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LETTER

A C-type lectin from *Bothrops jararacussu* venom reprograms endothelial cell biology

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Abstract

Snake venoms are intricate mixtures of enzymes and bioactive factors that induce a range of detrimental effects in afflicted hosts. Certain Viperids, including *Bothrops jararacussu*, harbor C-type lectins (CTLs) known for their modulation of a variety of host cellular responses. In this study, we isolated and purified BjcuL, a CTL from *B. jararacussu* venom and investigated its impact on endothelial cell behavior, contrasting it with human galectin-1 (Gal-1), a prototype member of the galectin family with shared β-galactoside-binding activity. We found that BjcuL binds to human dermal microvascular endothelial cells (HMECs) in a concentration- and carbohydrate-dependent fashion and reprograms the function of these cells, favoring a pro-inflammatory and pro-coagulant endothelial phenotype. In light of the quest for universal antagonists capable of mitigating the harmful consequences of snake venoms, BjcuL emerges as a promising target to be blocked in order to regulate pathological endothelial cell responses.

Keywords C-type lectin · Snake venom · Galectin-1 · Endothelial cells · Inflammation

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Dear Editor,

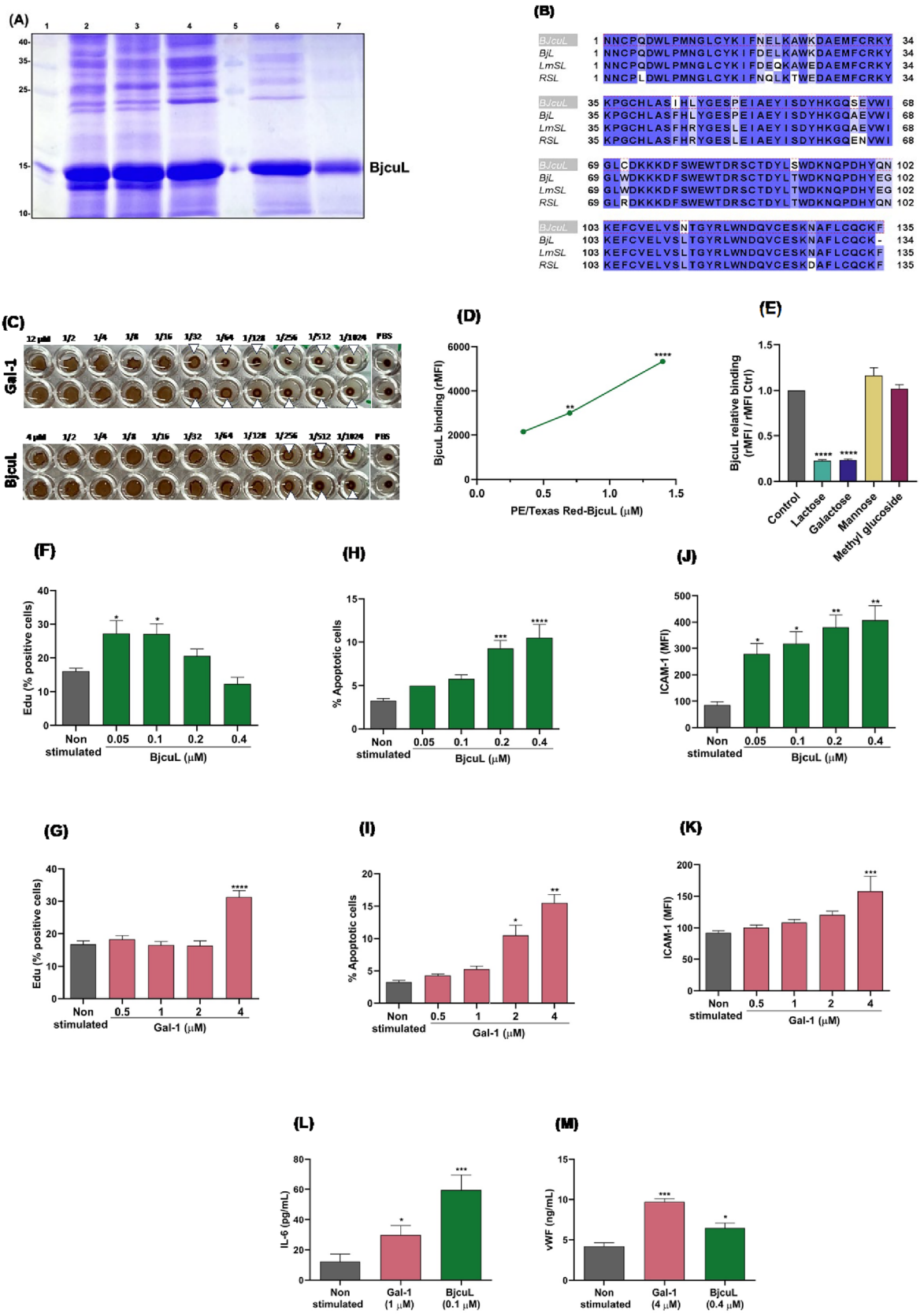
Snake envenomation poses a significant global health challenge, especially in developing nations. Statistics suggest that annually, between 421,000 and 1.8 million individuals fall victim to envenomation, leading to a staggering 81,000–138,000 fatalities and 400,000 cases of disability. Consequently, there is an urgent need to investigate novel therapeutic strategies to mitigate the detrimental and often fatal consequences of these venomous encounters [1].

Snake venoms contain a complex mixture of toxic bioactive factors that exert wide-ranging pathological effects on vital functions of the prey organism, including those involving the nervous and cardiovascular systems. The action of venoms from viperid snakes is mainly directed at blood coagulation [2]. In fact, hemohistotoxic viperid envenomation usually leads to local tissue damage and severe systemic hemorrhage, inflammation, consumption coagulopathy, or cardiovascular shock. In addition, it can impair the functions of the central nervous system by interfering with the blood clotting system and platelet aggregation, and by damaging the vascular endothelium, leading to severe intracranial bleeding and/or cerebral infarction [2].

Hemohistotoxic venoms comprise a spectrum of components, encompassing enzymes like serine and metalloproteinases, alongside non-enzymatic proteins such as disintegrins and C-type lectins (CTLs), a family of calcium-dependent extracellular glycan-binding proteins. Notably, CTLs constitute around 5–10% of certain viper venom constituents and are believed to disrupt cellular programs, including those critical for immune and endothelial cell functions [3, 4]. Likewise, galectin-1 (Gal-1), a member of the mammalian galectin family with β-galactoside-binding activity, plays a pivotal role in regulating immune and vascular processes [5]. In our quest to identify β-galactoside-binding proteins within the hemohistotoxic venom of *B. jararacussu*, which could potentially impact pro-coagulant and pro-inflammatory endothelial cell reactions, we successfully isolated BjcuL, a CTL known for its significant involvement in immune cell activation and apoptosis [4]. We purified BjcuL from the crude venom of *B. jararacussu* by single-step affinity chromatography using a lactosyl-Sepharose affinity column. SDS-PAGE analysis performed under reducing conditions yielded a single 15-kDa protein band (Fig. 1A). N-terminal amino acid sequencing identified the eluted protein as BjcuL, a CTL with high homology to lectin sequences from other viper venoms, as shown by BLAST analysis (Fig. 1B). Notably, BjcuL induced agglutination of rabbit erythrocytes yet, with higher activity than that triggered by human recombinant Gal-1 (Fig. 1C). To analyze the glycan-binding capacity of BjcuL, we exposed human dermal microvascular endothelial cells (HMECs) to this lectin. We found that BjcuL binds to HMECs in a concentration- and saccharide-dependent manner, as binding was specifically prevented by lactose and galactose (Fig. 1D, E).

Fig. 1 Characterization of endothelial cell responses triggered by BjcuL, a C-type lectin (CTL) purified from *Bothrops jararacussu* venom in comparison with those triggered by human recombinant Gal-1. **A** SDS-PAGE analysis. Lane 1: MW markers; lane 2: whole venom lysates; lane 3: venom solution seeded on lactosyl-Sepharose column; lane 4: eluate; lane 5: MW markers; lane 6: affinity-purified BjcuL; lane 7: purified BjcuL after dialysis and concentration. **B** Alignment of the protein sequence of BjcuL with sequences of other CTLs from snake venoms [4] (*Bjl*: *Bothrops jararaca* lectin; *LmSL*: *Lachesis muta stenophrys* venom lectin; *RSL*: rattlesnake venom lectin). Sequences are colored according to percentage of identity, ranging from blue meaning high identity and white meaning low identity. **C** Hemagglutinating activity of BjcuL or human recombinant Gal-1 on trypsinized rabbit erythrocytes. White arrowheads indicate absence of hemagglutination. **D** Flow cytometry analysis of PE/Texas Red-labeled BjcuL binding to HMECs (**$p < 0.01$, and ****$p < 0.0001$. BjcuL 0.70 µM and 1.40 µM versus BjcuL 0.35 µM). **E** Flow cytometry of PE/Texas Red-labeled BjcuL (0.70 µM) binding to HMECs in the absence or presence of specific (lactose and galactose) and non-specific (mannose, methylglucoside) saccharides (30 mM) (****$p < 0.0001$ versus BjcuL alone). **F**,**G** Flow cytometry analysis of EdU incorporation into viable HMECs triggered by exposure to BjcuL (**F**) or human recombinant Gal-1 (**G**) for 24 h. Percentage of apoptotic HMECs triggered by BjcuL (**H**) or human recombinant Gal-1 (**I**), determined by fluorescence microscopy following staining with acridine orange and ethidium bromide. Flow cytometry analysis of ICAM-1 expression induced by stimulation with BjcuL (**J**) or human recombinant Gal-1 (**K**) for 24 h. ELISA of IL-6 (**L**) and vWF (**M**) in HMEC supernatants after 24 h stimulation with BjcuL or human recombinant Gal-1 (**F**–**M**) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ versus non stimulated HMECs). Results represent the mean ± SEM of 3–4 independent experiments

Since vascular endothelium represents a primary target for both the hemohistotoxic and neurotoxic effects induced by viperid venoms [3], we next evaluated the impact of BjcuL on HMECs, in comparison with human recombinant Gal-1. We found that both lectins induce endothelial cell proliferation, but tenfold higher concentrations of Gal-1 were required to elicit similar responses as BjcuL (Fig. 1F, G). Moreover, at the highest BjcuL concentrations, we found that most cells were in suspension and the HMECs monolayer was completely disrupted (data not shown). Analysis of nuclear morphology and viability of these cells revealed an increased frequency of apoptotic HMECs triggered by BjcuL (Fig. 1H). Interestingly, treatment of HMECs with recombinant Gal-1 recapitulated these effects (Fig. 1I). However, neither BjcuL nor Gal-1 induced cellular necrosis (data not shown). The cytotoxic effect of BjcuL and Gal-1 is consistent with previous observations demonstrating the ability of these lectins to control cell viability [4–7]. Remarkably, non-apoptotic concentrations of BjcuL and Gal-1 increased basal expression of intercellular cell adhesion molecule-1 (ICAM-1) on HMECs (Fig. 1J, K) and triggered the synthesis and release of IL-6 (Fig. 1L), highlighting the ability


(A)
BjcuL
(B)
BjcuL
BjL
LmSL
RSL
(C)
Gal-1
BjcuL
PBS
(D)
BjcuL binding (rMFI)
PE/Texas Red-BjcuL (μM)
(E)
BjcuL relative binding (rMFI / rMFI Ctrl)
Control
Lactose
Galactose
Mannose
Methyl glucoside
(F)
Edu (% positive cells)
Non stimulated
BjcuL (μM)
(H)
% Apoptotic cells
(J)
ICAM-1 (MFI)
(G)
Gal-1 (μM)
(I)
% Apoptotic cells
(K)
ICAM-1 (MFI)
(L)
IL-6 (pg/mL)
Non stimulated
Gal-1 (1 μM)
BjcuL (0.1 μM)
(M)
vWF (ng/mL)
Gal-1 (4 μM)
BjcuL (0.4 μM)

of these lectins to foster pro-inflammatory endothelial cell responses. To evaluate the impact of BjcuL and Gal-1 on the pro-coagulant activity of endothelial cells, we finally examined their effects on constitutive secretion of von Willebrand factor (vWF) from storage granules. Although both lectins promoted the release of vWF, Gal-1 was more potent than BjcuL in contrast to the above-reported effects (Fig. 1M). Notably, none of these endothelial cell responses were inhibited or synergized when HMECs were treated simultaneously with both lectins (data not shown).

Overall, these findings highlight an activating role of BjcuL at the endothelium, which may trigger pro-inflammatory and pro-coagulant responses, and could be responsible, at least in part, for the deleterious effects of viper envenomation. Interestingly, endogenous Gal-1 could be potentially secreted in response to venom-driven inflammatory responses and contribute to these effects through binding to shared glyco-epitopes. In this regard, although Gal-1 exerts mainly anti-inflammatory and pro-resolving functions in several models of autoimmune and chronic inflammation [5], recent findings demonstrated pro-inflammatory activity of this lectin early during activation of the inflammatory cascade [8]. Thus, BjcuL emerges as a new potential therapeutic target in the control pathological endothelial responses triggered by *Bothrops jararacussu* or other viper envenomations. This effect could also be achieved by blocking endogenous Gal-1 in inflamed or damaged vascular tissues [9].

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Data availability All data are available in the text and supplementary material.

Declarations

Conflict of interest The authors declare no competing interests.

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