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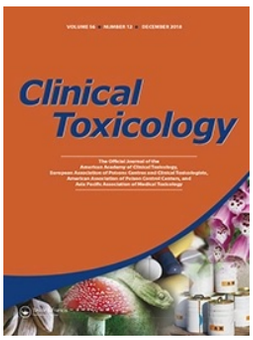

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REVIEW

The kiss of (cell) death: can venom-induced immune response contribute to dermal necrosis following arthropod envenomations?

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ABSTRACT

Introduction: Snakes, insects, arachnids and myriapods have been linked to necrosis following envenomation. However, the pathways involved in arthropod venom-induced necrosis remain a highly controversial topic among toxinologists, clinicians and the public. On the one hand, clinicians report on alleged envenomations based on symptoms and the victims' information. On the other hand, toxinologists and zoologists argue that symptoms are incompatible with the known venom activity of target species. This review draws from the literature on arthropod envenomations, snakebite, and inflammatory processes to suggest that envenomation by a range of organisms might trigger an intense inflammatory cascade that ultimately lead to necrosis. If confirmed, these processes would have important implications for the treatment of venom-induced necrosis.

Objectives: To describe two inflammatory pathways of regulated necrosis, tumour necrosis factor (necroptosis) and Neutrophil Extracellular Traps (NETosis); to discuss existing knowledge about snake venom and arachnid-induced necrosis demonstrating the involvement of tumour necrosis factor and neutrophils in the development of tissue necrosis following envenomation and to contribute to the understanding of venom-induced necrosis by arthropods and provide clinicians with an insight into little known inflammatory processes which may occur post envenomation.

Methods: ISI Web of Science databases were searched using the terms "spider bite necrosis", "arthropod envenomation necrosis", "venom necrosis", "venom immune response", "loxoscelism", "arachnidism", "necroptosis venom", "necroptosis dermatitis", "tumour necrosis factor TNF venom", "scorpionism", "scolopendrism", "centipede necrosis", "NETosis venom", "NETosis necrosis". Searches produced 1737 non-duplicate citations of which 74 were considered relevant to this manuscript. Non-peer-reviewed sources or absence of voucher material identifying the organism were excluded.

What is necrosis? Necrosis is the breakdown of cell membrane integrity followed by inflowing extracellular fluid, organelle swelling and the release of proteolytic enzymes into the cytosol. Necrosis was historically considered an unregulated process; however, recent studies demonstrate that necrosis can also be a programmed event resulting from a controlled immune response (necroptosis).

Tumour necrosis factor and the necroptosis pathway: Tumour necrosis factor is a pro-inflammatory cytokine involved in regulating immune response, inflammation and cell death/survival. The pro-inflammatory cytokine TNF-α participates in the development of necrosis after envenomation by vipers. Treatment with TNF-α-antibodies may significantly reduce the manifestation of necrosis.

Neutrophil Extracellular Traps and the NETosis pathway: The process by which neutrophils discharge a mesh of DNA strands in the extracellular matrix to entangle ("trap") pathogens, preventing them from disseminating. Neutrophil Extracellular Traps have been recently described as important in venom-induced necrosis. Trapped venom accumulates at the bite site, resulting in significant localized necrosis.

Arthropod venom driving necrosis: Insects, myriapods and arachnids can induce necrosis following envenomation. So far, the processes involved have only been investigated in two arachnids: *Loxosceles* spp. (recluse spiders) and *Hemiscorpius lepturus* (scorpion). *Loxosceles* venom contains phospholipases D which hydrolyse sphingomyelin, resulting in lysis of muscle fibers. Subsequently liberated ceramides act as intermediaries that regulate TNF-α and recruit neutrophils. Experiments show that immune-deficient mice injected with *Loxosceles* venom experience less venom-induced inflammatory response and survive longer than control mice. Necrosis following *Hemiscorpius lepturus* stings correlates with elevated concentrations of TNF-α. These observations suggest that necrosis may be indirectly triggered or worsened by pathways of regulated necrosis in addition to necrotic venom compounds.

Conclusions: Envenomation often induce an intense inflammatory cascade, which under certain circumstances may produce necrotic lesions independently from direct venom activity. This could explain the inconsistent and circumstantial occurrence of necrosis following envenomation by a range of organisms. Future research should focus on identifying pathways to regulated necrosis following envenomation and determining more efficient ways to manage inflammation. We suggest that clinicians should consider the victim's immune response as an integral part of the envenomation syndrome.

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Introduction

Venoms have evolved to fulfil mainly foraging and defensive functions. As such, envenomation typically results in neurotoxic symptoms which disable prey and enemies rapidly [1]. In some instances, however, envenomation leads to necrotic lesions in human and animal models, sometimes simultaneously with systemic organ injury [2]. Snakes [3–5], cnidarians [6,7], bees [2], wasps [8], ants [9], mosquitoes [10], beetles [11], centipedes [12,13], scorpions [14,15] and spiders [16,17] have all been linked to the formation of necrotic wounds in patients. The potentially lytic effects of jellyfish [6], coral [7] and snake [3–5] venoms are fairly well documented.

In regard to arthropods, however, and apart from the recluse spiders of the genus *Loxosceles* and the Asian scorpion *Hemiscorpius lepturus*, necrotic lesions are not consistent with the known, direct venom activity of the incriminated species. As a result, the occurrence of necrotic lesions following envenomation by arthropods remains a highly controversial topic among toxinologists, clinicians and the general public. This is particularly true in the case of spiders. While reports of alleged necrotic spider bites are on the rise, the actual spiders responsible for these lesions are rarely recovered and identified [18] and misdiagnoses are frequent [19] leading to a plethora of potentially misleading reports in the literature [19–21].

In the case of myriapods, insects and arachnids, venom compounds have been predominantly associated with neurotoxic function, although lesser known peptides may be capable of haemolytic and cytolytic activity [22–24]. Large centipedes of the *Scolopendridae* family have been shown to occasionally produce extensive dermonecrosis, sometimes in conjunction with organ failure and acute coronary ischemia [12,13,25].

Africanized bees *Apis mellifera scutellata* typically induce neurotoxic symptoms but cases involving a high number of stings have led to the development of localized necrosis [2]. Similarly, the stings from the Asian giant hornet *Vespa mandarinia* do not typically induce necrotic symptoms, however, cases involving multiple stings have led to systemic organ injury and tissue necrosis [8]. The tropical fire ant *Solenopsis geminata* typically induces pain and general discomfort following envenomation. However, in rare cases involving a large number of stings, victims might develop tissue necrosis [9].

The arthropods most commonly associated with necrotic lesions belong to the class Arachnida, and more specifically scorpions and spiders. Although venom compounds and their active pathways have not been identified yet, *Hemiscorpius lepturus,* a medically important scorpion from West Asia, is currently the only known species of scorpion to possess a necrotoxic venom [14,15,26]. In addition to inducing necrosis, other symptoms include renal failure and haemolysis [27] and they are responsible for fatal outcomes.

A plethora of reports are linking necrotic wounds to spider bites in both the scientific and popular literatures [19,28]. However, the alleged necrotic lesions produced by hobo spiders (genus *Tagenaria*) in the United States and white tail spiders (genus *Lampona*) in Australia have been systematically debunked [29,30]. Globally, confirmed spider-induced dermal necrosis can be considered a rare occurrence given the taxonomic diversity of spiders (over 45,000 species described so far), with confirmed cases involving only the Noble false widow spider *Steatoda nobilis* [17], the yellow sac spiders of the genus *Cheiracanthium* [19] and the recluse spiders of the genus *Loxosceles* [16,31–33].

In the case of *Steatoda nobilis*, reports of necrotic lesions following envenomation contrast sharply with the symptoms reported from envenomation by their relatives, the true black widows (genus *Latrodectus*) which produce exclusively neurotoxic symptoms [34,35]. Cases of venom-induced necrosis following bites from the genus *Cheiracanthium* have been reported frequently. However, an extensive review involving 59 verified bites showed that these claims were unwarranted with only a single case of minor necrosis confirmed in Europe [19]. The synanthropic spiders most commonly associated with necrotic lesions belong to the genus *Loxosceles* and are known to produce phospholipases D (formerly referred to in the literature as sphingomyelinases D), a group of enzymes which hydrolyse sphingomyelin [36].

With the exception of *Hemiscorpius lepturus* and *Loxosceles*, all the cases mentioned above are notable for their onset of necrotic lesions which are inconstant with known (typically neurotoxic) venom activity. However, neurotoxicity and necrotoxicity are not mutually exclusive. Necrotic lesions could develop in at least three independent, potentially overlapping ways:

1. direct action of venom toxins on cells;
2. bacterial infection (secondary or vector borne); and
3. strong immune response to the envenomation.

The former two have been discussed previously in the literature [37–41], but the latter remains a neglected topic despite earlier studies suggesting that the victim's immune response may contribute to dermonecrosis [3–5,27,42–47]. In the light of recent studies uncovering the inflammatory pathways leading to programmed necrosis (necroptosis), we postulate that when direct toxic activity from venom toxins occur simultaneously with an innate and adaptive immune response, inflammation may be an important driving force that results in symptoms superficially inconsistent with known, specific, venom activity.

While this review aims to shed light on the role of inflammation in arthropod envenomation, some studies carried out on snake venom are also discussed as they offer an insight into the behaviour of the immune system in response to venom toxins.

The cytokine tumour necrosis factor (TNF) is involved in regulating cell survival and cell death (apoptosis) and the more recently described pathway of regulated necrosis (necroptosis). Neutrophils are a phagocytic immune cell that typically engulf and digest foreign bodies, and once triggered by certain stimuli, they are also capable of rupturing and releasing their DNA contents which trap foreign bodies. These are known as Neutrophil Extracellular Traps (NETs) that can lead to necrosis (NETosis). Both TNF and neutrophils

have been linked to necrosis following envenomation by snakes and two medically important arthropods and will be discussed in this review.

We propose that the potential exists for necrosis to occur following arthropod envenomation via inflammatory pathways of regulated necrosis. We suggest two targetable mechanisms of inflammation, the TNF necroptosis and neutrophil NETosis pathways, as suitable candidates to study their potential involvement in the occurrence of necrotic lesions following arthropod envenomation. This hypothesis, which has been given little attention so far and has never been considered in the broader context for all arthropod envenomations, might explain the inconsistent, circumstantial occurrence of necrotic lesions following envenomation by a range of arthropods.

Objectives

1. To describe two inflammatory pathways of regulated necrosis: TNF (necroptosis) and NETosis.
2. To discuss the potential involvement of TNF and neutrophils in the development of tissue necrosis following envenomation by a range of arthropods, based on the existing literature on snake and arachnid-induced necrosis.
3. To contribute to the understanding of venom-induced necrotic lesions by arthropods and provide clinicians with an insight into little known inflammatory processes which may occur post-envenomation.

Methods

ISI Web of Science databases were searched using the terms "spider bite necrosis", "arthropod envenomation necrosis", "venom necrosis", "venom immune response", "loxoscelism", "arachnidism", "necroptosis venom", "necroptosis dermatitis", "tumour necrosis factor TNF venom", "scorpionism", "scolopendrism", "centipede necrosis", "NETosis venom", "NETosis necrosis". All citations ($N = 2{,}680$) were exported to EndNote™ version X7. The citation list was inspected and all duplicate entries ($N = 843$) were manually removed. Searches produced a total of 1,737 nonduplicate citations of which 74 were considered relevant to this manuscript. Because of the large volume of unsubstantiated reports on venom-induced necrotic lesions, non-peer-reviewed sources were excluded. Historical reports of suspected venom-induced necrosis published without voucher material clearly identifying the organism involved in the envenomation were also discarded.

What is necrosis?

Necrosis occurs when cells experience organelle swelling resulting from inflowing ions and fluid from the extracellular matrix after the breakdown of cell membrane integrity. The breakdown of organelle membranes releases proteolytic enzymes into the cytosol causing further degradation of the cells and subsequent release of the cell contents into the extracellular matrix, ultimately leading to cell death [48]. Necrosis typically result from infection, envenomation, injury, or deprivation of blood supply (ischemic necrosis) [49]. Consequences range from minor wounding causing discomfort, to extensive tissue necrosis, causing permanent disfigurement, impairment and disability [50].

Until the late 1980s, necrosis was considered an unregulated process, contrasting to the regulated, programmed cell death known as apoptosis [51]. Apoptosis occurs in individual cells (or sometimes small clusters) that are recognized as experiencing stress by either themselves (intrinsic) or by other nearby cells (extrinsic). The cell shrinks in size, the cellular components are degraded by enzymes, but cell membrane integrity is retained until the end, which avoids triggering an inflammatory response, and then signals for the recruitment of macrophages which engulf the cell. Apoptosis is a cell death process crucial for efficiently maintaining healthy tissue and regulating tissue growth [48]. However, the current consensus is that necrosis can also be a programmed event (necroptosis), resulting from a regulated process that can be favoured by the immune system over apoptosis [52–54].

Tumour necrosis factor and the necroptosis pathway

At the cellular level, cytokines, including interleukin and tumour necrosis factor (TNF), mediate communication through signalling pathways and play important roles as mediators in inflammatory cell death. As a result, the suppression of TNF-α activity using metalloproteinase inhibitors significantly reduces TNF-α associated pathologies [55].

TNF is a pro-inflammatory cytokine that plays a fundamental role in regulating immune response, inflammation and cell death/survival. It acts with other cytokines as important cell signalling molecules for recruiting immune cells from nearby blood vessels to the sites where foreign bodies occur [36]. TNF also plays an important role in determining the fate of affected cells, if it will live or die, and by which process. In recent years, studies have shed light on the processes (Figure 1(A)) by which necroptosis is the favoured outcome over apoptosis [52,56]. Following the binding of TNF with its corresponding receptors (TNFR1, TNFR2; Table 1) located on cell surface membranes, a cascade is set in motion which determines the life or death of the cell. Several molecules associate to form a large protein complex (complex I) [52,56] including TNFR1-associated death domain protein (TRADD), TNFR-associated factors (TRAF2 or TRAF5), inhibitor of apoptosis proteins (cIAP1 or cIAP2) which are linked by K63-linked ubiquitin molecule chains with linear ubiquitin chain assembly complex (LUBAC) and then with receptor-interacting serine/threonine-protein kinase (RIPK1) [52,56].

In living cells, survival is regulated by the transcription factor nuclear factor-κB (NF-κB) pathway. After the formation of complex I, ubiquitylation connects complex I to the IKK complex which activates NF-κB signalling, which in turn leads to the production of anti-apoptotic factors that prevent cell death and thus promotes cell survival. The cell death

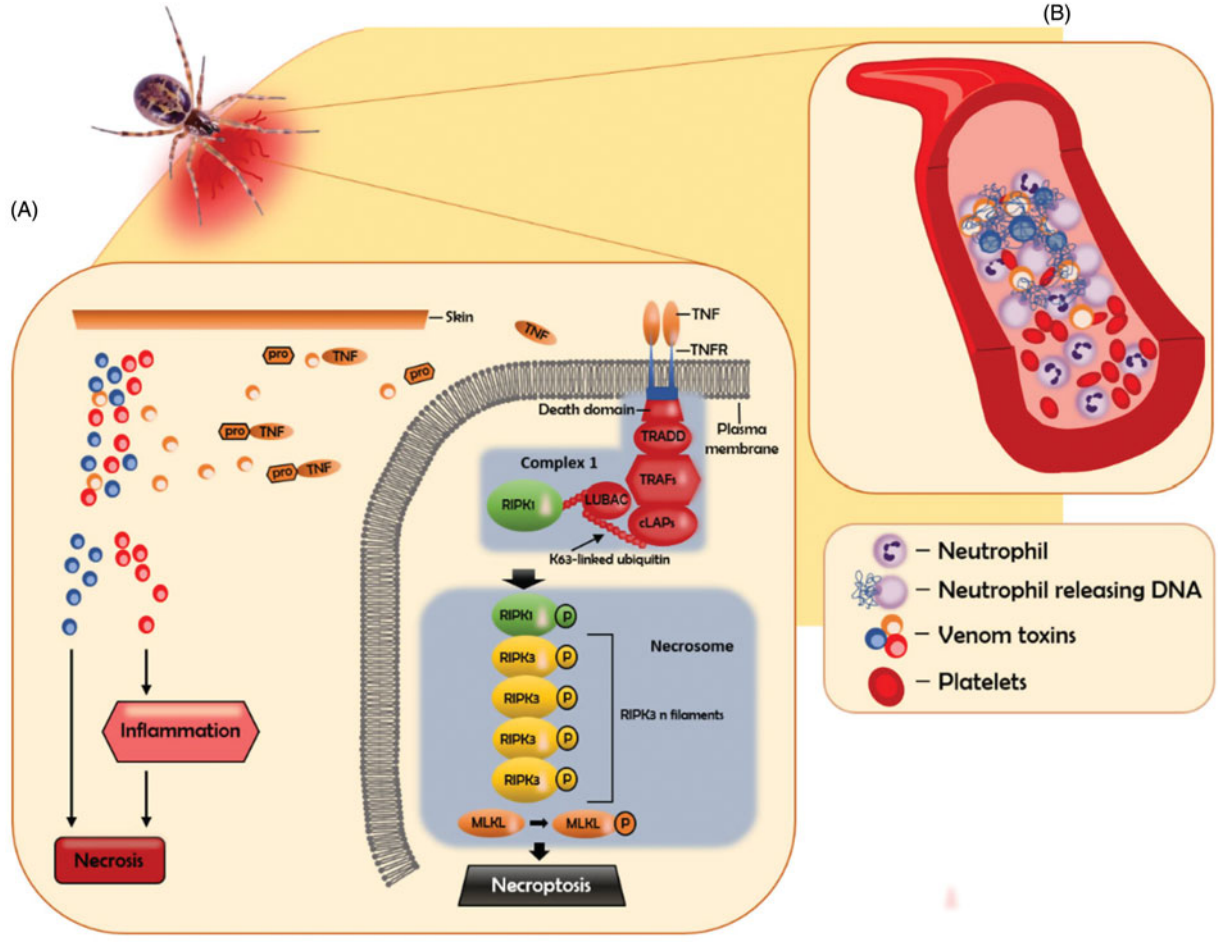

Figure 1. Potential inflammatory pathways to necrosis after envenomation. (A) Illustrates various ways different toxins may contribute to necrosis. Blue venom toxins represent direct lytic activity, red venom toxins represent trigger of inflammation which leads to necrosis (regulated necroptosis), and orange venom toxin represents the convertase activity suggested by Moura-da-Silva et al. [3] which converts inactive TNF to its active state and further contributing to the necroptosis pathway. Illustration adapted from (Brenner et al. [56] and Moura-da-Silva et al. [3]). (B) Blood vessel blockage resulting from NETs, illustrating the NET activity once triggered by certain toxins. Once NETs are activated they trap numerous molecules including venom toxins and platelets, potentially leading to vessel blockage causing ischemic necrosis or toxin concentration that could lead to localized activity of necrotic compounds.

process is initiated when non-ubiquitylated RIPK1 results in switching off TNF-driven NF-κB signalling, which in turn promotes TNF-driven apoptotic signalling. When the enzymatic activity of $FLIP_L$–pro-caspase 8 heterodimer is inhibited, it is unable to cleave RIPK1, and then, deubiquitylated RIPK1 can influence the cell death programme to favour the pathway for necroptosis over apoptosis. Non-unbiquitylated or deubiquitylated RIPK1 disassociates from complex I, which is bound to the membrane. After relocating in the cytosol, it forms an association with multiple RIPK3 complexes. These interactions trigger the formation of a new complex called the necrosome. The elevated concentrations of RIPK1/3 strongly influence necroptosis as the outcome [52,56].

TNF has been shown to play a significant role in snake venom-induced pathologies. Following inoculation with venom from the European asp (*Vipera aspis*), patients can experience cardiotoxic effects. A study suggested that this pathology is mediated by the systemic circulation of TNF. Prior treatment with TNF antibodies showed a significant decrease in cardiac pathology [44].

The development of necrosis in victims of envenomation by the South American pit viper *Bothrops jararaca* and the northern East African Saw-scaled viper *Echis pyrumidum leukeyi* has been shown to be directly influenced by TNF-α. Moura-da-Silva et al. [3] hypothesised that the pro-inflammatory cytokine TNF-α may participate to the development of tissue necrosis after envenomation by vipers. In addition to the direct potent action of venom toxins on cells, the authors [3] suggested that venom components such as metalloproteinases have a convertase activity that facilitates the hydrolysis of pro-TNF-α, converting it to its active state which further intensifies the level of necrosis in the victim (Figure 1(A)). In agreement with this, the authors demonstrated that treatment with TNF-α-antibodies would significantly reduce the manifestation of necrosis.

In concurrence with this hypothesis, a subsequent study by Laing et al. [4] showed that, when Balb C and C57 mouse KO strains deficient in TNF receptors (TNFR1 and TNFR2) and IL-6 were injected with the venom metalloproteinase jararhagin, necrosis did not manifest. While this venom toxin typically induces haemorrhage, oedema and necrosis in bite victims, in this study, haemorrhage and oedema continued to manifest in these KO strains at similar levels as to the WT control mice, but necrosis was consistently absent. This

Table 1. Nomenclature.

Protein	Abbreviation	Description
Transmembrane receptor molecules	TNFR1, TNFR2	Transmembrane receptor molecules that bind Tumour Necrosis Factor with cellular transduction pathways
TNFR1-associated death domain protein	TRADD	Intracellular adaptor protein that link proteins to create larger signalling complexes
TNFR-associated factors	TRAF2 or TRAF5	Adapter proteins that associate with specific tumour necrosis factor family receptors
Cellular inhibitor of apoptosis proteins	cIAP1 or cIAP3	Inhibitor of apoptosis protein that ubiquitinates RIP1
Linear ubiquitin chain assembly complex	LUBAC	Ubiquitin ligase complex that generates linear polyubiquitin chains and regulates the NF-kB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) pathway
Receptor-interacting serine/threonine-protein kinase	RIPK1, 3	Adaptor proteins whose kinase activity regulate eachothers phosphorylation through autotransphosphorylation
Inhibitor of kappa B	IKK complex	Protein that inactivates NF-κB transcription factor
FLICE-like inhibitory protein & cysteine-aspartic proteases	$FLIP_L$–pro-caspase 8 heterodimer	$FLIP_L$ is a regulatory protein of apoptosis, caspase 8 is a protease that regulates apoptosis. By forming a heterodimer, enzymatic activity is inhibited allowing for necroptosis to occur
Lysine 63-linked polyubiquitin chains	K63-linked ubiquitin molecule chains	Polyubiquitin chain linkages which are significant for linking substrates to distinct signal transduction pathways.

suggests that TNF might not be the sole target of jararhagin, and that cytokines other than TNF might be involved in the initiation of necrosis.

The inflammatory cascade and the role of important cytokines including TNF and interleukin in envenomation described by Moura-da-Silva et al. [3] and Laing et al. [4] tend to indicate that under certain circumstances the immune response can act independently against the host and contribute to venom-induced necrosis. In such cases, in addition to antivenom, TNF antibodies may be important in inhibiting the progression of tissue necrosis [50,57].

The influence of TNF in determining the outcome of a cell death event has also been described for bacterial pathogenesis. For example, in response to bacterial proliferation within phagocytic cells, the expression levels of TNF signalling play an important role in determining the three possible outcomes for the infected phagocyte. Low expressions of TNF signalling results in unregulated necrosis, whereas moderate expression levels of TNF result in the release of bactericidal molecules that ensure the survival of the cell. However, if the TNF expression levels are elevated, phagocytic cells can undergo another process via the RIPK1-RIPK3-mediated pathway which ultimately leads to necroptosis [53].

Neutrophil extracellular traps and the NETosis pathway

Upon activation of the immune system, some of the first line responders are leukocytes, for example, macrophages and neutrophils. Because infected macrophages can relocate and ultimately redistribute pathogens, the regulated necrosis of localized cells is considered a means to control the spread of infection at the expense of these local cells [53]. In addition to phagocytosis, neutrophils can also be triggered to undergo "cellular suicide" in a process during which the chromatin becomes untightened and the nucleus swells subsequently releasing nucleoplasm into the cytoplasm. The cell dies when its membrane ruptures and discharges its DNA contents known as neutrophil extracellular traps (NETs) in the extracellular matrix. NETs prevent bacteria disseminating farther by entangling them with a mesh of DNA strands [5,58]. Previously regarded as an NADPH oxidase–dependent cellular death process [59], recent studies indicate that NET discharge can occur independently of NADPH oxidase (NOX) and is now regarded as a non-universal pathway to cell death [58] because of its ability to be stimulated by either NOX-dependent, NOX-independent pathways, or even by both simultaneously [5].

NETs have been shown to be involved in coagulation and identified as potentially important in deep vein thrombosis due to their potential to interact with components of the blood clotting cascade [60]. As a result, NETs are a recognized target for treating deep vein thrombosis. In animal models, treatment with DNase 1, an enzyme that non-specifically cleaves DNA to release 5'-phosphorylated di-, tri-, and oligonucleotide products, inhibited the development of thrombus formation [61]. Pathogenic bacteria can express DNase which facilitates the denaturation of DNA strands, rendering the NETs inefficient [62]. It should also be noted that some snake venoms contain endonucleases [63]. In the event of NET initiation, the presence of snake venom endonucleases could restrict NET function and facilitate circulation of venom toxins from the bite site.

Envenomation by snakes such as *Echis* species often lead to complications such as coagulopathies, hemorrhage, and notably severe localized tissue necrosis at the bite site. Recent studies describe venom-induced NETs release, which subsequently limits the dissemination of venom toxins (Figure 1(B)) away from the bite site [5,64,65]. As a consequence, the venom toxins accumulate at the bite site, resulting in significant localized tissue damage. Moreover, NET activity is facilitated by the absence of endonucleases, which is a common constituent of other snake venoms. NETs have only been recently described as potentially significant in snake venom induced pathologies such as localised tissue necrosis and as a target for envenomation treatment using

nucleases such as DNase 1 [5]. It should also be noted that the release of extracellular traps is not limited to neutrophils and venom could potentially induce chromatin release from other cells [61].

The aforementioned studies demonstrate the potentially fundamental role of the immune system in determining the outcome of an envenomation. Within this paradigm, venom composition alone does not dictate the outcome of the bite, and intense inflammation can significantly contribute to the development of tissue necrosis.

Arthropod venom driving necrosis

The genus *Loxosceles* comprises approximately 130 species of medium-size spiders distributed globally. The neotropical species *L. laeta*, *L. intermedia*, *L. rufescens*, *L. gaucho* and *L. reclusa* are frequently involved in medically significant bites which result in localised and systemic pathological symptoms, including dermonecrosis. *Loxosceles* venom possess phospholipases D which causes hydrolysis of sphingomyelin resulting in the lysis of muscle fibres. However, while most studies tend to focus on the direct lytic activity of *Loxosceles* venom, few have assessed the immune status of the host.

Ribeiro et al. [45] investigated the toxicity of *Loxosceles intermedia* and the differential immune response of three mouse strains (C57, BalbC and the immune-deficient Swiss strain) to its venom. The authors demonstrated phospholipases D, hyaluronidase, metalloproteases, and serine proteases activity in the venom of *Loxosceles intermedia*. In addition to increasing venom potency, hyaluronidase facilitates the dissemination of venom from the bite site [66]. Phospholipases A2, which are thought to be the main compounds involved in the development of necrosis following snake bites [63] was not recovered. However, the authors suggested this could have been due to a low abundance of Phospholipase A2 within the pooled sample, and not their absence.

Following intradermic injection of *Loxosceles intermedia* venom, C57 and BalbC mouse strains experienced a differential inflammatory response. Immune-deficient Swiss mice showed no signs of venom-induced inflammatory response but some signs of vascular congestion.

All three mouse strains developed oedema, which persisted for 16hrs in Swiss, and 24hrs in C57 and BalbC mice. Dermal necrosis was not observed during this study, but intense inflammatory infiltrate was observed in C57 and BalbC mice [45]. All C57 and BalbC mice died between three and six days post-injection, but Swiss mice lived up to 30 days post-injection. Although unlikely, it is possible that dermal necrosis would have manifested itself if C57 and BalbC mice had survived longer. The longer survival rate and absence of inflammatory response in the Swiss strain may be due to the fact that relevant chemoattractant cytokines are expressed at low levels in Swiss mice [67]. Contrasting patterns of cell mobilization in the blood, bone marrow and spleen of C57 and BalbC mice indicated a venom induced innate and adaptive inflammatory response. It would have been interesting however, if the authors had included the Swiss mice when analysing cell mobilization. While no inflammatory reaction was observed in the histological analysis of the skin, it may have produced an interesting comparison to C57 and BalbC in the blood, bone marrow and spleen [45].

Phospholipases D from *Loxosceles laeta* venom stimulate the induction of intense inflammatory mediators which subsequently recruit monocytes to the bite site [36]. Following the cleavage of sphingomyelin by phospholipase D, the subsequent release of ceramide 1-phosphate (C1P) can act as a pro-inflammatory mediator [68]. Patel et al. [46] showed *in vitro* that the venom of *Loxosceles deserta* only leads to dermonecrosis when in the presence of infiltrating neutrophils, triggered indirectly by ceramides released from the breakdown products of sphingomyelin. The subsequent release of ceramides is significant during envenomation because they play an important role as intermediaries that regulate TNF-α and recruit neutrophils [47]. Previous studies [42] suggest that *Loxosceles* venom does not produce dermonecrosis in mice because the sphingomyelin in mice structurally differs from rabbits, guinea pigs and humans. In mice, the venom can diffuse farther, avoiding a localized buildup of venom at the bite site, but can result in a lethal effect, whereas sphingomyelin in rabbits, guinea pigs and humans reduces the spread of venom, causing venom to accumulate thus resulting in necrosis, but prolonging the animal's life. As a result, phospholipase D directly hydrolyses sphingomyelin, ultimately leading to the release of ceramides and the subsequent induction of inflammatory infiltrate which leads to localized necrosis. Domingos et al. [42] demonstrated using BALB/c mice that venom from *Loxosceles gaucho* only induced dermonecrosis at the bite site when co-administered with sphingomyelin or ceramide phosphate plus liposome. In this study, mice injected with only venom died, whereas although the addition of sphingomyelin or ceramide phosphate resulted in inflammatory response and subsequent dermonecrosis, these mice all lived.

Hemiscorpius lepturus is a medically important scorpion from West Asia that can induce local and systemic symptoms including dermonecrosis, renal failure and hemolysis [27] and is responsible for fatal outcomes. *Hemiscorpius lepturus* venom possesses heminecrolysin, a phospholipase D-like enzyme [69]. Studies have drawn on similarities between the molecular weights of venom compounds of *Hemiscorpius* and *Loxosceles* suggesting that symptoms including dermonecrosis may also be induced through activation of the immune system [70,71]. In a study by Jalali et al. [27], serum collected from 36 hospital patients in the southwest of Iran after being admitted following envenomation by *Hemiscorpius lepturus* showed that concentrations of cytokines IL-1, IL-6, IL-8 and TNF-α were higher than those in healthy, non-envenomated patients. The authors found that the severity of the envenomation correlated specifically with the elevated concentrations of TNF-α and suggests that these elevated cytokine concentrations and pathology are related. In the same study, patients stung by *Mesobuthus eupeus* also showed increased concentrations of interleukins but not TNF-α. Envenomation from the latter species is

considered typically mild and have never been associated with dermonecrosis.

Intense recruitment of inflammatory cells and mediators can potentially lead to disturbance in the blood [45]. Heavy traffic within the blood capillaries due to venom-induced inflammatory response could therefore potentially also lead to ischemic necrosis (Figure 1(B)). Necrotic lesions could therefore occur inconsistently and on an individual basis, being indirectly triggered by inflammation-inducing venom toxins rather than necrotic compounds; the outcome of envenomation would also depend on the immune status of the victim.

Variations in outcome after envenomation can also be explained by the volume of venom delivered. Snakes, spiders, centipedes and scorpions have been shown to adapt the amount of venom delivered according to the level of the perceived threat (cf. Venom Optimisation Hypothesis) [72–74]. In addition, venom glands may take several days or even weeks to replenish their stores and venom compounds may not be produced at the same rate. Therefore, envenomation by the same individual specimen may result in different subsequent immune response depending on the level of toxin replenishment and the quantity of venom injected [45]. This hypothesis is reinforced by the fact that in the case of envenomation by hymenopteran and dipteran, necrotic lesions developed only after multiple, simultaneous envenomations [2,8–10]. Regarding ischaemic necrosis, NETs could also potentially play a role in trapping material within blood capillaries and thus venom volume may have a critical role.

Conclusions

Arthropod venom can induce a complex and intense inflammatory response. Although typically most arthropod venoms are not associated with direct necrotic activity, it is proposed that envenomation could in some circumstances trigger an immune cascade ultimately leading to necrosis. This would explain the inconsistent and circumstantial occurrence of necrotic manifestations following envenomation by venomous arthropods. The role of TNF and neutrophil extracellular traps in snake venom-induced pathologies is remarkable, especially from the stand point of using TNF antibodies and DNase 1 as potential therapeutic agents to complement traditional antivenom treatment. Clinicians should consider the victim's immune response as an integral part of the envenomation syndrome. Focusing research into identifying TNF necroptosis, neutrophil NETosis or other pathways involved in regulated necrosis following envenomation may help determine more efficient ways to manage inflammation and potentially reduce the severity of symptoms.

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References

[1] Brodie ED. Toxins and venoms. Curr Biol. 2009;19:R931–R935.
[2] Mingomataj EC, Bakiri AH, Ibranji A. Unusual reactions to hymenoptera stings: what should we keep in mind? Clin Rev Allergy Immunol. 2014;47:91–99.
[3] Moura -da -Silva AM, et al. Processing of pro-tumor necrosis factor-α by venom metalloproteinases: a hypothesis explaining local tissue damage following snake bite. Eur J Immunol. 1996;26:2000–2005.
[4] Laing GD, Clissa PB, Theakston RDG, et al. Inflammatory pathogenesis of snake venom metalloproteinase-induced skin necrosis. Eur J Immunol. 2003;33:3458–3463.
[5] Katkar GD, Sundaram MS, NaveenKumar SK, et al. NETosis and lack of DNase activity are key factors in *Echis carinatus* venom-induced tissue destruction. Nat Commun. 2016;7:11361.
[6] Cegolon L, Heymann WC, Lange JH, et al. Jellyfish stings and their management: a review. Mar Drugs. 2013;11:523–550.
[7] García-Arredondo A, Rojas A, Iglesias-Prieto R, et al. Structure of nematocysts isolated from the fire corals *Millepora alcicornis* and *Millepora complanata* (Cnidaria: Hydrozoa). J Venom Anim Toxins Incl Trop Dis. 2012;18:109–115.
[8] Yanagawa Y, Morita K, Sugiura T, et al. Cutaneous hemorrhage or necrosis findings after Vespa mandarinia (wasp) stings may predict the occurrence of multiple organ injury: a case report and review of literature. Clin Toxicol (Phila). 2007;45:803–807.
[9] Knight D, Bangs MJ. Case report. Cutaneous allergic vasculitis due to *Solenopsis geminata* (Hymenoptera: Formicidae) envenomation in Indonesia. Southeast Asian J Trop Med Public Health. 2007;38:808.
[10] Chiu TM, Lin YM, Wang SC, et al. Hypersensitivity to mosquito bites as the primary clinical manifestation of an Epstein–Barr virus infection. J Microbiol Immunol Infect. 2016;49:613–616.
[11] Srihari S, Kombettu AP, Rudrappa KG, et al. Paederus dermatitis: a case series. Indian Dermatol Online J. 2017;8:361–364.
[12] Fung T, Lam SK, Wong OF. Centipede bite victims: a review of patients presenting to two emergency departments in Hong Kong. Hong Kong Med J. 2011;17:381–385.
[13] de Oliveira Pardal PP, Arraes JAA, Weekes KW, et al. Dermatitis caused by centipede envenomation: a case report. Rev Patol Trop. 2017;46:343–348.
[14] Chadha JS, Leviav A. Hemolysis, renal failure, and local necrosis following scorpion sting. JAMA. 1979;241:1038
[15] Radmanesh M. Cutaneous manifestations of the *Hemiscorpius lepturus* sting: a clinical study. Int J Dermatol. 1998;37:500–507.
[16] Boissiere F, Masson R, Fluieraru S, et al. Cutaneous loxoscelism, about an exceptional observation of 9 consecutive cases. Ann Chir Plast Esthet. 2016;61:811–819.
[17] Dunbar JP, Afoullouss S, Sulpice R, et al. Envenomation by the noble false widow spider *Steatoda nobilis* (Thorell, 1875)–five new

cases of steatodism from Ireland and Great Britain. Clin Toxicol. 2018;56:433–435.
[18] Stuber M, Nentwig W. How informative are case studies of spider bites in the medical literature? Toxicon. 2016;114:40–44.
[19] Vetter RS, Isbister GK, Bush SP, et al. Verified bites by yellow sac spiders (genus Cheiracanthium) in the United States and Australia: where is the necrosis? Am J Trop Med Hyg. 2006;74: 1043–1048.
[20] Nentwig W, Pantini P, Vetter RS. Distribution and medical aspects of *Loxosceles rufescens,* one of the most invasive spiders of the world (Araneae: Sicariidae). Toxicon. 2017;132:19–28.
[21] Vetter RS, Stoecker WV, Dart RC, Envenomations by widow, recluse, and medically implicated spiders. Dordrecht: Springer; 2018. p. 379–412.
[22] Saez NJ, Senff S, Jensen JE, et al. Spider-venom peptides as therapeutics. Toxins (Basel). 2010;2:2851–2871.
[23] Undheim EA, Jones A, Clauser KR, et al. Clawing through evolution: toxin diversification and convergence in the ancient lineage *Chilopoda* (Centipedes). Mol Biol Evol. 2014;31:2124–2148.
[24] Bawaskar HS, Bawaskar PH. Scorpion sting: update. J Assoc Phys India. 2012;60:46–55.
[25] Logan JL, Ogden DA. Rhabdomyolysis and acute renal failure following the bite of the giant desert centipede *Scolopendra heros*. West J Med. 1985;142:549
[26] Jalali A, Pipelzadeh MH, Sayedian R, et al. A review of epidemiological, clinical and in vitro physiological studies of envenomation by the scorpion *Hemiscorpius lepturus* (Hemiscorpiidae) in Iran. Toxicon. 2010;55:173–179.
[27] Jalali A, Pipelzadeh MH, Taraz M, et al. Serum TNF-α levels reflect the clinical severity of envenomation following a *Hemiscorpius lepturus* sting. Eur Cytokine Netw. 2011;22:5–10.
[28] Vetter RS. Clinical consequences of toxic envenomation by spiders. Toxicon. 2018;152:65–70.
[29] Gaver-Wainwright MM, Zack RS, Foradori MJ, et al. Misdiagnosis of spider bites: bacterial associates, mechanical pathogen transfer, and hemolytic potential of venom from the hobo spider, *Tegenaria agrestis* (Araneae: Agelenidae). J Med Entomol. 2011;48: 382–388.
[30] White J, Weinstein SA. A phoenix of clinical toxinology: White-tailed spider (*Lampona* spp.) bites. A case report and review of medical significance. Toxicon. 2014;87:76–80.
[31] Swanson DL, Vetter RS. Loxoscelism. Clin Dermatol. 2006;24: 213–221.
[32] Cohen N, Sarafian DA, Alon I, et al. Dermonecrotic loxoscelism in the Mediterranean region. Cutan Ocul Toxicol. 1999;18:75–83.
[33] Rubenstein E, Stoebner PE, Herlin C, et al. Documented cutaneous loxoscelism in the south of France: an unrecognized condition causing delay in diagnosis. Infection. 2016;44:383–387.
[34] Garb JE, González A, Gillespie RG. The black widow spider genus *Latrodectus* (Araneae: Theridiidae): phylogeny, biogeography, and invasion history. Mol Phylogenet Evol. 2004;31:1127–1142.
[35] Gendreau KL, Haney RA, Schwager EE, et al. House spider genome uncovers evolutionary shifts in the diversity and expression of black widow venom proteins associated with extreme toxicity. BMC Genomics. 2017;18:178.
[36] Rojas JM, Arán-Sekul T, Cortés E, et al. Phospholipase D from *Loxosceles laeta* spider venom induces IL-6, IL-8, CXCL1/GRO-α, and CCL2/MCP-1 production in human skin fibroblasts and stimulates monocytes migration. Toxins. 2017;9:125.
[37] Ahrens B, Crocker C. Bacterial etiology of necrotic arachnidism in black widow spider bites. J Clinic Toxicol. 2011;1:106.
[38] Atakuziev BU, Wright CE, Graudins A, et al. Efficacy of Australian red-back spider (*Latrodectus hasselti*) antivenom in the treatment of clinical envenomation by the cupboard spider *Steatoda capensis* (Theridiidae). Toxicon. 2014;86:68–78.
[39] Vetter RS, Swanson DL, Weinstein SA, et al. Do spiders vector bacteria during bites? The evidence indicates otherwise. Toxicon. 2015;93:171–174.
[40] Hauke TJ, Herzig V. Dangerous arachnids-Fake news or reality? Toxicon. 2017;138:173–183.
[41] Esmaeilishirazifard E, Usher L, Trim C, et al. Microbial adaptation to venom is common in snakes and spiders. bioRxiv. 2018;348: 433.
[42] Domingos MO, Barbaro KC, Tynan W, et al. Influence of sphingomyelin and TNF-alpha release on lethality and local inflammatory reaction induced by Loxosceles gaucho spider venom in mice . Toxicon. 2003;42:471–479.
[43] Hogan CJ, Barbaro KC, Winkel K. Loxoscelism: old obstacles, new directions. Ann Emerg Med. 2004;44:608–624.
[44] Szold O, Ben-Abraham R, Frolkis I, et al. Tumor necrosis factor as a mediator of cardiac toxicity following snake envenomation. Crit Care Med. 2003;31:1449–1453.
[45] Ribeiro MF, Oliveira FL, Monteiro-Machado M, et al. Pattern of inflammatory response to *Loxosceles intermedia* venom in distinct mouse strains: a key element to understand skin lesions and dermonecrosis by poisoning. Toxicon. 2015;96:10–23.
[46] Patel KD, Modur V, Zimmerman GA, et al. The necrotic venom of the brown recluse spider induces dysregulated endothelial cell-dependent neutrophil activation. Differential induction of GM-CSF, IL-8, and E-selectin expression . J Clin Invest. 1994;94: 631–642.
[47] Domingos MO, Tynan W, Barbaro KC, et al. Effect of *Loxosceles gaucho* venom on cell morphology and behaviour in vitro in the presence and absence of sphingomyelin. Toxicon. 2003;42: 439–445.
[48] Elmore SA, Dixon D, Hailey JR, et al. Recommendations from the INHAND apoptosis/necrosis working group. Toxicol Pathol. 2016; 44:173–188.
[49] Adigun R, Bhimji SS, Necrosis, cell (liquefactive, coagulative, caseous, fat, fibrinoid, and gangrenous). Treasure Island (FL): StatPearls Publishing; 2017.
[50] Harrison RA, Cook DA, Renjifo C, et al. Research strategies to improve snakebite treatment: Challenges and progress. J Proteomics. 2011;74:1768–1780.
[51] Vandenabeele P, Galluzzi L, Berghe TV, et al. Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat Rev Mol Cell Biol. 2010;11:700.
[52] Berghe TV, Linkermann A, Jouan-Lanhouet S, et al. Regulated necrosis: the expanding network of non-apoptotic cell death pathways. Nat Rev Mol Cell Biol. 2014;15:135–147.
[53] Blériot C, Lecuit M. The interplay between regulated necrosis and bacterial infection. Cell Mol Life Sci. 2016;73:2369–2378.
[54] Pasparakis M, Vandenabeele P. Necroptosis and its role in inflammation. Nature. 2015;517:311
[55] McGeehan GM, Becherer JD, Bast Jr RC, et al. Regulation of tumour necrosis factor-α processing by a metalloproteinase inhibitor. Nature. 1994;370:558.
[56] Brenner D, Blaser H, Mak TW. Regulation of tumour necrosis factor signalling: live or let die. Nat Rev Immunol. 2015;15:362
[57] Ferraz CR, Calixto-Campos C, Manchope MF, et al. Jararhagin-induced mechanical hyperalgesia depends on TNF-α, IL-1β and NFκB in mice. Toxicon. 2015;103:119–128.
[58] Yipp BG, Kubes P. NETosis: how vital is it? Blood. 2013;122: 2784–2794.
[59] Fuchs TA, Abed U, Goosmann C, et al. Novel cell death program leads to neutrophil extracellular traps. J Cell Biol. 2007;176: 231–241.
[60] Cimmino G, Salvatore F, Paolo G, The two faces of thrombosis: coagulation cascade and platelet aggregation. Are platelets the main therapeutic target? J Thrombo Cir. 2017;3:117.
[61] Brill A, Fuchs TA, Savchenko AS, et al. Neutrophil extracellular traps promote deep vein thrombosis in mice. J Thromb Haemost. 2012;10:136–144.
[62] Fuchs TA, Brill A, Wagner DD. Neutrophil extracellular trap (NET) impact on deep vein thrombosis. Arterioscler Thromb Vasc Biol. 2012;32:1777–1783.
[63] Dhananjaya BL, D Souza CJM. An overview on nucleases (DNase, RNase, and phosphodiesterase) in snake venoms. Biochemistry Mosc. 2010;75:1–6.

[64] Bennacef-Heffar N, Laraba-Djebari F. Beneficial effects of heparin and l arginine on dermonecrosis effect induced by *Vipera lebetina* venom: involvement of NO in skin regeneration. Acta Tropica. 2017;171:226–232.
[65] Slagboom J, Kool J, Harrison RA, et al. Haemotoxic snake venoms: their functional activity, impact on snakebite victims and pharmaceutical promise. Br J Haematol. 2017;177:947–959.
[66] Kemparaju K, Girish KS. Snake venom hyaluronidase: a therapeutic target. Cell Biochem Funct. 2006;24:7–12.
[67] Marques SM, Campos PP, Castro PR, et al. Genetic background determines mouse strain differences in inflammatory angiogenesis. Microvasc Res. 2011;82:246–252.
[68] Rivera IG, Ordoñez M, Presa N, et al. Sphingomyelinase D/ceramide 1-phosphate in cell survival and inflammation. Toxins (Basel). 2015;7:1457–1466.
[69] Khodadadi A, Pipelzadeh MH, Vazirianzadeh B, et al. An *in vitro* comparative study upon the toxic properties of the venoms from *Hemiscorpius lepturus*, *Androctonus crassicauda* and *Mesobuthus eupeus* scorpions. Toxicon. 2012;60:385–390.
[70] Zare MA. *Hemiscorpius lepturus* envenomation: manifestations and management with specific antivenom. Archiv Razi Inst. 2013; 68:91–99.
[71] Seyedian R, Pipelzadeh MH, Jalali A, et al. Enzymatic analysis of *Hemiscorpius lepturus* scorpion venom using zymography and venom-specific antivenin. Toxicon. 2010;56:521–525.
[72] Wigger E, Kuhn-Nentwig L, Nentwig W. The venom optimisation hypothesis: a spider injects large venom quantities only into difficult prey types. Toxicon. 2002;40:749–752.
[73] Dugon MM, Arthur W. Prey orientation and the role of venom availability in the predatory behaviour of the centipede *Scolopendra subspinipes mutilans* (Arthropoda: Chilopoda). J Insect Physiol. 2012;58:874–880.
[74] Morgenstern D, King GF. The venom optimization hypothesis revisited. Toxicon. 2013;63:120–128.