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Research Article

Immunomodulatory Effects of Nervonic Acid in RAW264.7 Macrophages

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The present work studies the immunomodulatory potential of nervonic acid (NA) on the growth and proliferation of murine RAW264.7 macrophages. The immunomodulatory effects of NA were assessed through the evaluation of nitric oxide (NO) levels, cytokine secretion, phagocytic activity, reactive oxygen species (ROS) generation, and nuclear factor-κB (NF-κB) signaling pathway of RAW264.7 macrophages. The methodology used included a cell counting kit-8 (CCK-8) technique, enzyme-linked immunosorbent assay (ELISA), neutral red phagocytosis test, flow cytometry, western blotting, and immunofluorescence, respectively. Obtained outcomes pointed to NA as a potential promoter of the production of NO, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and ROS in RAW264.7 cells. Meanwhile, the expression of iNOS, TNF-α, IL-1β, and IL-6 mRNA was upregulated. Besides, the expression of TLR4 protein and the phosphorylation level of NF-κB were increased in RAW264.7 cell lines in a concentration-dependent manner when compared against the control. As a conclusion, NA can activate the NF-κB signaling pathway through the TLR4 signaling pathway, which has immunomodulatory potential.

1. Introduction

Benefits associated with fatty acids, especially those containing some unsaturated position in their chemical formula, such as monounsaturated or polyunsaturated fatty acids (MUFAs and PUFAs, respectively), include immune-enhancement and improvement of chronic diseases such as cancer processes, cardiovascular diseases, and diabetes [1, 2]. Nervonic acid (NA) belongs to omega-9 long-chain MUFAs, and it was first found in mammalian nerve tissues [3]. In nature, NA is mainly obtained from plant seed oil, fungi, and microalgae [4, 5]. The role of NA, as a nutrient, is

key to human health since it is implied in the development of the brain and the biosynthesis of nerve cells. However, the daily intake of NA from diet is, in general terms, not enough to ensure an optimal regulation of the metabolism of lipid and energy [6]. Indeed, previous studies have shown that NA has the potential to protect brain tissue, prevent cardiovascular diseases, attack tumoral tissues, and enhance immunity [7, 8]. Therefore, scientific reports support the benefits that the correct diet supplementation of NA will trigger in human's health.

In recent decades, immunity has represented the target of multiple studies. The immune system is key to maintaining homeostasis and preventing diseases due to its capacity to neutralize external harmful substances/organisms and so minimizing harmful cell changes. In this context, macrophages play a relevant role as immunomodulatory cells associated with a variety of biological activities, such as bactericidal capacity, innate immunity, and adaptive immunity [9–11]. Macrophages usually exert these immunomodulatory functions by secreting cytokines, recruiting immune cells, and phagocytizing microorganisms. Toll-like family receptors (TLRs), a class of proteins, belong to the superfamily of pattern recognition receptors (PRRs), with special relevance for innate and adaptive immunity [12, 13]. TLRs get triggered by the reaction of infections or injuries caused in cells of the immunological system, and they activate nuclear factor-κB (NF-κB), as well as NF-κB activates, differentiates, and ensures the survival of immune cells [14–16]. When the NF-κB signaling pathway is activated, the expressions of cyclooxygenase-2 (COX-2) and nitric oxide synthase (iNOS) are triggered, as well as the production of IL-1β and TNF-α, are induced, which are essential cytokines in the development of inflammatory processes, and IL-6 is involved in B cells differentiation [17]. Reactive oxygen species (ROS) represent a cell defense mechanism which may modulate the inhibition or stimulation of NF-κB signaling pathways [18–20].

Nowadays, the regulatory effect and the mechanism of NA on immune activity are not clear yet. Therefore, the immunomodulatory effects of NA on RAW264.7 macrophages were assessed through the evaluation of nitric oxide (NO) levels, cytokine secretion, phagocytic activity, and ROS generation. The immunomodulatory mechanism of regulating the NF-κB signaling pathway was evaluation. Our study may provide a theoretical basis for the development of immune-related products based on the use of NA.

2. Materials and Methods

2.1. Materials. Macrophage RAW264.7 cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin, 1% L-glutamine and 1% penicillin-streptomycin, and phosphate buffered saline (PBS) were purchased from GIBCO BRL (Grand Island, NY, USA). Nervonic acid standard (NA, N1514, purity >99%), dimethyl sulfoxide (DMSO), and lipopolysaccharides (LPS, L3024) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Cell counting kit-8 (CCK-8), nitric oxide (NO) assay kit, enzyme-linked immunosorbent (ELISA) assay kit, TNF-α, IL-1β, and IL-6 detection kits, reactive oxygen species (ROS) assay kit, immunofluorescence detection kit, BCA protein assay kit, radio immunoprecipitation (RIPA) assay kit, enhanced chemiluminescent (ECL), and polyvinylidene difluoride (PVDF) membranes were obtained from Beyotime Biotechnology and the neutral red from Maclin Biochemical Co., Ltd. (Shanghai, China). TRIzol, first-strand cDNA synthesis kit, and quantitative PCR kit were purchased from Vazyme Biotech Co., Ltd. (Nanjing, China). Antibodies for inducible nitric oxide synthase (iNOS, 13120S), toll-like receptor 4 (TLR4, 38519S), IκBα (4812S), phospho-IκBα (p-IκBα, 2859S), NF-κB-p65 (8242S), and GAPDH (8884S) were obtained from Cell Signaling Technology (CST, Danvers, MA, USA). Horseradish peroxidase-conjugated secondary antibodies (AS014) were purchased from ABclonal Biotechnology Co., Ltd. (Wuhan, China).

2.2. Cell Culture. RAW264.7 macrophages were cultured using DMEM enriched with 10% (v/v) FBS, and the antibiotics penicillin (100 units/mL) and streptomycin (100 μg/mL). Cell cultures were incubated at 5% CO_2 and 37°C.

2.3. Cell Viability. Macrophage RAW264.7 cells at the logarithmic growth stage were inoculated into 96-well flat-bottomed microplates at a density of 1×10^5 cells/well. Then, the cells were incubated overnight at 37°C before administration. Different concentrations (6.25, 12.50, 25.00, 50.00, 75.00, 100.00, and 200.00 μmol/L) of NA were administrated to the treated group, and the enriched DMEM was used for the control group as a blank control. Six multiwells were set up in each group and incubated for another 24 hours. Then, a fresh medium containing 10% CCK-8 was added into each well for 2 h. Absorbance was simultaneously measured at 450 nm in each well using a microplate reader (Varioskan Flash, Thermo Scientific, MA, USA), and cell viability was expressed as a percentage of the control group.

2.4. ELISA Assays. Macrophage RAW 264.7 cells were seeded in 6-well plates at a density of 1×10^6 cells/well and incubated for 24 h at 37°C. Then, the cells were treated using various concentrations (12.50, 25.00, and 50.00 μmol/L) of NA. The positive control group was treated with 1 μg/mL LPS, and DMEM was used as a blank control instead of the stimulus. After incubation for 24 h, the culture medium was harvested and the secretion of cytokines TNF-α, IL-1β, and IL-6 was measured by using ELISA kits. Absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). The production of NO was detected using the Griess reagent and measuring the absorbance at 540 nm.

2.5. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). The isolation of the total RNA of RAW264.7 cells was performed using TRIzol reagent. Then, the first-strand

cDNA synthesis kit assisted the reverse transcription of RNA into cDNA. Later, the Biomiga SYBR qPCR mix kit allowed it to determine the relative mRNA expression levels using an ABI 7500 qRT-PCR instrument. Results are presented as $2^{-\triangle\triangle ct}$. Primer sequences of IL-1β, IL-6, TNF-α, iNOS, and GAPDH for qRT-PCR are presented in Table 1.

2.6. Neutral Red Phagocytosis Assay. The macrophages cells were inoculated into 96-well plates at a density of 1×10^5 cells/well for 24 h. Then, different concentrations (12.50, 25.00, and 50.00 μmol/L) of NA or 1 μg/mL LPS were added to the cells and incubated for another 24 h at 37°C. Later, the supernatant was discarded with a pipette, a volume of 100 μL of 0.1% neutral red solution was added to each well, incubated for another 1 h, and washed 3 times with PBS, and then, 100 μL of 1% acetic acid (50% ethanol, V/V) was added. Absorbance was simultaneously measured at 540 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

2.7. Flow Cytometry Analysis of ROS Generation. The production of ROS in macrophages was assessed using a specific ROS kit (Beyotime Biotechnology, Shanghai, China) following the manufacturer's protocols. Briefly, after treatments with NA, macrophages cells were incubated with oxidation-sensitive fluorogenic probe 2′, 7′-dichlorofluorescin diacetate (DCFH-DA) and the ROS-positive cell proportion was identified with a flow cytometer (Beckman Coulter, FL, USA).

2.8. Western Blot Analysis. Macrophage cells were inoculated into 6-well plates at a density of 1×10^6 cells per well and incubated at 37°C for 24 h. After that, the cells were treated using the same NA concentrations as for previous assays (12.50, 25.00, and 50.00 μmol/L of NA). The positive control group was treated with 1 μg/mL LPS, and the medium was used as a blank control instead of the stimulus. Later, after an incubation period of 24 h, the cells collected from 6-well plates were lysed using RIPA buffer with 10 mM phenylmethanesulfonyl fluoride (PMSF). Protein concentration of samples was determined with a BCA kit. The prepared samples separated using a 10% SDS-PAGE gel were later transferred into a PVDF membrane which was blocked for 2 h using a solution of 5% skim milk at room temperature. After that, the membrane was incubated with primary antibodies of TLR4, IκBα, and p-IκBα (1 : 1000 dilutions with TBST) at 4°C overnight. The membrane was washed with TBST thrice and incubated with a horseradish peroxidase-conjugated secondary antibody (1 : 2000 dilution with TBST) for 2 h at room temperature. After rinsing blots thrice using TBST, target bands were identified by dropping ECL hypersensitive chemiluminescence solution and scanned by using a Bio-Rad chemiluminescence imaging system. ImageJ was used to quantify the gray level of protein bands.

2.9. Immunofluorescence Analysis. The nuclear translocation of *p*65 was measured using immunofluorescence application solutions kit following the manufacturer's protocols. First, the cells, in their logarithmic growth stage, were inoculated into 6-well plates (glass slides were placed at the bottom of each well). The treatment of the cells using different concentrations of NA or 1 μg/mL LPS was followed by the removal of the supernatant. After the cells were washed thrice with PBS, a 4% paraformaldehyde solution was applied to fix cells which were labeled with a fluorescence kit. Photographs were taken with a fluorescence microscope.

2.10. Statistical Analysis. All experimental results presented along this study were obtained from at least the performance of three independent experiments. The results were statistically analyzed using OriginPro 2022 (OriginLab, Northampton, Massachusetts, USA). Differences between groups were considered significant at $p < 0.05$.

3. Results

3.1. Cytotoxicity Assay of NA. The cytotoxicity of NA on RAW264.7 cells was evaluated using a CCK-8 assay which allowed it to determine cell viability. The results (see Figure 1) showed that the treatment with concentrations in the range of 0–50 μmol/L of NA had no significant toxicity effects on RAW264.7 cells when compared against the control group. Nevertheless, cell viability decreased less than 50% when NA concentration was 75 μmol/L or higher. Therefore, the concentrations 12.5, 25, and 50 μmol/L of NA, within the safe range, were selected for the subsequent experiments.

3.2. Effects of NA on NO, TNF-α, IL-6, and IL-1β in RAW264.7 Cells. As important bioactive molecules in the body, NO, TNF-α, IL-1β, and IL-6 are closely related to various functions in the process of immune regulation, such as inflammatory response and immune-related diseases [21, 22]. The results (see Figure 2) showed that NA stimulation significantly increased the levels of these proinflammatory cytokines TNF-α, IL-1β, and IL-6 and the mediator NO in RAW264.7 cells with dose dependence when compared against the control group. In a similar way, the expression levels of TNF-α, IL-1β, IL-6, and iNOS were significantly increased in NA-treated groups ($p < 0.01$) when compared against the control group. The concentration and expression levels of proinflammatory cytokines in RAW264.7 cells of the positive control group were both increased significantly ($p < 0.001$). These results indicate that the expression of TNF-α, IL-1β, IL-6, and iNOS and the generation of cytokines TNF-α, IL-1β, and IL-6 and mediator NO in RAW264.7 cells could be regulated in NA-treated RAW264.7 cells.

3.3. Effects of NA on Phagocytosis Ability of RAW264.7 Cells. Endocytosis is the cellular process which helps macrophages exert their immune function by degrading small molecules, so it can be used as an indicator to evaluate the immune effect of macrophages [23]. To determine this activity, it demonstrated the ability of treated cells to intake neutral red. Data (see Figure 3) showed that RAW264.7 cells treated with

Table 1: Primers nomenclatures and sequences used for qRT-PCR.

Name	Species	Sense	Antisense
iNOS	Mouse	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT
TNF-α	Mouse	ATGTCTCAGCCTCTTCTCATTCCT	GGGTCTGGGCCATAGAACTGA
IL-1β	Mouse	TGTCCTGATGAGAGCATCC	AAGGTCCACGGGAAAGAC
IL-6	Mouse	CCCCAATTTCCAATGCTCTCC	CGCACTAGGTTTGCCGAGTA
GAPDH	Mouse	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT

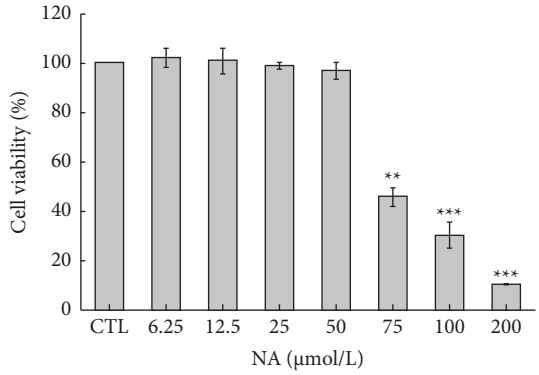

Figure 1: Effect of different concentrations of NA on the viability of RAW264.7 macrophage cells. RAW264.7 cells (1.0×10^5 cells/well) were treated with NA using a series of concentrations (6.25–200 μmol/L) in 96-well flat-bottomed microplates for 24 h. Only medium was added to the control group (CTL). Data are shown as the mean ± S.D. of three independent experiments. $^{**}p < 0.01$ or $^{***}p < 0.001$ vs. the control group.

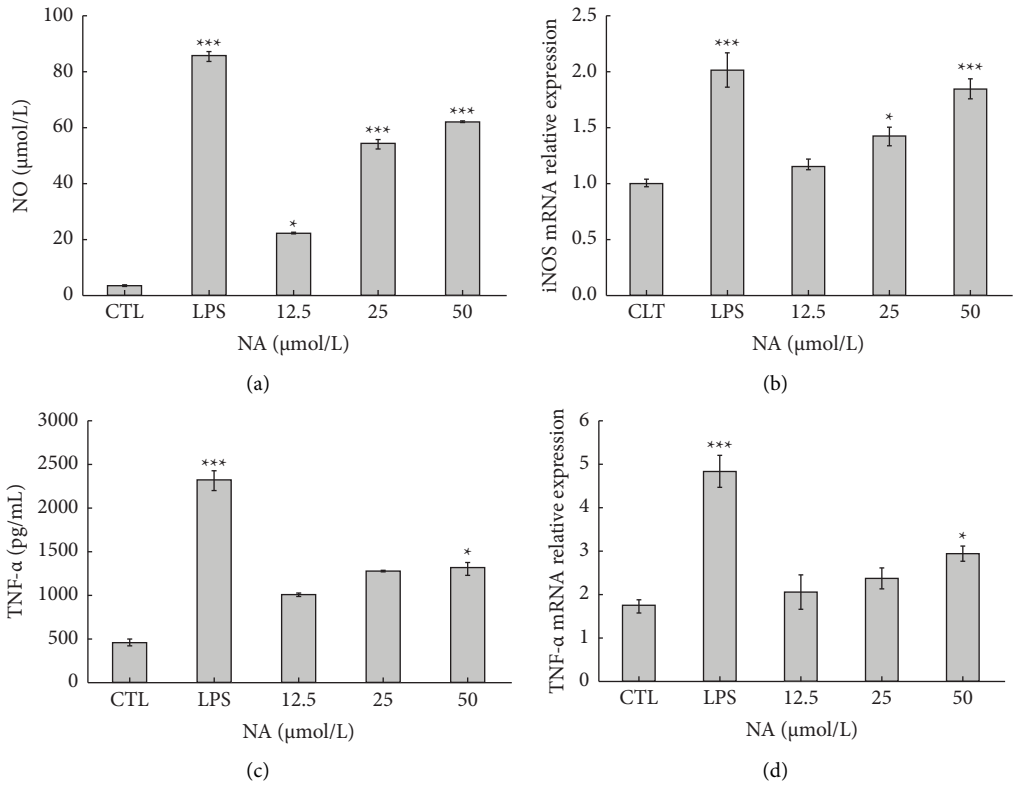

Figure 2: Continued.

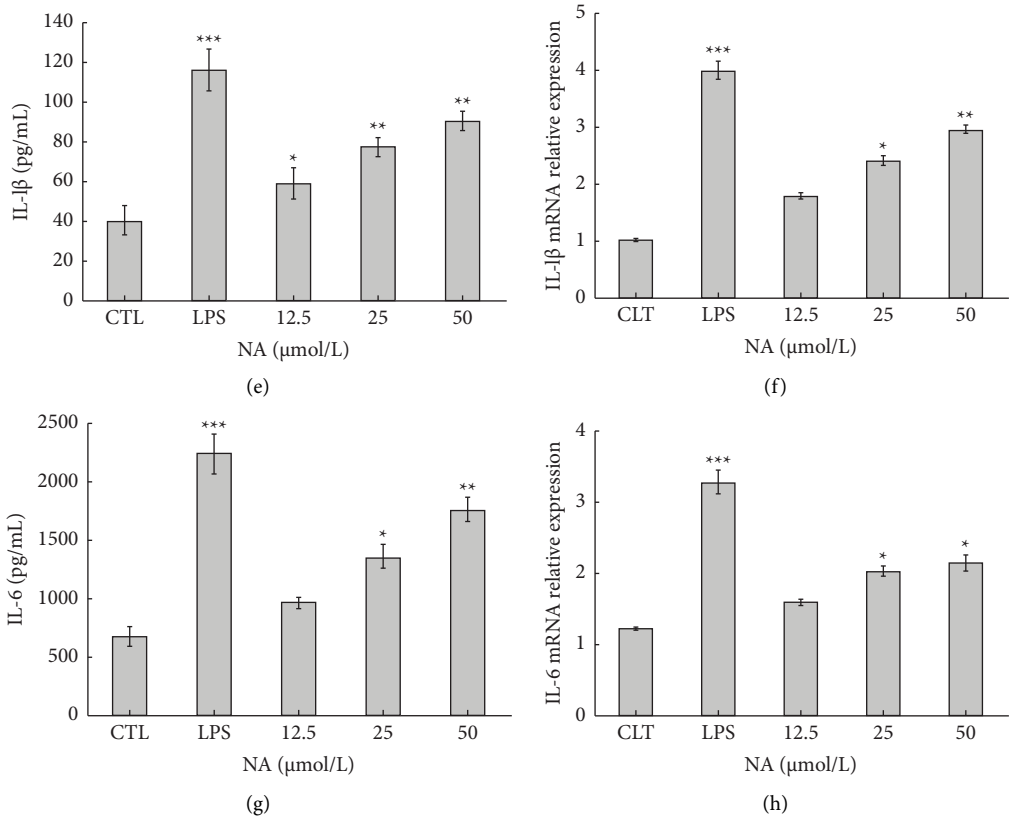

Figure 2: Effect of NA on cytokines, NO content, and gene expression in RAW264.7 macrophage cells. RAW264.7 cells were treated with 12.5, 25, and 50 μmol/L NA or 1 μg/mL LPS. The content NO (a) and mRNA levels of iNOS (b); content (c) and mRNA levels (d) of TNF-α; content (e) and mRNA levels (f) of IL-1β; content (g) and mRNA levels of IL-6 (h). Data are shown as the mean ± S.D. of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, or $^{***}p < 0.001$ vs. the control group.

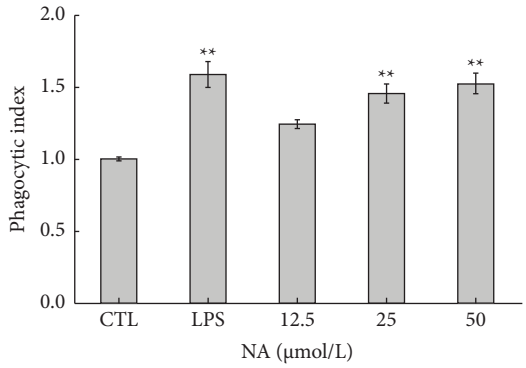

Figure 3: Effect of NA on cell phagocytosis in RAW264.7 macrophage cells. RAW264.7 cells (1.0×10^5 cells/well) were treated using three NA concentrations (12.5, 25, and 50 μmol/mL) or with LPS (1 μg/mL) in 96-well flat-bottom plates for 24 h. The supernatant was discarded for the measurement of macrophage cell phagocytosis. Data are shown as the mean ± S.D. of three independent experiments. $^{*}p < 0.05$ or $^{**}p < 0.01$ vs. the control group.

25 and 50 μmol/L NA had a significantly higher ability to incorporate neutral red than control ones ($p < 0.05$) but lower than that of the LPS-treated group ($p < 0.05$), which indicates that NA could promote the phagocytosis ability of macrophages.

3.4. Effects of NA on ROS Generation in RAW264.7 Cells. ROS is a kind of natural by-products of normal oxygen metabolism in organisms and plays an important role in immune regulation [24]. Results (see Figure 4) showed that ROS secretion in LPS-treated RAW264.7 cells was

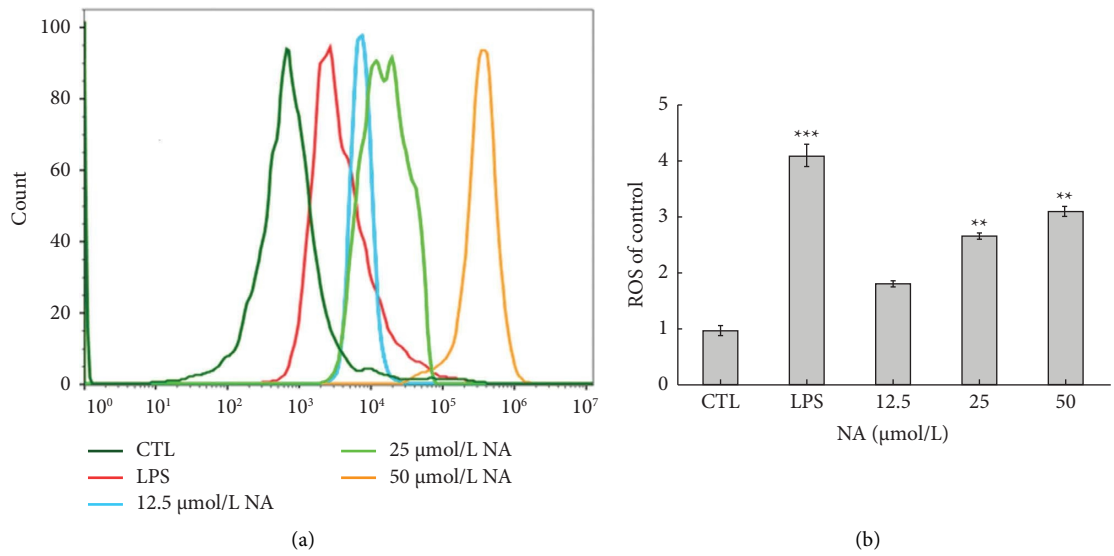

Figure 4: Effect of NA on ROS generation in RAW264.7 macrophage cells. (a) RAW264.7 cells (2×10^6 cells/well) were treated using three NA concentrations (12.5, 25, and 50 μmol/mL) or with LPS (1 μg/mL) in 6-well flat-bottom plates for 24 h. ROS generation was detected by flow cytometry. (b) Relative fluorescence intensity. Data are shown as the mean ± S.D. of three independent experiments. $^*p < 0.05$ or $^{**}p < 0.01$ vs. the control group.

significantly increased compared with that of the control group ($p < 0.05$) detected by flow cytometry. Similarly, ROS secretion in cells treated with different concentrations of NA increased significantly ($p < 0.05$) and showed a concentration-dependent trend compared to the control group, but the increase was less effective than with the LPS-treated group ($p < 0.05$), which indicates that NA could regulate the generation of ROS in RAW264.7 cells.

3.5. Effects of NA on the NF-κB Pathway in RAW264.7 Cells. To explore the mechanism of NA in regulating the immune activity of macrophages, the expression levels of TLR4, IκBα, and p-IκBα and the nuclear translocation of *p*65 in RAW264.7 cells were detected by western blotting and immunofluorescence [25, 26]. The cell stimulation with LPS triggers the regulation of TLR4 cellular immune responses and activates the NF-κB signaling pathway which enhances the secretion of proinflammatory factors [27, 28]. As presented in Figure 5, the incubation of RAW264.7 cells with LPS activated the expression of TLR4, and the ratio of p-IκBα/IκBα was significantly increased ($p < 0.05$) when p-IκBα was dephosphorylated compared with that of the control group. Meanwhile, the incubation of RAW264.7 cells with NA also activated the NF-κB signaling pathway. However, the expression of TLR4 was decreased when RAW264.7 cells were treated with various concentrations of NA, and the ratio of p-IκBα/IκBα was significantly increased ($p < 0.05$) when IκBα was phosphorylated, especially under the concentrations of 25 μmol/L and 50 μmol/L NA, compared with that of the LPS-treated group.

The upstream regulatory elements of targeted genes, which can be bound by transcription factors, will be exposed when p-IκBα is dephosphorylated. It will become easy for *p*65 to bind with the exposed regulatory elements of target genes after *p*65 is translocated into the nucleus from the cytoplasm; then, the transcription of the targeted gene will start, which will trigger the uncontrolled release of inflammatory factors. The presented results (Figure 5(d)) showed that the nuclear translocation of *p*65 was significantly increased when RAW264.7 cells were treated with LPS, while the nuclear translocation of *p*65 was decreased when RAW264.7 cells were treated with different concentrations of NA. Therefore, it could be suggested that the decrease in the expression of inflammatory factor genes delayed the release of inflammatory factors in NA treated RAW264.7 cells. In conclusion, NA could exert its immunomodulatory effects by regulating the expression of NF-κB signaling pathway-related proteins in RAW264.7 cells.

4. Discussion

Macrophages represent the cellular barrier of the innate immune system since they are responsible for phagocytizing bacteria, dead cells, debris, tumor cells, foreign materials etc. Besides, they secrete proinflammatory and antimicrobial mediators; therefore, their role as immunomodulators is crucial for maintaining the homeostasis of the body. When macrophages are stimulated by external factors, they can rapidly activate different signaling pathways and increase immune responses by releasing various cytokines [29–31]. That is the reason why macrophages are the preferred cell lines to evaluate agents involved in immunomodulation and oxidative stress pathways.

Although the excessive production of NO and proinflammatory cytokines during inflammation leads to DNA damage and mutations in healthy cells [32], NO, as an important bioactive molecule in vivo, is also an important mediator in the regulation of the immune response [33]. Similarly, proinflammatory cytokines, such as TNF-α, IL-1β,

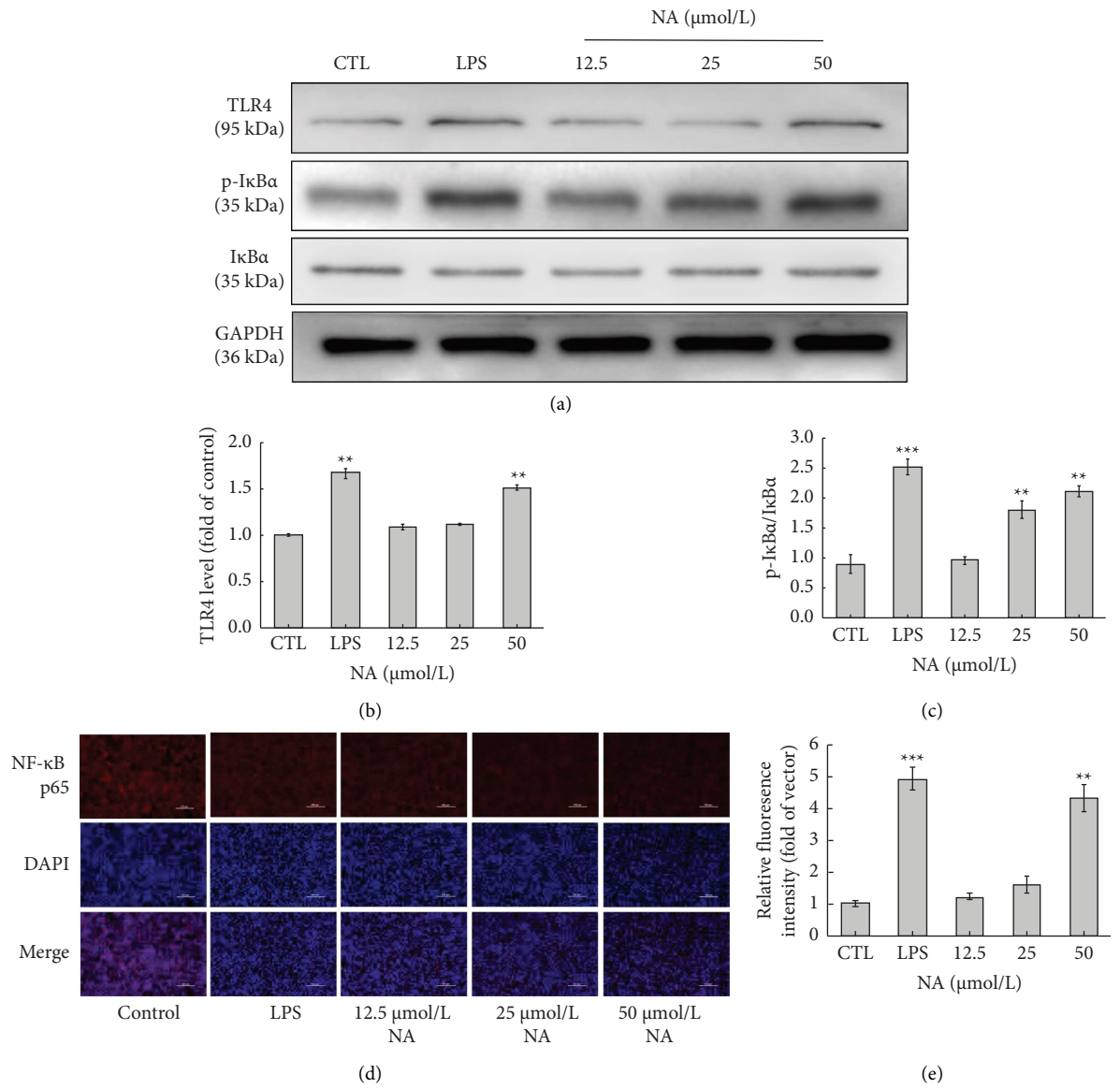

Figure 5: Effects of NA on the expression of NF-κB pathway-related proteins and nuclear translocation of *p*65 in RAW264.7 cells. (a) RAW264.7 cells (2×10^6 cells/well) were treated with three NA concentrations (12.5, 25, and 50 μmol/L) or with LPS (1 μg/mL) in 6-well flat-bottom plates for 24 h. CTL; control, LPS; lipopolysaccharide. Western blots showed the expression of the representative proteins. The bar graphs, respectively, showed density ratios of TLR4 (b) and p-IκBα to total IκBα (c) as analyzed using ImageJ software. Nuclear NF-κB *p*65 translocation (d) was observed by CLSM (×200, scale bar = 100 μm). Relative fluorescence intensity (e). Data are shown as the mean ± S.D. of three independent experiments. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. the control group.

and IL-6, induce severe inflammatory immune responses in tissue cells. Among them, NO, a highly reactive free radical, is usually produced in macrophages by the conversion of nitric oxide synthase (iNOS) for killing virus-infected cells and tumor cells, invading microorganisms and parasites, thus protecting the organism from external adverse reactions and incursion [34]. IL-1β is involved in the promotion of cell growth, differentiation, and apoptosis and triggers inflammation [35]. IL-6 prompts lymphocyte differentiation and proliferation, and it is also responsible for the pathogenic microorganisms' removal, prevents cellular injuries due to endotoxin exposition, and minimizes cell death by the downregulation of inflammatory mediators' expression [36]. TNF-α is commonly used as a drug target for the treatment of inflammatory diseases since it is in charge of the promotion of the expression of vascular endothelial cells at the site of infection, stimulates the secretion of growth factors and chemokines, and induces the elimination of bacteria and white blood cells together at the inflammation site and serum [37, 38]. These proinflammatory cytokines are crucial agents as immunomodulators in the inflammatory response of tissue cells. ROS acts as an important signaling molecule for immune regulation in macrophages, and its production is closely related to the NF-κB signaling pathway [39]. It is produced by activated macrophages and can trigger the inhibitory IκB kinase,

leading to the phosphorylation of IκB and to the formation of heterodimer NF-κB which will cross the nuclear membrane to bind the genomic region and promote the generation of inflammatory factors, thus playing an essential role in the regulation of the immune signal transduction network [40]. In this study, it was demonstrated that the production of cytokines and NO, the expression of TNF-α, IL-1β, IL-6, and iNOS on transcriptional levels, and ROS fluorescence were increased significantly in NA-treated macrophages. Therefore, NA plays an important regulatory role in the enhancement of immune activity in macrophages.

As explained above, endocytosis is a cellular mechanism involved in absorbing macromolecular substances from the extracellular medium, and so it represents a universal physiological phenomenon in the protection of eukaryote cells. Macrophages are widely involved in the body's immune response, immune effects, and immune regulation and can maintain the stability of the body's environment. The activation of macrophages can increase the body's resistance to infection, thus enhancing the body's nonspecific immune function, and their endocytosis is closely related to the immune response, cell signaling, or the metabolic balance of cells and tissues [41, 42]. In this study, by detecting the uptake capacity of macrophages to neutral red, it was found that NA enhances this uptake ability which may indicate that NA can exert its immune function by increasing the endocytosis ability of macrophages.

TLR4 is a transmembrane protein, and the LPS-activated TLR4 induces downstream myeloid differentiation factor-(MyD88-) dependent signal transduction; thereby, the NF-κB signaling pathway is activated [43, 44]. NF-κB, a ubiquitous transcription factor in eukaryotes, is key to host defense since it regulates the expression of multiple inflammatory and immune genes [40]. In the cytoplasm of unstimulated macrophages, NF-κB binds to inhibitory protein (IκB) to form an inactive trimer ($p50$-$p65$-IκBα). However, in stimulated macrophages, IκB kinase is activated and catalyzes the phosphorylation of IκB, leading to the ubiquitination of IκBα, which is then degraded, resulting in IκBα degradation and NF-κB activation. Two subunits of $p50$-$p65$ change from the inactive status to active status, and they are transferred from the cytoplasm to the nucleus (especially $p65$, which is first NF-κ found to be phosphorylated after IκBα degradation B subunits), where they bind to the upstream homologous regulatory regions of multiple genes [45, 46]. In this study, the capacity of NA to promote the expression of TLR4 protein, the phosphorylation of IκBα, and the translocation of $p65$ into the nucleus was observed. Therefore, RAW264.7 cells seem to respond to NA through a classic NF-κB signaling pathway.

5. Conclusions

This study has evaluated the immunomodulatory effect of NA on RAW 264.7 macrophages. The obtained results point to NA as a potential modulator of the immune activity of RAW264.7 macrophages, and the mechanism involved in this ability seems to be exerted through the regulation of the TLR4/NF-κB signal pathway. Therefore, the outcomes obtained in the present work suggested that NA has great potential in immune regulation, which provides a theoretical basis for the development of efficient functional NA-based products. To the best of our knowledge, this is the first study that NA has the documented immunomodulatory potential in a cellular model and the underlying molecular mechanisms of its effects.

Data Availability

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Sheng-Nan Yuan and Cai-Yun Feng contributed equally to this work.

Acknowledgments

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Supplementary Materials

The graphical abstract includes the immunomodulatory potential of nervonic acid (NA) on the growth and proliferation of murine RAW264.7 macrophages. (*Supplementary Materials*)

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