Two Immunoregulatory Peptides with Antioxidant Activity from Tick Salivary Glands*†‡§1, 2

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Ticks are blood-feeding arthropods that may secrete immunosuppressive molecules, which inhibit host inflammatory and immune responses and provide survival advantages to pathogens at tick bleeding sites in hosts. In the current work, two families of immunoregulatory peptides, hyalomin-A and -B, were first identified from salivary glands of hard tick Hyalomma asiaticum asiaticum. Three copies of hyalomin-A are encoded by an identical gene and released from the same protein precursor. Both hyalomin-A and -B can exert significant anti-inflammatory functions, either by directly inhibiting host secretion of inflammatory factors such as tumor necrosis factor-α, monocyte chemotactic protein-1, and interferon-γ or by indirectly increasing the secretion of immunosuppressive cytokine of interleukin-10. Hyalomin-A and -B were both found to potently scavenge free radical in vitro in a rapid manner and inhibited adjuvant-induced inflammation in mouse models in vivo. The JNK/SAPK subgroup of the MAPK signaling pathway was involved in such immunoregulatory functions of hyalomin-A and -B. These results showed that immunoregulatory peptides of tick salivary glands suppress host inflammatory response by modulating cytokine secretion and detoxifying reactive oxygen species.

Ticks are parasites that feed on the blood of their hosts. Their salivary glands can secrete various immunomodulatory molecules to inhibit host inflammatory and immune responses (1–7). Ticks are second only to mosquitoes as vectors of disease-causing agents to humans and are the most important arthropod capable of transmitting pathogens to other animal species (8–13). Medically important tick-borne diseases include Lyme disease (14, 15), tick-borne encephalitis, granulocytic ehrlichiosis (8–13), babesiosis (2, 7, 12), and Crimean-Congo hemorrhagic fever, which occurs sporadically throughout much of Africa, Asia, and Europe and results in an ~30% fatality rate (16–19).

Many reports have showed that proteins extracted from saliva and salivary glands of several ticks may inhibit humoral immunity and the B and T cell responses to tick-transmitted pathogens (1, 20–26) and, furthermore, may facilitate the pathogen transmission and infection to their hosts by down-regulating host immunity (27–34), altering blood flow (30, 35, 36) and inhibiting inflammation (37–41). For example, tick saliva-enhanced transmission has also been demonstrated for several viral and bacterial pathogens, including tick-borne encephalitis virus and Borrelia burgdorferi spp., the causative agent of Lyme disease (11, 22, 38). It has been suggested that the anti-inflammation mechanism is well conserved among tick species, in spite of the presence of the marked molecular polymorphism in the protein profile of salivary glands from individual ticks (11). Much of the literature has shown that saliva and salivary gland extracts of ticks can inhibit host inflammatory responses by modulating its cytokine secretion or directly blocking cytokine activities via extract-cytokine interactions (1, 20, 24, 26, 27, 32). Some cytokine-binding peptides and proteins have been identified from tick salivary glands, but so far no regulatory peptides affecting cytokine secretions have ever been characterized (27, 39–41). In the current work, two families of immunoregulatory peptides, hyalomin-A and -B, were identified from salivary glands of hard tick Hyalomma asiaticum. Hyalomin-A and -B were shown to suppress host inflammatory response by modulating cytokine secretion and detoxifying reactive oxygen species.

**EXPERIMENTAL PROCEDURES**

**Salivary Gland Dissection**—Partially fed adult hard ticks (H. asiaticum) of both sexes (n = 2300) were collected from the Xinjiang Province of China (May to July, detached from camels). The ticks were glued to the bottom of a Petri dish and placed on ice for 20 min. They were then incised along the dorsal-lateral margin, and the dorsal integument was removed. The salivary gland was isolated and transferred into 0.1M phosphate-buffered solution (PBS) containing protease inhibitor mixture (Sigma; P2714), pH 6.0, and kept at −20 °C.

**Peptide Purification**—The salivary glands from ticks were homogenized using a glass homogenizer in 0.1 M PBS, pH 6.0,
containing a protease inhibitor mixture. The homogenate was centrifuged at 10,000 rpm. The supernatant (10 ml) was applied to a Sephadex G-50 (Superfine; Amersham Biosciences; 2.6 × 100 cm) gel filtration column equilibrated with 0.1 M PBS, pH 6.0. Elution was performed with the same buffer, collecting fractions of 3.0 ml, whereas the absorbance was monitored at 280 nm. The effects of eluted fractions on interferon-γ (IFN-γ) secretion were determined as described below. The fractions with the desired activity were pooled (~20 ml), lyophilized, resuspended in 2 ml of 0.1 M PBS, and purified further by C18 RP-HPLC (Hypersil BDS C18, 30 × 0.46 cm) as illustrated in supplemental Fig. S1B.

Structural Analysis—Purified peptides were subjected to complete peptide sequencing by Edman degradation on an Applied Biosystems pulsed liquid phase sequencer (model 491). Mass spectrometry analysis was performed by using a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS) AXIMA CFR (Kratos Analytical) in positive ion and linear mode. The operating parameters were as follows: the ion acceleration voltage was 20 kV, and the accumulating time of single scanning was 50 s, using polypeptide mass standard (Kratos Analytical) as an external standard. The accuracy of mass determinations was within 0.01%.

Construction and Screening of cDNA Library—mRNAs were prepared from the total RNA of *H. asiaticum* salivary glands by oligo(dT) cellulose chromatography. A cDNA library was constructed using a SMART™ PCR cDNA synthesis kit (Clontech, Palo Alto, CA) as described (34). The first strand was synthesized by using cDNA 3′ SMART CDS Primer II A and SMART II oligonucleotide provided by the kit. The second strand was amplified using Advantage polymerase by 5′ PCR primer II A provided by kit. All of the PCR conditions are according to the manufacturer’s instructions. A directional cDNA library was constructed with a plasmid cloning kit (SuperScript™ plasmid system; Invitrogen) according to the instructions of the manufacturer, producing a library of ∼2.3 × 10^5 independent colonies.

Two pairs of oligonucleotide primers, S1 (5′-CARACNNCC-NXGNACNATNGGN-3′, in the sense direction, where N is A, T, C, or G, R is A or G, and X is A or C)/Primer IIA (in the antisense direction) and S2 (5′-ACNCTNXGNACNACNGAY-3′, in the sense direction, where Y is T or C)/Primer IIA (in the antisense direction) were used to screen hyalomin-A and -B, respectively. S1 and S2 are specific primers designed according to the amino acid sequence determined by Edman degradation. All of the oligonucleotide primers for PCR were prepared with a DNA synthesizer (model 381A; Applied Biosystems). The PCR conditions were: 2 min at 94 °C, followed by 30 cycles of 10 s at 92 °C, 30 s at 50 °C, and 40 s at 72 °C. DNA sequencing was performed on an Applied Biosystems DNA sequencer (model ABI PRISM 377).

Stimulation of Rat Spleenocytes and Cytokine Assays—A suspension of spleenocytes from Wistar rats in RPMI 1640 medium (Invitrogen) supplemented with 5% fetal bovine serum and 100 units/ml penicillin and streptomycin was seeded to the wells of a 96-well plate. A total of 6 × 10^6 cells (100 μl/well) were incubated at 37 °C and 3.5% CO₂, 20-μl samples dissolved in RPMI 1640 medium with different concentrations and lipopolysaccharide (final concentration, 2 μg/ml; Sigma) were co-cultured with splenocytes. Supernatants of the cultures were harvested after 48 h of cultivation and stored at −70 °C. All of the combinations were set up in triplicate. Cytokines assays for IL-10, IFN-γ, and MCP-1 were detected using antibody sandwich enzyme-linked immunosorbent assays using kits from Adlit- teram Diagnostic Laboratories, Inc. according the manufacturer’s instructions.

Free Radical Scavenging Activity—The free radical scavenging activity of hyalomin-A1/B was assayed by measuring the reduction of cationic radical 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) according to the manufacturer’s instructions of the kit GMS10114.4 (Genmed Sciences, Inc., Shanghai Branch). The total formation of products (i.e. the reduced form of ABTS and the purple hyalomin-A1/B modification) and the total consumption of ABTS radical during the initial, rapid reaction phase were determined by linear regression analysis. The concentrations of ABTS and ABTS⁺ were calculated by using ε_{340} = 4.8 × 10^3 M⁻¹ cm⁻¹ and ε_{415} = 3.6 × 10^4 M⁻¹ cm⁻¹, respectively (18, 19). The purple hyalomin-A1/B modification was monitored at 550 nm (18). The purple end product of hyalomin-A1/B with ABTS⁺ was purified by a Sephadex G-25 Fine column (Volume, 30 × 1.6 cm) eluted with 2 mM NH₄HCO₃, pH 8.5, at a flow rate of 0.3 ml/min, and 0.3-ml fractions were collected manually (supplemental Fig. S2). The absorbances at 280, 340, 415, and 550 nm were monitored. The fraction containing purple hyalomin-A1/B modification was purified further by RP-HPLC using C18 column and then subject to MALDI-TOF-MS analysis.

Effects of Hyalomin-A1/B on Proliferation of Mouse Spleenocytes in Vitro—Spleenocytes from Kunning mouse were harvested and cultured in RPMI 1640 medium supplemented with 5% fetal calf serum, and 1.5 × 10⁶ cells/0.2 ml were plated in a 96-well plate. Tested sample hyalomin-A1/B was added at final concentrations of 5, 10, 20, 40, and 80 μg/ml in the presence of 4 μg/ml concanavalin A. After a 44-h incubation at 37 °C in a humidified 5% CO₂ atmosphere, 20 μl of 3-(4–5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (5 mg/ml) was added to each well for a 4-h incubation. Finally, each well was added 100 μl of acidified isopropylalcohol, and the cells were homogenized for at least 10 min to fully dissolve the colored material. The absorbance at 570 nm was measured in an enzyme-linked immunosorbent assay reader.

Macrophage Cell Proliferation and Viability Measurement—Raw 264.7 murine macrophage cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; catalog number 11960-044; Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 units/ml of streptomycin in a humidified 5% CO₂ atmosphere at 37 °C. 2 × 10⁵ raw 264.7 murine macrophage cells/well (180 μl) were plated into a 96-well plate. After overnight incubation, raw 264.7 murine macrophage cells were adhered to the plate, and then 20 μl of tested samples dissolved in DMEM were added to the wells for 20 and 44 h of incubation, using the same volume of DMEM as blank control. At the end of incubation, 20 μl of 3-(4–5-di methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (5 mg/ml) was added to each well, and the cells were further incubated for 4 h at 37 °C. The cells were solved in 200 μl of
Me$_2$SO, and the absorbance at 570 nm was measured in an enzyme-linked immunosorbent assay reader. The viability of the treated group was expressed as the percentage of the control group, which was assumed to be 100%.

Western Blot Analysis—Raw 264.7 murine macrophage cells (1/H11003 10$^6$/well) were plated and adhered to a 24-well culture plate. The cells were then transferred to serum-free DMEM for an 18-h incubation. The cells were pretreated by peptide samples of various concentrations (2, 4, and 8/H9262 g/ml) or blank for 1 h before the addition of lipopolysaccharide (LPS) (1/H9262 g/ml). After incubation for 15 min, the cells were collected by centrifugation and washed twice with ice-cold PBS. The washed cell pellets were resuspended in 150/H9262 l of extraction lysis buffer (50 mM Tris-HCl, pH 7.4; 1% Nonidet P-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM phenylmethylsulfonyl fluoride; 1 g/ml each of aprotinin, leupeptin, and pepstatin; 1 mM sodium orthovanadate; and 1 mM NaF) and incubated for 30 min at 4 °C. Cell debris was removed by centrifugation, followed by quick freezing of the supernatants. The protein concentration was determined using the Bradford protein assay. Forty micrograms of cellular protein from treated and untreated cell extracts were separated on a 12% SDS-PAGE gel and electroblotted onto a polyvinylidene difluoride membrane. The immunoblot was incubated with blocking solution (5% skim milk) at room temperature for 3 h, followed by incubation overnight with a primary antibody against the phosphorylated forms of Erk1/2, SAPK/JNK, or p38 MAPK at 4 °C, respectively. The blots were washed three times with Tween 20-Tris-buffered saline (TBST) and incubated with a 1:1000 dilution of horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. The blots were again washed three times with TBST and then developed by enhanced chemiluminescence (Tiangen Biotech).

For the Western blotting of pc-Jun, p-ATF-2, and proliferating cell nuclear antigen, cellular nuclear extracts were prepared as described below. The cells were washed twice with ice-cold PBS and resuspended in hypotonic buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1.5 mM MgCl$_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 mM NaF, and 1 mM Na$_3$VO$_4$). After the addition of 0.5% (v/v) Nonidet P-40 and vigorous vortexing for 10 s, the nuclei were pelleted by centrifugation (12,000 g for 1 min at 4 °C). The collected nucleus pellet was resuspended in extraction buffer (20 mM HEPES, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM NaF, and 1 mM Na$_3$VO$_4$). After centrifugation (12,000 × g for 10 min at 4 °C), the supernatant was collected and stored at −70 °C. Twenty micrograms of cellular protein from treated and untreated cell extracts was electroblotted onto a polyvinylidene difluoride membrane following separation on a 12% SDS-PAGE gel. The procedure of the immunoblotting was the same as above.

Effects of Hyalomin-A1/B1 on Adjuvant-induced Arthritis in Mice—20 μl of vehicle (saline) or Freund’s complete adjuvant (Sigma) was administered into the plantar surface of the right hind paw of male Kunming mice (12/group). The basal footpad thickness of each mouse was measured with a vernier caliper at the beginning of the experiment. Hyalomin-A1/B1 (1.25, 2.5, and 5 mg/kg) were administered into the muscles of hind leg root alternatively from day 1 after injection of Freund’s complete adjuvant to day 14. Control groups of mice received the same volume of saline (vehicle). As an index of inflammation, right hind paw thickness was measured with a vernier caliper at day 1, 5, 9, 11, 15, and 21, respectively. The experimental pro-
Peptide Synthesis—Hyalomin-A1 and -B1 were synthesized by solid phase synthesis on an Applied Biosystems model 433A peptide synthesizer. After the cleavage and deprotection of side chain, the crude synthetic peptide was purified on a Vydac C18 RP-HPLC column (25 × 1 cm), eluting at a flow rate of 1 ml/min by a linear gradient of acetonitrile in 0.1% trifluoroacetic acid in water. The identity of the peptide was confirmed by automated Edman degradation with a protein sequencer and mass spectrometry analysis.

Statistics—Data analysis was performed using the Statistical Package for Social Science (SPSS 11.5). The statistical analysis data are presented as means ± S.D.

RESULTS

Purification and Structural Analysis of Peptides from Tick Salivary Gland Extracts—The crude extracts from the tick salivary glands have been divided into several factions by Sephadex G-50 gel filtration, and the fraction indicated by a bar was found to inhibit IFN-γ secretion as illustrated in supplemental Fig. S1, top panel. The sample with IFN-γ secretion inhibitory activity was pooled and further purified by C18 RP-HPLC. Two peptides were purified from this step as indicated in supplemental Fig. S1, bottom panel. They are named hyalomin-A1 and -B1, respectively. The complete amino acid sequences by Edman degradation of hyalomin-A1 and -B1 were determined as QTP-RTIGPPYT and TLRTTTGYWTTVEKGNFGPAANSTEKGNR-PYGR, respectively. The observed [M+H]⁺ values from MALDI-TOF-MS were 1231.19 and 3688.27 (supplemental Fig. S3), which matched well with the calculated [M+H]⁺ of 1231.39 and 3688.00, respectively. The amino acid sequences of hyalomin-A1 and -B1 were further confirmed by the cDNA cloning as described below.

cDNA Cloning—As illustrated in Fig. 1A, a cDNA of 801 bp (GenBank™ accession GU828034) was cloned from the cDNA library of salivary glands of tick *Hyalomma asiaticum asiaticum*. This cDNA encodes a precursor protein composed of 221 amino acid residues. It was found that both hyalomin-A1 and -B1 share the same 221-amino acid precursor.
protein. In the sequences of the 221-amino acid precursor, there are three copies of hyalomin-A1 and three homologs of hyalomin-B1 (B1–B3; Fig. 1B). Hyalomin-A1 is composed of 11 amino acid residues. Both hyalomin-B1 and -B2 are composed of 34 residues, whereas hyalomin-B3 is composed of 32 residues. All of the sequences of mature hyalomin-A and -B peptides are flanked by the possible enzymatic processing site of -RR- (Fig. 1A).

Effects on Cytokine Secretion—We tested the effects of synthesized hyalomin-A1 and -B1 on IL-10, IFN-γ, MCP-1, and tumor necrosis factor-α secretion induced by LPS in mouse splenocytes. Four concentrations (0, 2, 4, and 8 µg/ml) of hyalomin-A1 and -B1 were used. LPS alone could induce IL-10, IFN-γ, and MCP-1 secretion. As illustrated in Fig. 2, both hyalomin-A1 and -B1 could markedly inhibit secretions of IFN-γ, MCP-1, and tumor necrosis factor-α induced by LPS in a dose-dependent manner (Fig. 2, A, C, and D). The secretion of IL-10 could be significantly increased by hyalomin-B1. Hyalomin-A1 could only slightly increase the IL-10 secretion even at the concentration of 8 µg/ml (Fig. 2B).

Free Radical Scavenging Activity—Because of the good performance with respect to the stability, maneuverability, and reproducibility, the ABTS+ radical scavenging assay was commonly used to evaluate the antioxidant capacity of biomolecules (33). The decrease in absorbance at 734 nm was monitored at 1-min intervals for 30 min. The absorbance of control and samples was monitored simultaneously. Both hyalomin-A1 and -B1 could react with ABTS+ and convert it into colorless compounds. As illustrated in Fig. 3, hyalomin-A1 and -B1 could rapidly scavenge ABTS+ in a time-dependent manner. The overlaid scans of the solution were performed to monitor the decrease of the ABTS radical-specific 415- and 735-nm peaks and a concomitant increase of the ABTS-specific peak at 340 nm. Although the ABTS+ displayed a slender auto-scavenging activity (Fig. 3A), both hyalomin-A1 and -B1 could significantly catalyze this process (Fig. 3, C and D), and the catalysis rate is even much faster than that of commercial antioxidant factor (butylated hydroxytoluene) (Fig. 3B). The reduction processes by hyalomin-A1 and -B1 were two-phase reactions. An initial faster phase over several seconds was followed by a second slower phase that was still ongoing after 10 min (Fig. 3). Most of the ABTS radical was reduced after 5 s. Two end products of reduced ABTS were found, represented by the 340-nm peak and a novel peak at 550 nm (Fig. 3, C and D, insets). No product with the absorbance at 550 nm was found in the butylated hydroxytoluene-ABTS react system (Fig. 3B), indicating that butylated hydroxytoluene might have a different radical scavenging mechanism from these tick peptides.

It has been proved that phenols can react with ABTS radicals to form purple compounds with a broad absorbance of ~550 nm (32, 39, 40). Among the essential amino acids, only tyrosine contains phenol side chain. One and two tyrosine residues are found in hyalomin-A1 (Tyr10) and -B1 (Tyr8 and Tyr32), respectively, and they possibly react with ABTS radicals to form hyalomin-A1/B1-ABTS purple adducts. The observed masses of hyalomin-A1 and -B1-ABTS purple adducts were 1498.1 and 3954.9, respectively, which were two mass units less than the total masses of hyalomin-A1/B1 plus ABTS free radical (1500.2 and 3956.8). The two mass units account for the oxidized tyrosine residue as confirmed by previous report (32). As listed in supplemental Table S1, Tyr10 and Tyr8 replacement in hyalomin-A1 and -B1 decreased their antioxidant capabilities extremely, whereas Tyr32 replacement in hyalomin-B1 had only a slight effect on the antioxidant capability, suggesting that Tyr32 did not take part in the reaction with ABTS radicals.

Effects on Cell Proliferation and Cell Viability—The effects of hyalomin-A1 and -B1 on cell proliferation were tested by coculturing with mouse splenocytes in RPMI 1640 medium supplemented with 5% fetal calf serum (supplemental Fig. S2). Five sample concentrations (5, 10, 20, 40, and 80 µg/ml) were used. Interestingly, all five concentrations of hyalomin-A1 could slightly (~20%) inhibit splenocyte proliferation in a dose-independent manner. Regarding the hyalomin-B1, low concentrations (5, 10, and 20 µg/ml) showed the same cell proliferation inhibitory effects as hyalomin-A1, whereas high concentrations (40 and 80 µg/ml) had no effect on splenocyte proliferation.

FIGURE 3. Antioxidant functions of hyalomin-A1 and -B1. ABTS+ was scavenged by hyalomin-A1 and -B1 in a time-dependent manner. Water (A), butylated hydroxytoluene (B), or hyalomin-A1 (C) and -B1 (D) was added to standard ABTS+ solutions, respectively, to a final concentration of 3 µM. The absorbance spectrum was read at 0 s, 2 s, 30 s, 2 min, 5 min, 10 min, and 20 min, and finally the end point scan was read immediately after the addition of sodium azide.
The effects of hyalomin-A1 and -B1 on cell viability were assayed by using Raw264.7 murine macrophages grown in DMEM supplemented with 10% fetal bovine serum and antibiotics. All of the tested concentrations (10, 20, 40, 80, and 160 µg/ml) of hyalomin-A1 and low concentrations (10 and 20 µg/ml) of hyalomin-B1 have little effect on the cell viability, but high concentrations (40, 80, and 160 µg/ml) of hyalomin-B1 could increase the cell viability by 10–20% (supplemental Fig. S2B).

**Influence on MAPK Signaling Pathway**—The MAPKs play important roles in the regulation of cell growth and differentiation and as well the control of cellular responses to proinflammatory cytokines and environmental stresses. The effects of hyalomin-A1 and -B1 on the LPS-stimulated phosphorylations of Erk1/2, SAPK/JNK, and p38 MAPK kinases in RAW 264.7 macrophage cells were examined using Western immunoblot analysis. As shown in Fig. 4, both hyalomin-A1 and -B1 inhibited LPS-induced degradation of IxB-α. Hyalomin-A1 could inhibit the LPS-induced activation of p38 MAPK, whereas hyalomin-B1 had no obvious effects on p38 MAPK. Neither hyalomin-A1 nor -B1 had a marked effect on the LPS-induced activation of Erk1/2 MAPK; however, with respect to the LPS-induced activation of JNK MAPK, both hyalomin-A1 and -B1 indeed showed significant suppression in a dose-dependent manner. The phosphorylations of JNK1 (p46) and JNK2 (p54) were blocked by both hyalomin-A1 and -B1, and especially, the LPS-induced phosphorylation of JNK1 could be completely blocked at sample concentrations above 8 µg/ml. To further confirm whether hyalomin-A1/B1 mainly act on JNK/SAPK signaling pathway, we examined the effects of hyalomin-A1 and -B1 on upstream and downstream kinases of JNK. It was found that the phosphorylations of MKK4 (the upstream kinase of JNK), c-Jun, and ATF-2 were all inhibited by hyalomin-A1/B1 in a dose-dependent manner.

**Inhibition of Adjuvant-induced Inflammation in Mouse**—Considering that both hyalomin-A1 and -B1 could inhibit proinflammatory cytokine secretion by acting on the JNK/SAPK signaling pathway and could enable free radical scavenging, Freund’s complete adjuvant-induced inflammation model was used to evaluate the potential anti-inflammatory ability of hyalomin-A1 and -B1. As illustrated in Fig. 5, they could inhibit hind paw inflammation in mouse in a dose-dependent manner. Such anti-inflammatory functions were present significantly after 9 days of administration. At the dose of 5 mg/kg of body weight, inflamma-
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In contrast to mouse cells, the majority of human cells were not affected by the addition of hyalomin-A1/B1. The addition of hyalomin-A1/B1 did not affect the proliferation of cells of BHK-21 cells. A statistically significant reduction in the proliferation of cells of BHK-21 cells could be observed for most of the hyalomin-A/B1 concentrations tested (supplemental Fig. S2A). Cell viability has not been affected by hyalomin-A1/B1, indicating that the immunoregulatory properties of hyalomin-A1/B1 are not related with cytotoxicity.

In addition to the investigation of antioxidant and anti-inflammatory functions at molecular and cell levels, adjuvant-induced inflammatory models in mice were also exploited to evaluate the roles that hyalomin-A1/B1 play in acute inflammation. A concentration-dependent inhibition of inflammation was observed. This further reveals the biological significance of these antioxidant peptides derived from tick salivary glands, depending on which of the ticks could suppress host inflammatory responses and successfully get a blood meal.

The current work identified two families of small peptides other than big proteins with antioxidant and anti-inflammatory activities from hard tick salivary glands. Also, the possible mechanisms underlying their immunoregulatory functions were fully discussed.

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