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A tick B-cell inhibitory protein from salivary glands of the hard tick, *Hyalomma asiaticum asiaticum*

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Abstract

Some studies done to date suggest that B-cell inhibitory factor occurred in tick saliva. In this study, a novel protein having B-cell inhibitory activity was purified and characterized from the salivary glands of the hard tick, *Hyalomma asiaticum asiaticum*. This protein was named B-cell inhibitory factor (BIF). The cDNA encoding BIF was cloned by cDNA library screening. The predicted protein from the cDNA sequence is composed of 138 amino acids including the mature BIF. No similarity was found by Blast search. The lipopolysaccharide-induced B-cell proliferation was inhibited by BIF. This is the first report of the identification and characterization of B-cell inhibitory protein from tick. The current study facilitates the study of identifying the interaction among tick, *Borrelia burgdorferi*, the causative agent of Lyme disease, and host.

Keywords: Tick; B-cell inhibitor; Salivary glands; Lyme disease; *Hyalomma asiaticum asiaticum*; *Borrelia burgdorferi*

Ticks are obligatory blood-feeding arthropods that secrete various immunomodulatory molecules to antagonize host inflammatory and immune responses [1,2]. They are second only to mosquitoes as vectors of disease-causing agents to humans, and the most important arthropod capable of transmitting pathogens to other animal species [3]. Medically important tick-borne diseases include tick-borne encephalitis, granulocytic ehrlichiosis, babesiosis, and Lyme disease, which is the most common vector-borne disease in Europe and North America [4,5]. It has been shown that saliva and salivary gland extracts from several ticks may affect humoral immunity and the B- and T-cell response to tick-transmitted pathogens [1,6], and furthermore, may downregulate both the innate and adaptive arms of host immunity, alter blood flow and inflammation, and directly promote pathogen growth in vitro [7–10]. Although the presence of marked molecular polymorphism has been demonstrated in the protein profile of salivary glands from individual ticks [11], these anti-inflammation phenomena appear to be well conserved among tick species [12]. Tick saliva-enhanced transmission has also been demonstrated for several viral and bacterial pathogens, including tick-borne encephalitis virus, *Borrelia burgdorferi* spp., and the causative agent of Lyme disease [13,14]. Taken together, salivary gland extract and secreted salivary proteins may facilitate the ability of tick to successfully feed on the host and aid

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on the immune evasion of pathogens by downregulating both the innate and adaptive arms of host immunity, and thus enhance pathogen transmission.

B lymphocytes play a crucial role in antimicrobial immunity. Immediately after the pathogen entry, circulating natural antibodies contribute to the effective elimination of most of the circulating antigens by rapid exotoxin neutralization and enhancing opsonization [15,16]. The following studies were carried out to identify and characterize the B-cell inhibitory factor from the hard tick, *Hyalomma asiaticum asiaticum*, and its immunosuppressive functions.

Materials and methods

Animals. Unfed and fed adult hard ticks of both sexes (*H. asiaticum asiaticum*), kept in the laboratory according to the method of Kaufman and Phillips [17,18], and were maintained at 26 °C and >90% humidity.

Salivary gland dissection. 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 excised and transferred into 0.1 M phosphate buffer solution, pH 6.0, and kept in the same solution at −20 °C.

Protein purification. The salivary glands from 200 g tick were homogenized by glass homogenizer in 0.1 M phosphate buffer solution, pH 6.0, containing protease inhibitor cocktail (Sigma). The homogenate

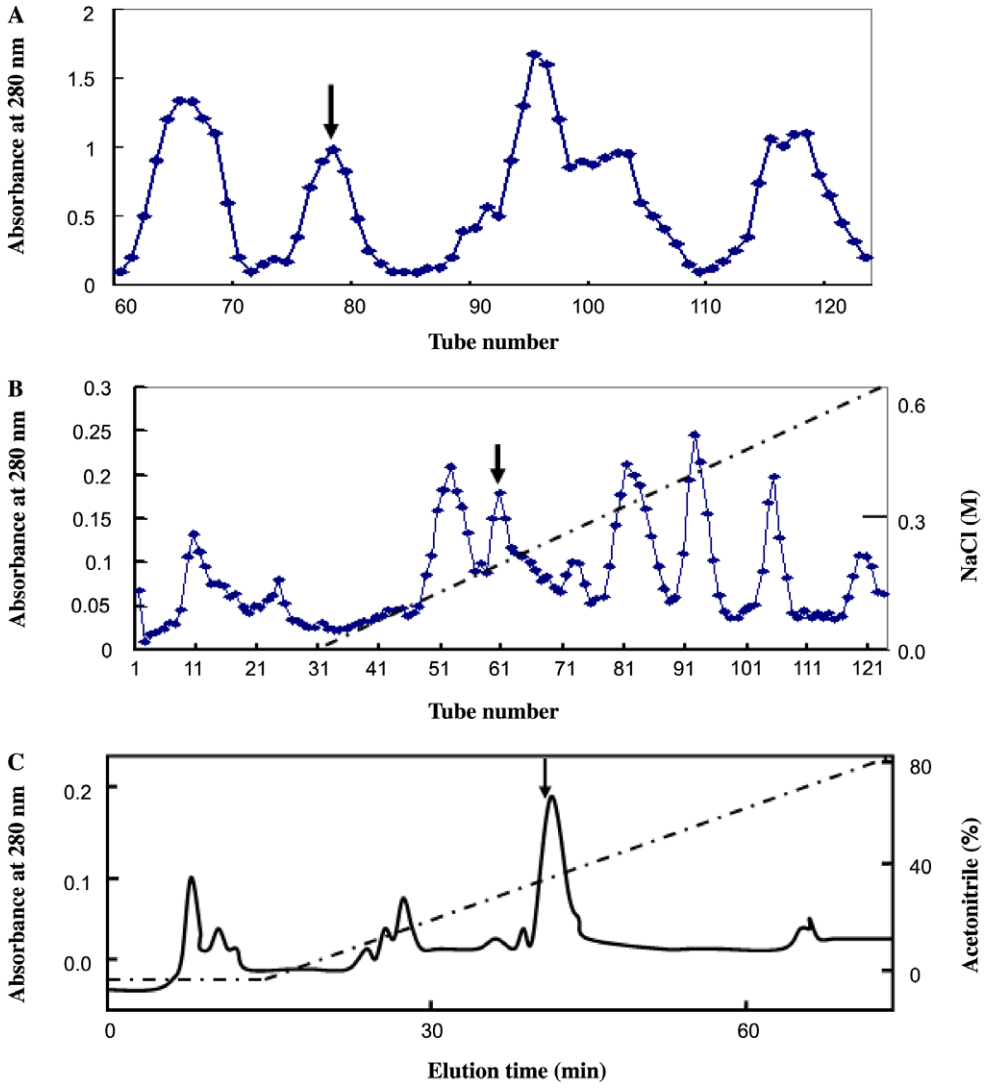

Fig. 1. Isolation of ixosin antimicrobial peptide from *I. sinensis* salivary glands. (A) Sephadex G-50 gel filtration of salivary gland extracts of *H. asiaticum asiaticum*. Salivary gland extract was applied to a Sephadex G-50 (Superfine, Amersham Biosciences, 2.6 × 100 cm) column equilibrated with 0.1 M phosphate buffer solution, pH 6.0. Elution was performed with the same buffer, collecting fractions of 3.0 ml (A). The peak (indicated by an arrow) with B-cell inhibitory activity from Sephadex G-50 was further purified on a DEAE-Sephadex A-50 anion exchange column (2.6 × 50 cm) equilibrated with 0.05 M Tris–HCl buffer (pH 7.8). The elution was performed at a flow rate of 30 ml/h with the indicated NaCl gradient in (B). The fractions containing B-cell inhibitory activity from DEAE Sephadex A-50 column were applied on a Hypersil BDS C_{18} RP-HPLC column (30 × 0.46 cm) equilibrated with 0.1% (v/v) trifluoroacetic acid/water. The elution was performed with the indicated gradient of acetonitrile in (C) at a flow rate of 0.7 ml/min, and the B-cell inhibitory protein is indicated by an arrow (C).