL5KLK with deoxyinosine/deoxycytosine mixtures elicit Th1-type cellular and humoral responses [31-32]. Although accumulating evidence demonstrates that antimicrobial peptides influence innate and adaptive immunity, the underlying mechanisms remain poorly understood. Current evidence suggests antimicrobial peptides may activate innate immunity to initiate adaptive responses by inducing dendritic cell and macrophage differentiation [33-35], or directly regulate adaptive immunity by acting on T and B cells [6]. In vivo studies indicate antimicrobial peptides may enhance adaptive immunity by improving antigen delivery to antigen-presenting cells (APCs) or prolonging antigen retention at injection sites [7]. Fritz et al. [7] found that synthetic antimicrobial peptide KLKL5KLK enhanced OVA presentation to the monocyte-macrophage cell line P388D1 and prolonged OVA retention at injection sites. While our study demonstrates that Sublancin enhances antigen-specific cellular and humoral immune responses with mixed Th1/Th2 polarization, the precise mechanisms require further investigation.

Conclusion

Sublancin can induce mixed Th1 and Th2 immune responses in OVA-immunized mice, thereby enhancing both humoral and cellular immune responses.

References

[1] Zasloff M. Antimicrobial peptides of multicellular organisms[J]. Nature, 2002, 415(6870): 389-395.
[2] Lai Y P, Gallo R L. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense[J]. Trends in Immunology, 2009, 30(3): 131-141.
[3] Nguyen L T, Haney E F, Vogel H J. The expanding scope of antimicrobial peptide structures and their modes of action[J]. Trends in Biotechnology, 2011, 29(9): 464-472.
[4] Zhang L J, Gallo R L. Antimicrobial peptides[J]. Current Biology, 2016, 26: R14-R19.
[5] Baltzer S A, Brown M H. Antimicrobial peptides-promising alternatives to conventional antibiotics[J]. Journal of Molecular Microbiology and Biotechnology, 2011, 20(4): 228-235.
[6] Mansour S C, Pena O M, Hancock R E W. Host defense peptides: front-line immunomodulators[J]. Trends in Immunology, 2014, 35(9): 443-450.
[7] Fritz J H, Brunner S, Birnstiel M L, et al. The artificial antimicrobial peptide KLKLLLLLKLK induces predominantly a TH2-type immune response to co-injected antigens[J]. Vaccine, 2004, 22(25/26): 3274-3284.
[8] Zhang H H, Yang X M, Xie Q M, et al. The potent adjuvant effects of chicken -defensin-1 when genetically fused with infectious bursal disease virus

VP2 gene[J]. Veterinary Immunology and Immunopathology, 2010, 136(1/2): 92-97.
[9] Acosta J, Carpio Y, Valdes I, et al. Co-administration of tilapia alpha-helical antimicrobial peptides with subunit antigens boost immunogenicity in mice and tilapia (*Oreochromis niloticus*)[J]. Vaccine, 2014, 32(2): 223-229.
[10] Oman T J, Boettcher J M, Wang H, et al. Sublancin is not a lantibiotic but an S-linked glycopeptide[J]. Nature Chemical Biology, 2011, 7(2): 78-80.
[11] Stepper J, Shastri S, Loo T S, et al. Cysteine S-glycosylation, a new post-translational modification found in glycopeptide bacteriocins[J]. FEBS Letters, 2011, 585(4): 645-650.
[12] Ji S Y, Li W L, Baloch A R, et al. Improved production of sublancin via introduction of three characteristic promoters into operon clusters responsible for this novel distinct glycopeptide biosynthesis[J]. Microbial Cell Factories, 2015, 14: 17.
[13] Paik S H, Chakicherla A, Hansen J N. Identification and characterization of the structural and transporter genes for, and the chemical and biological properties of Sublancin 168, a novel lantibiotic produced by *Bacillus subtilis* 168[J]. The Journal of Biological Chemistry, 1998, 273(36): 23134-23142.
[14] Wang Q W, Zeng X F, Wang S, et al. The bacteriocin Sublancin attenuates intestinal injury in young mice infected with *Staphylococcus aureus*[J]. Anatomical Record, 2014, 297(8): 1454-1461.
[15] Wang G S, Li X, Wang Z. APD3: the antimicrobial peptide database as a tool for research and education[J]. Nucleic Acids Research, 2015, 44: D1087-D1093.
[16] Kouwen T R H M, Trip E N, Denham E L, et al. The large mechanosensitive channel MscL determines bacterial susceptibility to the bacteriocin Sublancin 168[J]. Antimicrobial Agents and Chemotherapy, 2009, 53(11): 4702-4711.
[17] Yang D, Biragyn A, Hoover D M, et al. Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense[J]. Annual Review of Immunology, 2004, 22(1): 181-215.
[18] Nicholls E F, Madera L, Hancock R E W. Immunomodulators as adjuvants for vaccines and antimicrobial therapy[J]. Annals of the New York Academy of Sciences, 2010, 1213: 46-61.
[19] Bommineni Y R, Pham G H, Sunkara L T, et al. Immune regulatory activities of fowlicidin-1, a cathelicidin defense peptide[J]. Molecular Immunology, 2014, 59(1): 55-63.
[20] Cao X T. Medical Immunology[M]. 6th ed. Beijing: People' s Medical Publishing House, 2013: 49-110.
[21] Gong F L. Medical Immunology[M]. 3rd ed. Beijing: Science Press, 2009: 167-168.
[22] Finkelman F D, Holmes J, Katona I M, et al. Lymphokine control of in vivo immunoglobulin isotype selection[J]. Annual Review of Immunology, 1990, 8(1): 303-333.
[23] Mohan T, Sharma C, Bhat A A, et al. Modulation of HIV peptide antigen specific cellular immune response by synthetic - and -defensin peptides[J]. Vaccine, 2013, 31(13): 1707-1716.

[24] Kang Y M, Jin H L, Zheng G X, et al. The adjuvant effect of levamisole on killed viral vaccines[J]. Vaccine, 2005, 23(48/49): 5543-5550.
[25] Malik F, Singh J, Khajuria A, et al. A standardized root extract of *Withania somnifera* and its major constituent withanolide-A elicit humoral and cell-mediated immune responses by up-regulation of Th1-dominant polarization in BALB/c mice[J]. Life Sciences, 2007, 80(16): 1525-1538.
[26] Gautam M, Saha S, Bani S, et al. Immunomodulatory activity of *Asparagus racemosus* on systemic Th1/Th2 immunity: implications for immunoadjuvant potential[J]. Journal of Ethnopharmacology, 2009, 121(2): 241-247.
[27] Zhai L J, Li Y T, Wang W Y, et al. Effect of oral administration of ginseng stem-and-leaf saponins (GSLS) on immune responses to Newcastle disease vaccine in chickens[J]. Vaccine, 2011, 29(31): 5007-5014.
[28] Hancock R E W, Diamond G. The role of cationic antimicrobial peptides in innate host defenses[J]. Trends in Microbiology, 2000, 8(9): 402-410.
[29] Lillard J W, Boyaka P N, Chertov O, et al. Mechanisms for induction of acquired host immunity by neutrophil peptide defensins[J]. Proceedings of the National Academy of Sciences of the United States of America, 1999, 96(2): 651-656.
[30] Tani K, Murphy W J, Chertov O, et al. Defensins act as potent adjuvants that promote cellular and humoral immune responses in mice to a lymphoma idiotype and carrier antigens[J]. International Immunology, 2000, 12(5): 691-700.
[31] Agger E M, Rosenkrands I, Olsen A W, et al. Protective immunity to tuberculosis with Ag85B-ESAT-6 and a synthetic cationic adjuvant system IC31[J]. Vaccine, 2006, 24(26): 5452-5460.
[32] De Gonzalo C V G, Zhu L Y, Oman T J, et al. NMR structure of the S-linked glycopeptide sublancin 168[J]. ACS Chemical Biology, 2014, 9(3): 796-801.
[33] Biragyn A, Ruffini P A, Leifer C A, et al. Toll-like receptor 4-dependent activation of dendritic cells by -defensin 2[J]. Science, 2002, 298(5595): 1025-1029.
[34] Van der Does A M, Joosten S A, Vroomans E, et al. The antimicrobial peptide hLF1-11 drives monocyte-dendritic cell differentiation toward dendritic cells that promote antifungal responses and enhance Th1 polarization[J]. Journal of Innate Immunity, 2012, 4(3): 284-292.
[35] Pena O M, Afacan N, Pistolic J, et al. Synthetic cationic peptide IDR-1018 modulates human macrophage differentiation[J]. PLoS One, 2013, 8(1): e52449.

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