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Mini review

Scorpion envenomation and inflammation: Beyond neurotoxic effects

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A B S T R A C T

Scorpion envenomation results in a wide range of clinical manifestations that are mostly attributed to the activation of the autonomic nervous system by venom toxins. In fact, sympathetic and parasympathetic disturbances play important roles during poisoning. However, scorpion venom also induces a complex hyperinflammatory state that occurs parallel to systemic inflammatory response syndrome and acute sepsis. After a scorpion sting, innate immune cells are exposed to the venom molecules, which bind to pattern recognition receptors and activate pro-inflammatory pathways that contribute toward the promotion of severe symptoms, such as pulmonary edema, and eventually lead to death. In this review, we highlight studies that pointed out inflammation as a major pathological facet of scorpion envenomation, so as to provide novel targets to improve therapeutics for scorpionism.

1. Introduction

Scorpionism is a neglected public health problem worldwide. The high prevalence of scorpions in endemic areas is in part because there are no effective chemicals to control these arthropods (Daar et al., 1997), but also because some species reproduce via parthenogenesis (Chippaux and Goyffon, 2008). Scorpion sting causes variable and complex clinical manifestations, which range from a local effect to intense autonomic nervous system responses and systemic inflammatory reaction, similar to those associated with systemic inflammatory response syndrome and acute sepsis (Fukuhara et al., 2003; Zoccal et al., 2016). These manifestations often progress to severe cardiac and pulmonary alterations that may culminate in fatal outcomes, especially in children and elderly subjects (Isbister and Bawaskar, 2014).

Independently of the species, scorpion venom is composed of a mixture of molecules, including ion channel–modulating toxins (Pucca et al., 2015a, 2015b, 2015c), and inflammatory toxins. Acting on sodium, potassium, calcium, and chloride channels, these toxins induce a "neurotransmitter storm" that is considered the major driver of pathology after a scorpion sting (Cupo, 2015; Isbister and Bawaskar, 2014). Additionally, over the years, several studies have demonstrated that venoms and toxins from different scorpions are potent activators of the immune system, changing the concept that perturbations of the autonomic nervous system are the sole drivers of pathology in scorpionism. In fact, many of the toxins that affect ion channels also induce exacerbated inflammatory responses, which is a research area that has been intensively explored by us and other researchers employing the venom of the Brazilian scorpion *Tityus serrulatus* (Fialho et al., 2011; Paneque Peres et al., 2009; Pessini et al., 2003; Pucca et al., 2015a, 2015b, 2015c; Van Fraga et al., 2015; Zoccal et al., 2011, 2018, 2016, 2015, 2014, 2013). Other scorpion species also stimulate host immune responses; however, there are very few studies addressing the whole magnitude of immunopathological mechanisms during scorpion poisoning (Cupo, 2015; FUNASA, 2001). In this review, we summarize the findings pointing to scorpion venom as a potent activator of the innate immune response, and as a very valuable model to understand the mechanisms triggered in sterile inflammation. Furthermore, we discuss the importance of the inflammatory response to the clinical manifestations caused by scorpion venoms.

2. Local symptoms induced by scorpion sting

Most cases of scorpionism are characterized by the development of local symptoms after venom inoculation in the skin (Abourazzak et al.,

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2009). Clinical manifestations associated with different species of scorpion include intense pain, with an incidence of approximately 81%, followed by hyperemia, scarification, and itching (Abourazzak et al., 2009). Interestingly, previous exposure to *Centruroides vittatus* scorpion venom has been reported to predispose individuals to increased dermal manifestations 48 h after clinical injection of the venom, diluted at 1:100,000, whereas naïve subjects have not been reported to show local symptoms as severe as those observed in pre-exposed subjects (Demain and Goetz, 1995). The reaction to the venom is very similar to that driven by type IV or delayed-type hypersensitivity, which is mediated by antigen-specific memory T cells, suggesting an influence of the immune system in promoting local symptoms.

The local effects of scorpion sting are dictated by venom composition. For example, hyaluronidase and metalloproteinases act as spreading factors by degrading the extracellular matrix of the skin and promote hyperemia and intense pain (Pucca et al., 2015a, 2015b, 2015c). Moreover, the presence of vasoactive amines is associated with increased blood flow to the bite region, thereby enhancing redness and edema (Abourazzak et al., 2009; Demain and Goetz, 1995; Pucca et al., 2015a, 2015b, 2015c; Rahmani and Jalali, 2012). Actually, if accidents with scorpions were limited to the local effects, such as pain and some discomfort after a few hours of the sting, the impact on public health would be different. However, this is not the case, as the severity of envenomation is tightly associated with factors such as age, weight, and development of systemic manifestations.

3. Systemic inflammatory manifestations

The scorpion usually injects the content of the venom gland into the subcutaneous region of the skin. Thereafter, hyaluronidases and other enzymes enhance tissue permeability of the toxins that reach the circulation and distribute the toxins to the whole body. The number of organs in which the venom accumulates is variable among some studies, but they converge in pointing out that the kidney, blood, liver, lung, heart, and spleen exhibit higher venom concentrations (Nunan et al., 2003; Revelo et al., 1996). High venom concentrations in the kidneys are related to the excretion of toxins, which usually occurs until 24 h after the sting. Other organs such as the lung and heart are highly irrigated and affected early after injection. Organs, such as the liver, might also participate in the oxidation and detoxification of diverse compounds composing scorpion venoms; however, further studies are needed to elucidate these processes.

Systemic manifestations of scorpionism are characterized by moderate and severe manifestations. They are mainly described as consequences of the hyperactivation of the autonomic nervous system, due to direct effects of toxins, and are correlated with clinical symptoms such as hyperglycemia, priapism in male children, arrhythmia, tachycardia, bradycardia, and hypotension (Cupo, 2015). However, recent studies point out that components of the inflammatory response also control the development of systemic effects and severe clinical symptoms, which could also affect the autonomic nervous system. The management of systemic manifestations of scorpionism requires the administration of antivenom serum and supportive treatment for severe symptoms, especially pulmonary and cardiovascular disturbances (Isbister and Bawaskar, 2014). Herein, we summarize and discuss the evidence for these effects during envenomation with different species of scorpion.

3.1. Inflammation induced by Tityus sp. venoms

The *Tityus* sp. scorpion species *are* the most prevalent in South America, whereas the species *Tityus serrulatus* (Ts) causes the majority of accidents induced by poisonous animals, accounting for more cases than all other poisonous animals together in Brazil (FUNASA, 2001; Reckziegel and Pinto, 2014). Ts venom (TsV) evokes distinct clinical symptoms that are classified as local, mild, or severe manifestations (Khattabi et al., 2011). In most cases, local effects are the only clinical consequences due to scorpion sting, which include skin edema, intense pain, erythema, itching, and tingling (Khattabi et al., 2011). Mild manifestations include local symptoms and autonomic nervous system excitation, culminating symptoms including nausea, vomiting, intense sweating, priapism, diarrhea, and confusion (Cupo, 2015; Isbister and Bawaskar, 2014). Severe manifestations consist mainly of serious cardiac function alterations such as bradycardia/tachycardia and disturbance in arterial pressure. These symptoms are a consequence of pulmonary edema and cardiac dysfunction (Cupo, 2015; Isbister and Bawaskar, 2014), and are potentially fatal.

First described in 1999 (Magalhães et al., 1999), the increased serum levels of inflammatory mediators in patients envenomed by Ts encouraged further research into immunological mechanisms associated with severe clinical manifestations. Although many case reports have described the clinical manifestations of envenomation by Ts, only few studies have addressed the potential link between inflammation and the pathology of scorpionism, the common findings of which included blood leukocytosis and neutrophil entrapment in the lungs (Cupo et al., 1994; Kumar et al., 2012). Furthermore, there is evidence that envenomation by Ts induces a “cytokine storm” and systemic inflammation, reflected by elevated plasma levels of interleukin-1α (IL-1α), IL-1β, tumor necrosis factor-α (TNF-α), IL-6, IL-8, interferon-γ (IFN-γ), granulocyte-macrophage colony stimulating factor (GM-CSF), and IL-10 (Fukuhara et al., 2003; Magalhães et al., 1999).

Activation of innate immune cells, such as macrophages, is often initiated with the interaction between pattern recognition receptors (PRRs) and ligands, triggering intracellular signaling cascades and production of inflammatory mediators (Medzhitov, 2001). The first study to address isolated macrophage responses to TsV showed that the crude venom extract induced a dose-dependent production of interferon-γ (IFN-γ), IL-6, and nitric oxide (NO) independently of the cellular necrosis caused by increasing concentrations of TsV (Petricevich, 2002). Further studies described the production of other pro-inflammatory and anti-inflammatory cytokines by TsV-stimulated macrophages, including IL-1α, IL-1β, and IL-10 (Petricevich et al., 2007; Petricevich and Lebrun, 2005). Macrophages also increase phagocytosis and vacuole formation upon stimulation with Ts1, a purified TsV toxin (Petricevich et al., 2008). Indeed, Ts1 is highly relevant in the context of macrophage activation by TsV. Zoccal et al. found that J774.1 murine macrophages responded to Ts1 and Ts6 by producing pro-inflammatory mediators, while Ts2 induced contrasting responses (Zoccal et al., 2011). Importantly, Ts2 and Ts6 induced the production of bioactive lipids involved in the inflammatory response, such as prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) (Zoccal et al., 2013).

TsV is recognized by surface PRRs, such as toll-like receptor 2 (TLR2), TLR4, and CD14 (Zoccal et al., 2014) (Fig. 1). After the stimulus with TsV, macrophages up-regulate the expression of these receptors, which promote the activation of transcription factors targeting the genes that code for inflammatory mediators. Such pathways include Myd88-dependent signaling and nuclear factor kappa B (NF-κB) activation, as well as Myd88-independent c-Jun (AP1) activation (Zoccal et al., 2014) (Fig. 1). In parallel with the definition of pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) as ligands of PRRs, the term venom-associated molecular patterns (VAMPs) was introduced to define molecules composing venoms that serve as ligands for PRRs and induce inflammatory responses (Zoccal et al., 2014). Stimulus with TsV also activates peroxisome proliferator-activated receptor gamma (PPAR-γ) (Zoccal et al., 2015) (Fig. 1). Activation of this transcription factor depends on TLR2/TLR4 signaling and controls the formation of lipid bodies in macrophages, a phenomenon that is tightly associated with the production of arachidonic acid (AA)-derived bioactive lipids or eicosanoids (Alvarez et al., 2010).

Due to its pro-inflammatory properties, TsV is known to induce cellular recruitment to the site of injection, including an intense

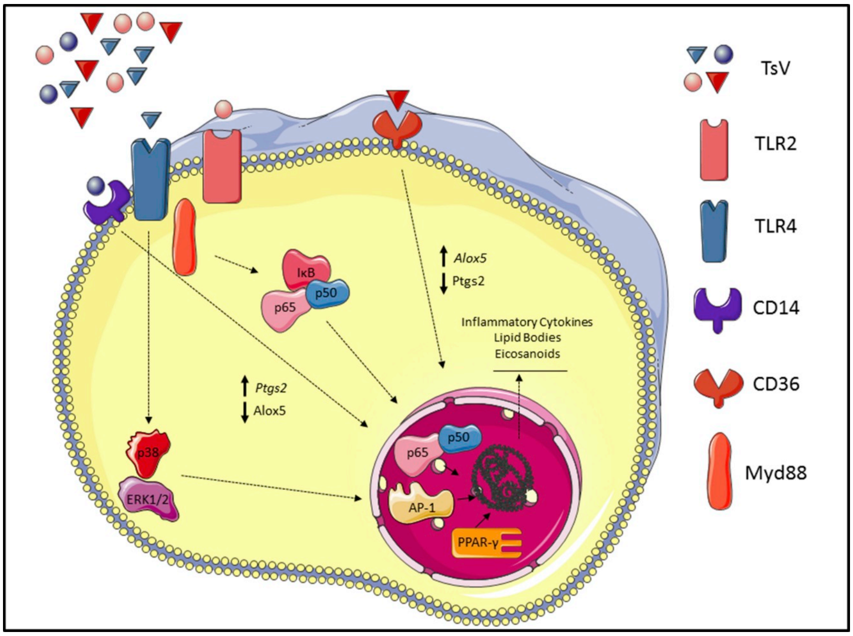

Fig. 1. Activation of macrophages by *Tityus serrulatus* venom. After stimulation with *Tityus serrulatus* venom (TsV), macrophages up-regulate pattern recognition receptors (TLR2, TLR4, and CD14) and scavenger receptor CD36, which recognize the venom and activate transcriptional factors such as AP-1, NF-κB, and PPAR-γ, which are correlated with cytokine/eicosanoid production and lipid body formation.

accumulation of neutrophils and macrophages early after inoculation and of lymphocytes at later time points (Zoccal et al., 2013). Interestingly, prostaglandins and leukotrienes promote the recruitment of CD4 and CD8 lymphocytes to the peritoneal cavity of mice injected with TsV, because reduced numbers of these cells were recovered after pharmacological inhibition of eicosanoids (Zoccal et al., 2013). *In vitro* studies using human lymphocytes isolated from peripheral blood mononuclear cells (PBMC) showed that TsV is not cytotoxic to these cells, but the venom inhibited lymphocyte proliferation while promoting the release of IL-6 (Casella-Martins et al., 2015). Using isolated TsV toxins, Pucca et al. described a highly suppressive property of Ts6 and Ts15 on T-cell proliferation and IFN-γ production (Pucca et al., 2015a, 2015b, 2015c). Specifically, they observed that Ts6 inhibited the proliferation of effector memory T cells, while Ts15 repressed the proliferation of naïve, effector, central memory and effector memory $CD4^+$ T-cell subsets. Strikingly, Ts6 and Ts15 completely reversed the BSA-induced delayed-type hypersensitivity in a murine model (Pucca et al., 2015a, 2015b, 2015c). Curiously, another investigation indicated that crude TsV is completely excreted in the first 24 h after inoculation (Revelo et al., 1996), thus suggesting that TsV may only interact with naïve and/or resident memory lymphocytes *in vivo*. Despite these findings, immunosuppressive effects of TsV toxins on different $CD4^+$ T-cell subsets offer a new avenue for exploring the therapeutic activity of isolated toxins in $CD4^+$ T lymphocyte-mediated diseases.

Lung edema and cardiac dysfunction are the main causes of death during scorpionism (Ingelfinger et al., 2014). Recently, we demonstrated that TsV-induced pulmonary edema is a major consequence of inflammatory response to the venom (Zoccal et al., 2016). Indeed, TsV induces K^+ efflux and activates the multimeric platform NLRP3 inflammasome, which activates caspase-1 to process the immature form of the highly inflammatory cytokine, IL-1β. Increased levels of IL-1β in the lungs promote neutrophil influx and edema that culminate in the death of mice injected with a lethal dose of TsV. We also found that PGE_2 increases intracellular cyclic adenosine monophosphate (cAMP) levels via EP2/4 receptors, activating protein kinase A, and increasing NF-κB activity and IL-1β production by macrophages. In contrast, LTB_4 decreases cAMP levels via the BLT1 receptor and shuts down the signaling pathway that controls IL-1β levels, inflammation, and mortality caused by TsV (Zoccal et al., 2016). In a follow-up study, we identified the innate immune receptors involved in the opposing roles played by PGE_2 and LTB_4 (Zoccal et al., 2018). We demonstrated that CD14 induces a pro-inflammatory pathway by promoting a massive PGE_2 release by macrophages, culminating in elevated cAMP and IL-1β production. In addition to CD14, TLR4 contributes to the phenomenon (Zoccal et al., 2018). In contrast, CD36 was the sole receptor involved with LTB_4 production, which suppressed inflammation by decreasing intracellular cAMP via the BLT1 receptor (Zoccal et al., 2018). Collectively, these studies pointed to potential targets for the development of innovative therapeutic strategies. This hypothesis has been tested in a proof-of-concept study using EP80317, a CD36 receptor ligand (Zoccal et al., 2019). Treatment with this peptide limited the mortality caused by Ts envenomation by promoting LTB_4 production and diminishing the inflammatory response (Zoccal et al., 2019).

In addition to the stimulation of cellular immune responses, TsV also activates the complement system (CS). A study by Peres et al. described increased levels of CS components immediately after envenomation (1 h). This event was reflected by a decrease in hematocrit over time and an increase in the lytic activity of classical and alternative CS pathways (Bertazzi et al., 2003). In addition, there was evidence for the synthesis of CS components even after 24 h of envenomation, a period in which the venom was depurated and secreted (Bertazzi et al., 2003; Santana et al., 1996). Bertazzi et al. also showed that TsV directly activated factor B and C3 cleavage, generating anaphylatoxins that increased inflammation due to their chemotactic properties (Bertazzi et al., 2005).

Other scorpions of *Tityus* sp. genus include *Tityus discrepans*, a common scorpion in South America, whose venom induces an acute inflammatory response in rams. The inflammatory response was characterized by activation of macrophages and neutrophils, and increased plasma levels of IL-6 and TNF-α (D'Suze et al., 2004). *Tityus stigmurus* is an endemic scorpion in northeastern Brazil and produces venom with mitogenic properties in murine macrophages (Daniele-Silva et al., 2016). Interestingly, *Tityus bahiensis* venom promotes inflammatory infiltrate in the hippocampus of rats, accompanied by increased intracerebral levels of IL-6 and TNF-α (Beraldo Neto et al., 2018).

3.2. Inflammation induced by Androctonus australis hector

Host response has been investigated during envenomation with other scorpion species, including *Androctonus australis hector* (Aah), the main cause of fatality in some regions of Africa and Asia (Table 1) (Adi-Bessalem et al., 2008). Aah venom (AahV) causes clinical symptoms

Table 1
Effects of the venoms of different scorpion species on the immune system.

Species	Geographic distribution	Immunological consequences	Reference
Tityus serrulatus	Brazil	↑ Cytokines	(Fukuhara et al., 2003; Magalhães et al., 1999; Petricevich, 2002; Zoccal et al., 2018, 2016, 2015, 2014, 2013, 2011)
		↑ Lymphocyte suppression	(Casella-Martins et al., 2015; Pucca et al., 2015a, 2015b, 2015c)
		↑ NO production	(Zoccal et al., 2014, 2011)
		↑ Prostaglandins and leukotrienes	(Zoccal et al., 2018, 2016, 2015, 2014, 2013, 2011)
		↑ NF-κB activation	(Zoccal et al., 2016, 2014)
		↑ PPAR-γ activation	Zoccal et al. (2015)
		↑ COX-2 expression	(Zoccal et al., 2018, 2016, 2015, 2014, 2013, 2011)
		↑ Complement activation	Bertazzi et al. (2003)
		↑ C3 and factor B cleavage	Bertazzi et al. (2005)
Tityus discrepans	Brazil, Suriname, Venezuela, Guyana, and Trinidad and Tobago	↑ IL-6 and IL-10 levels	D'Suze et al. (2004)
Tityus stigmurus	Brazil	↑ Mitogenic activity on macrophages	Daniele-Silva et al. (2016)
		↑ IL-6, IL-10, and NO levels	Daniele-Silva et al. (2016)
Tityus bahiensis	Brazil	↑ Intracerebral IL-6 and IL-10 levels	(Beraldo Neto et al., 2018)
Androctonus australis hector	Africa (Algeria, Chad, Egypt, Mauritania, Somalia, Sudan, Tunisia) and Asia (India, Israel, Pakistan, Saudi Arabia, Yemen)	↑ Cytokines	(Ait-Lounis and Laraba-Djebari, 2012; Haddad-Ishak-boushaki and Laraba-Djebari, 2017; Liu et al., 2007; Raouraoua-Boukari et al., 2012)
		↑ MPO and EPO activity	(Adi-Bessalem et al., 2012; Raouraoua-Boukari et al., 2012)
		↑ NO production	Raouraoua-Boukari et al. (2012)
		↑ Histamine release	Raouraoua-Boukari et al. (2012)
		↑ Polarization to M1 macrophages	(Liu et al., 2007)
Buthus martensii	China, Japan, Mongolia, Korea	↑ Mast cell degranulation	(Liu et al., 2007)
		↑ Inflammatory pain	(Bai et al., 2006; Chen et al., 2002)
		↑ Histamine release	(Liu et al., 2007)
Leiurus quinquestriatus	Sahara, Arabian Desert, Thar Desert, Algeria, Mali, Egypt, Ethiopia, Arabian Peninsula, India	↑ Neutrophil recruitment	Abdoon and Fatani (2009)
		↑ IL-8, TNF-α, and NO levels	Abdoon and Fatani (2009)
Centruroides noxius	Mexico	↑ IL-6, IL-10, TNF-α, and IFN-γ levels	Petricevich (2006)

similar to those observed during envenomation by Ts. *In vivo* studies showed an intense inflammatory response, reflected by increased plasma levels of IL-4, IL-6, IL-10, TNF-α, and IL-1, as well as hemolytic activity and blood leukocytosis (Adi-Bessalem et al., 2008). AahV also elevates serum and pulmonary NO, histamine, and myeloperoxidase (MPO) activity and ICAM-1 expression, correlating with increased neutrophilia in blood and lungs (Raouraoua-Boukari et al., 2012). AahV also induces lung eosinophilia with increased peripheral blood levels of IL-4, IL-5, and IgE (Adi-Bessalem et al., 2012). It has been shown to induce hyperglycemia via an increase in TNF-α levels in adipose tissue, whereas TNF-α inhibitor restored the glucose balance (Ait-Lounis and Laraba-Djebari, 2012). Another study has shown that parasympathetic responses regulated the production of pro-inflammatory cytokines in the lungs during envenomation with Aah (Saidi et al., 2013).

AahV (crude venom) and its main toxins AahI and AahII also activate the CS in the liver, and promote the production pro-inflammatory cytokines in the organ (Bekkari et al., 2015). This venom is also a strong inducer of macrophage M1 and M2 polarization. Stimulus with AahV reduces the expression of M2-associated genes *Arg1* and *Il10* and increases the expression of inflammatory genes such as *Il1b*, *Il23*, and *Nos2* (Ait-Lounis and Laraba-djebari, 2015). AahV also induces lung alterations such and hemorrhage and activation of alveolar macrophages and secretion of inflammatory mediators (Saidi et al., 2018).

3.3. Inflammation induced by *Buthus martensii* venom

Buthus martensii (Karsch), also known as gold scorpion or Chinese scorpion, is a very common species in China, Mongolia, Japan, Korea, and other Asian countries (Qi et al., 2003). Peptides from *B. martensii* venom (BmV) exhibit analgesic properties and have been used in traditional Chinese Medicine (Goudet et al., 2002; Shao et al., 2007). Many of these peptides have already been characterized, and one of them exhibits high potential for application as a new analgesic compound (Santa et al., 2008). Despite these analgesic properties, the sting of this scorpion is known to be highly painful. These effects are linked to the immunomodulatory properties of BmV, whose injection in rats induced an intense dose-dependent edematogenic response (Bai et al., 2006). Interestingly, this response was reversed by suppressing the production of prostaglandins by using indomethacin, a COX inhibitor (Bai et al., 2006). Besides causing pain via the production of prostanoids, BmV also induced mast cell degranulation and histamine release, whose inhibition reversed pain in rats (Jiang et al., 2007). However, knowledge about the inflammatory response induced by BmV remains limited, and future studies are needed to understand the molecular mechanisms inducing immunopathology.

3.4. Other scorpion species

Leiurus quinquestriatus, also known as Palestinian yellow scorpion, is common in deserts and distributed between the Sahara to the Arabian Peninsula. *L. quinquestriatus* venom induces a large range of immunological alterations, including elevated serum levels of IL-8, TNF-α, and NO, which are associated with blood leukocytosis, specifically of neutrophils (Abdoon and Fatani, 2009). *Centruroides noxius,* a scorpion species found in Mexico, also induces an increase in the serum levels of TNF-α, IL-6, IL-10, and IFN-γ (Petricevich, 2006). The main alterations induced by the venom of different species are shown in Table 1.

4. Concluding remarks and future directions

Due to limited knowledge about the effects of venoms on the immune system, only recently have Immunology books described venoms as inducers of sterile inflammation. However, it has long been very well accepted that venoms from different scorpion species induce intense inflammatory responses that are strongly associated with pathology and mortality. Innate immune cells play important roles during these processes, in which PRRs recognize VAMPs promoting the activation of signaling cascades culminating in the production of inflammatory mediators.

Although the clinical treatment of scorpionism is quite effective, cardiac manifestations might progress to fatal outcomes even after antivenom serum therapy. Therefore, it is possible that the host response to the venom is perpetuated by molecular mechanisms functioning after the venom toxins have been neutralized, which could promote further pathology. Filling the gaps in knowledge about the influence of the immune system during scorpion poisoning may result in supportive therapies that could improve survival and prevent comorbidities. Indeed, detailed investigations are needed to elucidate the potential effects of the immune system on the nervous and cardiovascular systems. Immune cells could also be part of the detoxification response in the liver or influence secretion in the kidneys. The inflammatory response could also be involved in perturbations in the pancreas, which is also exposed to high concentrations of venom in the first 24 h after the accident.

Therefore, pharmacological antagonisms of innate immune receptors and/or inhibition of inflammatory enzymes provide avenues for the development of novel therapeutic approaches aimed at controlling excessive inflammation that occurs even in the presence of antibodies used to neutralize the venom during scorpionism.

Author contributions

MR reviewed the literature, conceptualized the figure and table, and wrote the first draft. KZ, LG, and LF discussed and revised the manuscript. All authors read and approved the final version.

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