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Immunological Responses to Envenomation

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Venoms are complex mixtures of toxic compounds delivered by bite or sting. In humans, the consequences of envenomation range from self-limiting to lethal. Critical host defence against envenomation comprises innate and adaptive immune strategies targeted towards venom detection, neutralisation, detoxification, and symptom resolution. In some instances, venoms mediate immune dysregulation that contributes to symptom severity. This review details the involvement of immune cell subtypes and mediators, particularly of the dermis, in host resistance and venom-induced immunopathology. We further discuss established venom-associated immunopathology, including allergy and systemic inflammation, and investigate Irukandji syndrome as a potential systemic inflammatory response. Finally, this review characterises venom-derived compounds as a source of immune modulating drugs for treatment of disease.

Keywords: venom, detoxification, innate immunity, adaptive immunity, immunopathology, Irukandji syndrome, venom allergy, systemic inflammation

INTRODUCTION

Venoms are complex mixtures of proteins, peptides, biogenic amines, and salts produced by a diverse range of animals for predation, protection, and competition (1–4). In humans, needle-like stinging apparatuses inject venom compounds into dermal-epidermal junctions, capillary vessels, and skeletal muscle fibres (5). The consequences of envenomation range from innocuous to lethal (6, 7).

As the initial site of venom's interaction with the immune system, the dermis provides protection through physical, chemical, and cellular defence mechanisms (8, 9). Prominent defenders in the dermal immune network include keratinocytes, endothelial cells, and tissue-resident and infiltrating immune cells for fast and non-specific responses (innate) and acquired long term protection (adaptive) (8, 9). The primary role of these cells is host defence. However, venom-mediated immune dysregulation can contribute to envenomation severity (10). Accordingly, this review discusses both the protective and pathological responses of barrier cells and the immune system towards venom compounds.

INNATE RESPONSES TO ENVENOMATION

Defence against envenomation requires an acute response achieved by the body's innate immune system. Innate mechanisms comprise barrier and cellular defences for immediate but non-specific resistance to foreign bodies (such as venom compounds), injuries, and pathogens. Physical barriers (skin and mucosal membranes) and secretions (chemical substances and enzymes) along with resident and infiltrating immune cells provide readily available protection without requiring prior exposure to the damaging compounds (11). Instead, sentinel and scavenger cells express receptors that sense evolutionarily conserved structures common to microbes, cellular stress, and harmful substances (12).

A wide diversity of innate signalling receptor and response types is responsible for efficient detection and neutralisation/elimination of various host threats (12). The detection of danger or stress signals initiates proinflammatory events. Broadly, these include the production of cytokines and chemokines for immune cell recruitment/activation, the release of antimicrobial peptides that directly kill pathogens, the phagocytosis and destruction of foreign particles and microbes, the generation of reactive oxygen species (ROS), reactive oxygen intermediates, and reactive nitrogen intermediates, and the release of enzymes with potent protein degrading and microbicidal properties (11).

Regulated innate effector functions are also critical for tissue repair and homeostasis (13). In addition, the presentation of foreign macromolecules, required for the establishment of acquired (adaptive) immune responses, is achieved by innate antigen-presenting cells (APC), including dendritic cells (DCs), monocytes (MNCs), and macrophages (MΦ) (11). Likewise, plasma proteins, including those of the complement system (an ancient protein defensive system), promote inflammation or directly kill pathogens (14).

Detection of venom compounds by innate mechanisms initiates inflammatory reactions critical to host protection, venom detoxification, and ultimately the resolution of symptoms (15, 16). Participation by the epidermis, endothelium, neutrophils, MNCs, MΦs, mast cells, and soluble effector mediators increases host resistance to the damaging events of bites and stings. Yet, as discussed below, many venom constituents can augment the activity of these components leading to venom-induced, immune-mediated host damage.

Epidermis (Keratinocytes)

The epidermis, the outermost layer of the skin, comprises 95% keratinocytes arranged in four layers (17). Tight junctions formed by keratinocyte-derived proteins provide a physical barrier from the external environment and structural support for Langerhans cells (epidermal-resident DCs), melanocytes, Merkel cells (tactile epithelial cells), and sensory neurons (18). Keratinocytes serve important sentinel and proinflammatory functions, where cross-talk between keratinocytes and cells of the dermal-epidermal junction direct immune cell function and maturation during both initial and late phases of inflammation (19). Like cells of the immune system, keratinocytes express cytokine receptors and pattern-recognition receptors (PRRs), enabling the detection of pathogen-, damage-, and venom-associated molecular patterns (PAMPs, DAMPs, and VAMPS) (20, 21). Activation of keratinocyte proinflammatory genes, such as by venom compounds, initiates the synthesis and release of cytokines, nitric oxide (NO), and alarmins, stimulating resident immune cells and attracting immune cell infiltration (20, 21).

To counteract this defensive barrier, many animal venoms contain matrix metalloproteinase (MMP) and L-Amino acid oxidase (LAAO) enzymes (22–25). Venom-derived MMPs and LAAOs can induce keratinocyte cell death by autophagy, apoptosis, or necrosis (22–25). Proteolytic degradation of the dermis facilitates access of venom-derived toxins to the circulation, lymphatics, and target organs for prey/predator immobilisation (22–25). In some instances, the induction of apoptosis stimulates the overexpression of endogenous MMPs, indirectly triggering tissue destruction (26). For example, brown recluse spider (*Loxosceles rufescens*) bites cause significant dermonecrotic effects, systemic inflammation, and potentially death in children (26). Interestingly, the molecular mechanism underpinning the initiation of cutaneous necrosis (a common reaction in loxoscelism) involves keratinocyte-derived enzymes (26). Induction of apoptosis by *Loxosceles* sphingomyelinase D, the main component of *Loxosceles* venom, stimulates the expression/activation of secreted and membrane-bound MMP-2 and MMP-9 in keratinocyte cultures (26). It has been shown that the augmented expression of MMPs has a role in the necrotic skin lesions associated with *L. rufescens* envenoming (26). Hence, tetracycline has shown protective effect against venom-induced cell death by inhibiting the activation of MMP proenzyme precursors and MMP enzymatic activity (26).

Endothelium

Throughout the vascular system, endothelial cells (ECs) line the interior surface of blood and lymphatic vessels (27). Although once considered bystanders in the inflammatory process, ECs can dictate inflammatory responses under homeostatic and pathophysiological settings (28). As a primary point of contact for bloodborne pathogens and other host assaults, including toxins, ECs play an important sentinel role (29–32). Expression of numerous PRRs, including toll-like receptors (TLRs) and receptors for tumour necrosis factor (TNF) and interleukin (IL)-1β, enables the intravascular detection of harmful compounds, the activation of proinflammatory genes, and the alteration of the microenvironment (29–32). ECs also express major histocompatibility complex (MHC) molecules, classes I and II, and costimulatory molecules, such as the CD40 ligand (CD40L) that allows intravascular antigen presentation and EC-mediated activation of effector memory T cells (T_{EM}) (33). However, ECs principally modulate immune function by directing leukocyte trafficking and distribution (34–36). Leukocyte tethering, rolling, and extravasation occurs in response to highly selective expression of cell adhesion molecules (CAMs), such as intercellular adhesion molecule-1 (ICAM-1) and selectins, on the apical surface of ECs. CAMs are essential for the homing and migration of immune cells towards secondary lymphoid tissue and inflammatory foci (34–36). The

powerful influence of ECs on immune function has led to hypothesise that immune dysregulation, such as seen in systemic inflammation, might be partially mediated by the endothelium (37). As venom compounds from different species can modulate EC function (described below), this may have important implications in venom-induced systemic inflammation or allergy.

Venom from different species can induce EC perturbations, including distortions in cellular function, morphology, cytoskeletal organisation, and cell viability (38–41). Collectively these actions alter vascular permeability and blood vessel stability (38–41). Additionally, snake and spider venoms are highly proinflammatory in EC cultures, commonly provoking the secretion of IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1/CCL2), and Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES/CCL5) (42). Together, these events modify the extracellular environment and leukocyte activity in local and systemic compartments, which may have important implications for the pathology of some envenomations (41, 42). For instance, though neutrophil depletion abrogates *Loxosceles* venom-induced necrotic lesions, neutrophils are not a direct target of *Loxosceles* venom-derived toxins (43). While neutrophils are likely the proximal cause of inflammation and tissue destruction, direct exposure to venom does not provoke this response (43). Instead, research has shown that the venom contains EC agonists that elicit dysregulated activation and cellular damage (43). *Loxosceles* venom strongly stimulates EC-secretion of IL-8, a potent neutrophil chemoattractant, and low-level surface expression of E-selectin (43). Researchers have noticed an unusual activation response in neutrophils to these venom-mediated proinflammatory signals (43). Specifically, neutrophils adhere to venom-stimulated ECs *via* selectin-mediated tethering in a time- and dose-dependent manner, yet without transmigration (43). In culture, these sequestered leukocytes rapidly increase intracellular Ca^{2+} levels and release primary and secondary granules containing the lytic enzymes responsible for tissue degradation (43). Accordingly, the initiation of *Loxosceles* necrotic lesions appears to be dependent upon toxin-mediated EC responses (43). These findings, further to work by Paixão-Cavalcante and colleagues, suggest a role for immune-targeted (in addition to toxin-targeted) therapeutic strategies for envenomation (26).

Mononuclear Phagocytic System

MNCs and MΦs form a crucial phagocytic component of innate immunity. Both MNCs and MΦs are highly migratory, enabling tissue surveillance, antigen capture, and migration to draining lymph nodes for antigen presentation to adaptive immune cells (44, 45). As such, they function primarily as sentinel phagocytes and regulators of immunity (46). MNCs and MΦs secrete a wide range of cytokines and chemokines that modulate immune cell function and are potent mediators of neutrophil recruitment (47).

Many animal venoms can modulate the metabolism and function of MNCs and MΦs. For example, *Crotalus durissus terrificus* venom (CDTV) significantly inhibits the trafficking and phagocytic capacity of rat peritoneal-resident, thioglycollate-elicited, and *Mycobacterium bovis* strain bacille Calmette Guérin (BCG)-activated MΦs, without affecting cell viability at 2 h, 4 days, or 7 days post intraperitoneal administration (48). In contrast to these immunosuppressive effects, CDTV can enhance the production of hydrogen peroxide (H_2O_2) and NO from phorbol 12-myristate 13-acetate-stimulated resident, elicited, and activated MΦs (48). Further, CDTV-treatment augments cellular metabolism *ex vivo*. Extracted peritoneal cells showed upregulated glucose and glutamine usage and increased maximal activity of hexokinase, glucose-6-phosphate dehydrogenase, citrate synthase, and phosphate-dependent glutaminase (48). These venom-mediated actions result in amplified MΦs candidacidal activity and decreased phagocytosis potential (48).

Comparably, venom from the pit viper, *Bothrops alternatus* (BAV), stimulates increased production of superoxide anion (O_2^-) from isolated thioglycollate-elicited MΦs (49). Again, BAV-treatment showed a limited impact on MΦ viability, as evaluated by Trypan blue exclusion, and did not interfere with MΦ's adhesion or detachment capacity up to 100 μg/mL BAV (49). Pretreatment with the protein kinase C inhibitor, staurosporine (14 nM/mL), suppressed O_2^- production and phagocytosis, suggesting the involvement of a PKC-dependent signalling pathway (49). However, unlike CDTV, Setubal et al. observed increased MΦ complement receptor (CR3)-mediated phagocytosis following incubation with BAV (49). Phagocytosis of serum-opsonised zymosan particles was significantly higher in venom-stimulated MΦs compared to vehicle control (49). It was hypothesised that increased phagocytic activity and excessive release of superoxide might be involved in the local tissue destruction caused by *B. alternatus* snakebite (49).

Studies using human MNCs have revealed the potent proinflammatory properties of different venom compounds. For example, venom from the *Androctonus crassicauda* scorpion induces IL-12p40 mRNA expression and protein secretion from purified MNCs (50). However, venom exposure also produced concentration- and time-dependent cytotoxicity, as evidenced by significant LDH release in MNC cultures (50). Further examples include a C-type lectin (BjcuL) isolated from *Bothrops jararacussu* snake venom that induces TNF production from resting $CD14^+$ cells without stimulating proliferation (51). Phospholipase D from *Loxosceles laeta* spider venom promotes MNC migration in THP-1 cell cultures and cytokine release from skin fibroblasts (52). *Bothrops* snake venoms provoke the release of proinflammatory mediators, prostaglandin E_2 (PGE_2), macrophage inflammatory protein 1-alpha (MIP-1α/CCL3), and IL-1β, and induces activation of NF-κB in human MNCs (53). Given the immunostimulatory role of MNCs and MΦs on immune function, these data demonstrate the capacity of venom to induce systemic inflammatory responses.

Contrasting this research, Khemili et al. examined the immunosuppressive potential of ion channel modulators from scorpion venom using murine MΦs (54). Voltage-gated potassium channels (K_V) play a crucial role in calcium signalling and immune cell excitability (54). In the resting

state, murine MΦs predominantly express the $K_V1.5$ subunit of the $K_V1.5/K_V1.3$ heterotetrameric complex (54). Innate activation, including LPS stimulation, induces $K_V1.3$ overexpression (54). Using non-cytotoxic concentrations of *Androctonus australis hector* (Aah) venom, the authors observed a voltage-independent inhibition of K_V current amplitude in LPS-activated (M1) MΦs (54). On the contrary, venom perfusion did not significantly decrease K_V current amplitude in resting cells (54). These results suggest the presence of an ion channel blocker with a higher affinity for the $K_V1.3$ subunit, abundant on the cell surface of activated MΦs (54). However, as indicated by the authors, the downstream functional consequences of MΦ ion channel modulation requires further examination (54).

Additional immunomodulatory functions, such as TLR inhibition, have been identified using synthetic venom components (55). TLR signalling is a critical element in innate detection and MΦ activation (56). Many animal venoms contain VAMPs that strongly provoke immune stimulation *via* TLR engagement (56). Contrasting this, recombinant rhodostomin (Rn), a snake venom-derived disintegrin, exhibits potent TLR2 inhibition against lipopeptide-stimulated THP-1 cells (55). Incubation with Rn suppresses TNF, IL-1β, and IL-8 release and IκB degradation from Pam3CysSerLys4-activated cells (a TLR1/TLR2 agonist), in a dose-dependent manner (55). In THP-1 cell cultures, Rn reverses the phosphorylation of focal adhesion kinase downstream kinases, thereby inhibiting signal transduction (55). In the caecal ligation and puncture (CLP) model of sepsis, Rn significantly suppresses CLP-induced TNF, IL-6, and MCP-1 production and reduces animal mortality (55). Histology has also revealed that Rn significantly alleviates CLP-induced tissue-damage (55). Studies such as these highlight venom as a source of compounds for drug discovery.

Granulocytes (Neutrophils)

Neutrophils are the most abundant leukocyte, constituting 40-75% of circulating white blood cells (WBC) (57). Derived from pluripotent stem cells in the bone marrow, a segmented nucleus of three to five lobes and the presence of secretory vesicles/granules characterise mature cells (58). Although short-lived, estimates range from hours to several days, they are the first phagocyte recruited and mobilised from the bone marrow or periphery to the infection/injury (59, 60). Upon arrival, these granulocytes directly destroy pathogens, inactivate toxins, and mount inflammatory responses through oxidative and non-oxidative pathways (59, 60). Like other immune cells, neutrophils are prolific producers of cytokines and chemokines and can mount robust proinflammatory responses (61).

For non-infectious/sterile challenges, such as envenomation, exocytosis of granules/secretory vesicles releases up to 700 defensive proteins into the extracellular milieu (58). These proteins include defensins, serine proteases, neutrophil elastase, proteinase 3, cathepsin G, cytokines, and chemokines, some of which inactivate venom components through proteolytic degradation (62). An additional neutrophil defensive strategy, critical during envenomation, is the neutrophil extracellular trap or "NET". NET formation (NETosis) occurs through programmed self-destruction, whereby the release of nuclear DNA forms a sticky "net" of extracellular fibres, containing the dissemination of toxins, bacteria, and pathogens (63, 64). However, whether neutrophils protect against or promote venom injury is disputed. Certainly, the participation of neutrophils in venom-associated pathologies, such as dermonecrosis, has been well documented (26, 43). Nevertheless, neutrophilic functions, including toxin trapping and inactivation, provide critical defence against systemic injury and death (65). Additionally, neutrophil clearance of necrotic tissue is essential for muscle regeneration following snakebite (16).

Snake venom, such as from *Echis carinatus*, induces NETosis and ROS generation in a time- and dose-dependent manner in animal models and cell cultures (65). While these neutrophilic-defensive actions hinder venom's systemic dissemination, dense NET accumulation can block blood vessels, resulting in localised tissue damage and impeding antivenom's efficacy (65). Unfortunately, though research shows that co-treatment with DNase 1 prevents tail injury in *E. carinatus* experimentally envenomed rodents, mortality is significantly higher among these mice (65). Interestingly, follow-up work by Stackowicz et al. determined that localised tissue damage is neutrophil independent (66). Despite verifying that DNase-treatment does indeed reduce tail injury at the expense of survival, the study reported similar occurrences in both neutrophil-sufficient and deficient settings (66). These data suggest that extracellular DNA from multiple dying cell types, including neutrophils, mediate capillary obstruction following envenomation.

Regardless of DNA source, toxin retention inhibits systemic injury to the detriment of the localised compartments (66). NET formation and capillary obstruction can lead to severe consequences, such as amputation, which has devastating implications for victims' lives (67). Accordingly, there is an urgent need for effective therapeutics that minimise tissue necrosis and facilitate antivenom efficacy. However, given that neutrophil participation is critical in tissue repair post-envenomation, neutrophil-targeting therapies may be counterproductive (16). Hence, further research is required.

Granulocytes (Mast Cells)

Mast cells (MCs) are long-lived, tissue-resident effector cells derived from a myeloid lineage and matured under the influence of stem cell factor and cytokines (68). MCs are positioned near entry points of mucosal, epithelial, and sub-endothelial connective tissue to provide innate defence and perform a wide range of physiological functions that maintain tissue homeostasis (68). MCs induce killing and assist in the clearance of parasites and pathogens. For venom/toxin defence, sequestering and neutralisation occur (69). Expression of multiple PRRs on the cell surface enables rapid detection and response to immune challenges, including venom toxins. Activation of PRRs induces *de novo* synthesis of cytokines, chemokines, and eicosanoids to attract and stimulate other effector cells (70). A classic feature of MCs are weaponised granules, containing preformed toxic inflammatory mediators, including enzymes (tryptase, chymase, and carboxypeptidase A3), amines (histamine and heparin), and cytokines (TNF). MC activation, mediated by immunoglobulin E (IgE)-bound

FcεRI, causes rapid degranulation potentially inducing a systemic proinflammatory response (71, 72).

Despite the widely recognised role of MCs in allergy and anaphylactic shock, animal models have provided evidence of MCs' protective function against envenomation (73, 74). As reviewed by Galli et al., functional MCs enhance the survival of mice challenged with sub-lethal doses of snake (*Atractaspis engaddensis; Daboia russelii)*, Gila monster *(Heloderma suspectum)*, European honey bee *(Apis mellifera)*, and scorpion (*Leiurus quinquestriatus hebraeus; Centruroides exilicauda)* venoms (75). The significantly higher mortality among MC-deficient mice has been attributed to the dysregulation of serine proteases (carboxypeptidase A3 and mast cell protease 4), which degrade peptides, and heparin and histamine (69, 75–77). In healthy individuals, the release of heparin and histamine can neutralise the effects of venom-derived toxins (69, 71). Adversely, the release of these amines provokes dangerous allergic symptoms in hypersensitive individuals, particularly in response to Hymenopteran venom (the venom of bees, wasps, and ants) (70).

Chemical Mediators

The immune network is vast and highly complex. Intercellular communication across the network requires small soluble protein effectors, known as cytokines (78). Cytokines (interferons, interleukins, chemokines, and growth factors) are secreted by cells to instruct and regulate the immune system's activity for protection against injury, infection, and disease (78). Biological functions include cellular activation, proliferation, differentiation, growth, and immune regulation (78). Further, as chemoattractant proteins, chemokines exert their effects *via* cell recruitment, migration, and adhesion (79). Like hormones, cytokines have autocrine, paracrine, or endocrine functions for localised or systemic effects (78). Broadly, they elicit either pro or anti-inflammatory action (80, 81). The reality, of course, is more complicated as many cytokines exhibit pleiotropic effects that are dependent on cellular source, target receptor, and the stage of the inflammatory process (80, 81). Additionally, immune cells adapt to the overall profile of the cytokine milieu they encounter (80, 81).

The expression and release of these potent chemical mediators are tightly regulated (82, 83). Nevertheless, infection, cancer, injury, disease (such as autoimmunity), and medical interventions (including drugs and organ transplant) can provoke dysregulation in cytokine levels resulting in devastating pathophysiological effects. Unchecked, cytokines and other proinflammatory mediators cause severe tissue destruction, systemic pathology, multiple organ failure, and potentially death (82, 83). Existing literature extensively describes diverse pathophysiology induced by dysregulated inflammatory mediators. These include cytokines (IL-1β, IL-6, TNF, IFN-γ, IL-10, IL-12, and GM-CSF) and chemokines (IL-8, MCP-1, eotaxin/CCL11, IP-10/CXCL10, MDC/CCL22, MIP-1α, and TARC/CCL17), as well as bradykinin, eicosanoids (prostaglandins and leukotrienes), cyclooxygenases, NO, and histamine (84). Dysregulation of these mediators is associated with inflammatory and neuropathic pain (85, 86), tissue destruction (87), systemic inflammation (88–90), autoimmunity, and allergic reactions (91). Unsurprisingly, the same proteins are detected in the serum of victims of envenomation, where pain and systemic injury occur (92, 93). Notably, similar secretion profiles are also present in experimentally envenomed animals and cell cultures (92). Additionally, venoms can have detrimental effects on platelet function and components of the complement system (94, 95). In particular, snake venoms can trigger critical pathologies, such as venom-induced consumption, thrombocytopenia, and hemorrhage (94, 95).

Cytokines and their respective receptors represent important immunotherapeutic targets for numerous conditions (96). Accordingly, it might seem plausible that targeting proinflammatory cytokines, chemokines, and small molecules (or their receptors) similarly represents novel therapeutic avenues for certain envenomations. However, research in this area is still in its infancy, and to date, studies have described both beneficial and detrimental outcomes of immunosuppression during experimental envenomation. For example, the detection of snake and bee venom toxins by NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome, triggers immune cell activation, potent IL-1β secretion, and neutrophil influx (15). Interestingly, Palm and Medzhitov showed that although inflammasome inhibition, such as seen in caspase-1-deficient mice, successfully inhibited cytokine release and leukocyte influx, it unexpectedly resulted in a higher susceptibility of the mice to the noxious effects of venoms, including mortality (15). Conversely, Zoccal et al. determined that using a hexapeptide ligand for the MΦ scavenger receptor (CD36) protected mice against a lethal dose of *T. serrulatus* scorpion venom through decreased production of IL-1β, IL-6, TNF, CCL3, and PGE2, and restrained lung inflammation (97). While reduced IL-1β secretion and neutrophil influx was observed in both models, together, these data demonstrate the importance of innate immunodetection in the defence against bites and stings.

Adaptive Responses to Envenomation

The immune system's adaptive arm is predominantly comprised of B cells and T cells. The primary effector function of B cells is the generation of antibodies (immunoglobulins; Ig) for humoral defence (98). T cell effector functions are produced by a range of subsets, including cytotoxic ($CD8^+$) T cells and helper ($CD4^+$) T cells (TH) cytotoxic ($CD8^+$) and helper ($CD4^+$) lymphocytes. Cytotoxic $CD8^+$ T cells protect against intracellular pathogens and suppress infectious disease and tumour growth, while $CD4^+$ T cells maintain homeostasis and shape proinflammatory and regulatory immune responses (99).

Bites, stings, and intentional venom inoculation stimulate the generation of venom protein-specific antibodies (100, 101). Antibody-mediated neutralisation effectively counteracts venom activity (102). However, a primary B cell response is slow (requiring days to weeks to become fully active), while defence against rapid venom action requires an immediate response (103). As an alternative to host antibodies, antivenom, produced in large mammals and purified for

medical purposes, can provide passive immunity to victims of life-threatening envenomation (104–106).

The following provides a simplified overview of a primary (thymus-dependent) humoral response towards envenomation. Following bite or sting, APCs, such as DCs, MNCs and MΦs, capture and process venom proteins at the site of injury, promoting maturation (100, 101). Matured APCs migrate to secondary lymphoid tissue to present venom antigen to naïve T_H cells *via* membrane-bound peptide-MHC II protein complexes (100, 101). In lymph nodes, engagement of a T cell receptor (TCR) with cognate peptide-MHC molecule initiates T_H activation (signal 1) (107). Critical secondary signals, required for complete T cell activation, are provided by APCs. APCs, especially DCs, highly express ligands (including CD80 and CD86) for T cell co-stimulatory molecules, such as CD28 (signal 2) (108). Next, APC-derived and circulating cytokines (as well as autocrine IL-2) induce T cell proliferation and differentiation (signal 3). For extracellular immune challenges, such as envenomation, $CD4^+$ T cells acquire a T_H2 phenotype with effector functions that include B cell activation (100, 101).

During a primary antibody response, B cells require multiple stimulatory signals. The first occurs when a B cell receptor (BCR) encounters its specific soluble or membrane-bound epitope (100, 101). The internalised antigen is processed and displayed on the B cell surface as a peptide-MHC complex for T_H presentation (100, 101). TCR binding triggers upregulation of co-stimulatory ligands, such as CD40L, and the production of proinflammatory cytokines, including IL-4 (107). CD40L engagement with B cell CD40 mediates the recruitment of intracellular adaptor proteins essential for propagating downstream signalling (107). Additionally, cytokines secreted by primed T_H2 cells provide B cells with accessory stimulation for the early (proliferation and clonal expansion) and later (differentiation, antibody production, and isotype switching) stages of B cell activation (109). Proliferating B cells form germinal centres (GCs) where memory B and antibody-secreting plasma cells develop. Here, B cells also undergo somatic hypermutation and isotype switching (IgM and IgD to IgG, IgE, or IgA) to generate high-affinity antibodies for robust immune responses (110).

Yet, critical though they may be, adaptive responses can also produce severe pathology (74). For example, IgE isotype switching following venom challenge can, in a percentage of hypersensitive individuals, lead to fatal allergic reactions (discussed below) (74). In addition to allergy, dysregulation of adaptive responses and loss of self-tolerance stimulate destructive auto-reactivity (111). As such, lymphocytes (T cells in particular) are a target for therapeutic modulation (111). Serendipitously, venoms can contain ligands for T cell ion channels and receptors, able to modulate immune function with high specificity (described below) (112–121).

VENOM-INDUCED IMMUNOPATHOLOGY

Cell-specific venom-mediated immune dysregulation is described above. The following sections discuss modes of immunopathology, including venom-induced allergic reaction and systemic inflammation.

Venom Allergy

Despite a notorious reputation for venomous snakes, spiders, and jellyfish, Australia's largest proportion of venom-related fatalities occur due to anaphylactic events (122). Reflecting a global trend, honey bee (*A. mellifera*) stings are a significant contributor to venom injury in Australia, representing 16.3% of anaphylactic fatalities between 1997 and 2013 (123).

Venom from stinging Hymenopterans is commonly associated with allergic reactions worldwide (123). While most sting responses are localised and self-limiting, fatality can occur due to immune-mediated respiratory and/or cardiovascular failure (124). In these incidences, systemic reactions (SR) are predominantly mediated by IgE-mechanisms; however, dose-dependent IgE-independent responses are also possible (7).

Among venom-sensitised individuals, SR's develop in 0.3% to 8.9% of cases (124, 125). Accordingly, Hymenopteran major allergens (antigens that bind IgE in greater than 50% of venom-sensitive individuals) have been well-characterised (7). For honey bee venom, these hypersensitivity-inducing proteins include phospholipase A2, hyaluronidase, acid phosphatase, and dipeptidylpeptidase (124). In vespid venom (wasp and yellow jacket), Antigen 5 and phospholipase A1 are the recognised major allergens (124).

Classic IgE-mediated allergic disease begins with a sensitisation process. Keratinocytes and resident immune cells detect damage induced by noxious substances, such as venom-derived compounds, stimulating the release of alarmins, cytokines (IL-4, IL-5, and IL-13), and other proinflammatory mediators required for antibody production (126, 127). DCs capture and process antigen for presentation to naïve T cells in draining lymph nodes, triggering events eventuating in plasma cell IgE antibody production (126, 127). Elevated IgE is a normal physiological response following a bite or sting and is not necessarily predictive of disease (69, 75, 124). Nevertheless, in some individuals, systemic IgE levels remain elevated longer term and can trigger SR, including anaphylactic shock, after multiple stings (7).

The symptoms of immediate (Type-1) allergic reactions occur during secondary antigen challenges. When IgE encounters its cognate antigen, crosslinking of FcεR1 on MCs and basophils in mucosal and epithelial tissues provoke activation and degranulation (128–130). Preformed inflammatory mediators, including histamine and proteases, are rapidly released from granules into the extracellular environment (128–130).

Histamine is chiefly responsible for the clinical consequences of Type-1 allergic reactions (131, 132). Histamine's protective functions include toxin binding and deactivation (69, 75). In allergic disease, histamine (acting upon H_1 and H_2 receptors) causes smooth muscle contraction, constriction of airways, swelling of the epiglottis, and increased vascular permeability. These events lead to dangerously low blood pressure, oedema and potentially death (131, 132). Further to this, proinflammatory genes, stimulated during the initial phase, induces *de novo* synthesis of the leukotrienes (particularly

LTB_4), cytokines, and chemokines responsible for the late phase (or delayed-type) symptoms (133, 134). These mediators are potent inducers of cell activation, migration, and the influx of lymphocytes and neutrophils (128, 134). The incidence and severity of biphasic anaphylaxis are highly variable, and fatalities can occur, necessitating continued patient observation following the resolution of initial symptoms (132, 133, 135).

The acute nature of fatal anaphylactic shock means death is more likely to occur in the home (87% of cases) than in the hospital (122, 123). Adrenaline autoinjectors (AAI) are an essential first-line treatment; however, death may still occur despite prompt administration (123, 136). Additional therapies include H_1 and H_2 antihistamines to counter the pathophysiological effects mediated through these receptors and critical supportive care (137, 138). For individuals with verified IgE-mediated allergy, venom immunotherapy (VIT) may generate a lifesaving tolerance to known allergens (139).

The pathogenic role of MCs and IgE-mediated granule release is well established (74). However, it has been postulated that allergy may be a barrier function disease in which cellular damage and perturbations of the epithelium and endothelium induce excessive proinflammatory responses from the resident immune cells (140). If this hypothesis is correct, the therapeutic modulation of these cells may correct the imbalanced proinflammatory response but this hypothesis has not been investigated for venom-associated allergy.

Hypersensitivity to Marine Stings

In Australia, contact with venomous marine animals and plants accounts for 9% of venom-related hospitalisations (122). The phylum *Cnidaria* (classes Hydrozoa, Scyphozoa, and Cubozoa) comprises approximately 10,000 jellyfish species distributed throughout the world (138, 141). Of these, ~1% are medically relevant (142). Jellyfish stings typically trigger local or large local responses, manifesting as pain, swelling, and erythema, but are usually not life-threatening (143, 144). However, severe delayed cutaneous reaction, allergy, and anaphylactic shock can occur (145–147).

It may not be surprising then that in 1902 the unexpected discovery of anaphylaxis by physiologists Charles Richet and Paul Portier involved marine venom from the Portuguese man-of-war (*Physalia physalis*) and sea anemone (148, 149). When attempting to immunize dogs against harmful venom effects, Richet and Portier found that rather than confer protection (phylaxis), a subsequent venom challenge resulted in death. Although allergy was yet to be characterised, the experiments recognised immune involvement and the term "anaphylaxis" (against protection) was coined. This discovery later won Richet the 1913 Nobel Prize in Physiology or Medicine (148, 149).

Jellyfish envenomation is the most common marine sting type, impacting fishers, surfers, and sea bathers globally. An estimated 150 million stings occur annually, with peak incidence coinciding with blooming (or swarming) seasons during warmer months (148). A characteristic feature of the phylum *Cnidaria* is specialised stinging organelles, known as nematocysts. Located within cnidocytes, nematocysts are explosive capsule organelles containing coiled, barbed and threadlike tubules coated in venom (150). High-velocity capsule release, triggered by physicochemical stimuli, causes inversion of tubules into harpoon-like threads able to puncture and penetrate prey and predators (151, 152). Through nematocysts, jellyfish venom, containing pore-forming compounds, metalloproteases, serine proteases, and phospholipases, is injected into the victim, causing paralysis of prey and in humans dysregulation of immune function, cardiac function, respiratory function, and potentially fatal outcomes (153).

Although jellyfish venom can cause severe immediate-phase and delayed-type allergic reactions, the causative allergens are mostly unknown. *Chironex yamaguchii* is the box jellyfish species responsible for 78% of reported stings in Japan (144). Recently, the N-linked glycoprotein, CqTX-A (a hemolytic toxin), was identified as a major allergen from this venom (144). While the underlying mechanism has yet to be elucidated, this finding has important implications as CqTX-A shares significant sequence homology with other lethal pore-forming jellyfish proteins, specifically, CfTX-1 and CfTX-2 (*Chironex fleckeri*), CrTX-A (*Carybdea rastoni*), CaTX-A (*Carybdea alata*) and CqTX-A (*Chiropsalmus quadrigatus Haeckel*) (154–157).

For Hydrozoa (which includes Portuguese man-of-war), Scyphozoa (true jellyfish), and Cubozoa (box jellyfish), nematocysts are located on the tentacles, oral arms, and in some instances, the bell of the jellyfish (158, 159). Contact with tentacles can result in inoculation from potentially millions of nematocysts (160). Application of acetic acid and careful removal of tentacles from the victim's skin prevents the further discharge of unfired nematocysts but does not deactivate the already injected toxins (161). Once stung, venom distribution occurs *via* capillaries and the lymphatic system to target organs, while the barbed tubules remain embedded in the skin until clearance (149, 160). Tubules are allergenic scaffolds comprising carbohydrates, proteins, chitin, and mini-collagen (148, 149, 162–164). As such, it has been suggested that impaired clearance, especially of chitin, may contribute to more severe outcomes of envenomation and hypersensitivity (149).

Beyond stings, there are recently described cases of severe allergic reactions to edible jellyfish consumption (147). It has also been reported that jellyfish stings, particularly among surfers, lead to sensitisation of foods containing gamma-glutamic acid, such as fermented soybean (165, 166). Collectively, these data highlight the antigenic properties within jellyfish nematocysts and tissue, in addition to their venom-derived destructive potential.

Systemic Inflammation

Systemic inflammation, including cytokine release syndrome (CRS), is a life-threatening immune condition triggered in response to endotoxemia, severe viral infections (including influenza), and immunotherapies (167–169). Clinical manifestations can include fever, nausea, tachycardia, dyspnea, headache, muscle and joint pain, and in severe cases, neurotoxicity, pulmonary oedema, respiratory failure, and death (167). Certain envenomations can similarly provoke a systemic inflammatory response, most notably is scorpionism (10).

Scorpion envenomation is another leading cause of venom-associated morbidity and mortality, affecting more than one million individuals per annum (6). Although most stings produce only local symptoms (81% of cases), envenoming by dangerous species can initiate a surge of endogenous neurotransmitters, adrenaline and noradrenaline, resulting in an autonomic storm and severe systemic effects (6, 10). Additionally, venom-derived toxins induce spontaneous acetylcholine (Ach) release from peripheral nerves, responsible for the life-threatening cardiac dysfunction seen in severe cases (170). Interestingly, these mediators are also implicated in the box jellyfish pathology, Irukandji syndrome (171).

Along with intense acute pain and distress, severe scorpion envenoming (grade III stings) produces complex pathophysiology in victims (172). Like CRS, symptoms can include respiratory distress, cardiac dysfunction, pulmonary oedema, multiple organ failure, and potentially death, especially among children and the elderly (173). These clinical consequences are principally mediated by neurotoxic peptides, able to cause hyperexcitability of the autonomic nervous system through Na^+, K^+, Ca^+ or Cl^- ion channel modulation (172). Ion channel modulation is also implicated in the development of pulmonary oedema, a symptom present in many fatal sting cases (174). Research led by Comellas et al. observed decreased lung fluid clearance in *Tityus serrulatus* envenomed rats, postulating venom-induced impairment of Na^+/K^+ ATPase in alveolar epithelial cells as the mechanism (174).

Beyond neurotoxic effect, the immune network plays a role in significant scorpion envenomations (175). Immune participation in SR is multifactorial, involving direct antigenic activation and indirect stimulation *via* the neuroendocrine-immune axis (176). In addition to neurotransmitters, stings induce a rapid release of proinflammatory mediators. Elevated IFN-γ, IL-1β, IL-6, IL-8, IL-10, and TNF have been detected in the plasma of sting patients and animal models of scorpionism (10). Scorpion venom also provokes hypersensitivity mediators, particularly histamine (177). Blockade of the histamine H1 receptor has shown to be protective in *Androctonus australis hector* envenomed mice (177). Specifically, pretreatment with hydroxyzine (H1 receptor antagonist) reduced immune cell infiltrate and oedema in the brain and spinal cord and diminished levels of circulating proinflammatory cytokines (177). Further, scorpion toxins activate components of the complement system, including the generation of anaphylatoxins, which are potent chemotactic proteins (10). As further evidence of immunological involvement, heightened dermal reactions are reported in individuals predisposed to scorpion venom, such as seen in delayed-type hypersensitivity reactions (10).

For direct immunological activation, the most extensively studied species is the Brazilian scorpion, *T. serrulatus* (10). Whole *T. serrulatus* venom (TsV) and select purified toxins are potent stimulators of innate immune cells, including MΦs (56). *In vitro* assays have revealed that surface receptors TLR2, TLR4, CD14, and CD36 recognise TsV compounds, triggering cellular activation and production of cytokines and lipid mediators (56). Engagement of CD14 and co-receptor TLR4 promotes NF-κB and AP-1 signalling pathways and transcription of potent proinflammatory genes, including IL-1β (10). Consequently, TsV stimulates cytokine release from innate immune cells in a time- and dose-dependent manner, independent from cytotoxic effect (10). In addition, NF-κB signalling regulates cyclooxygenase-2 (COX-2) expression and the secretion of eicosanoid, PGE_2 (178). PGE_2 is a lipid mediator with pleiotropic roles in the initiation and resolution of inflammation, particularly inflammatory pain (179). Among its diverse biological functions, PGE_2 activates IL-1β, MCP-1, and IL-6 pathways *via* prostaglandin EP4 receptor signalling (179). Accordingly, IL-1β and its receptor (IL-1R) are strongly suppressed by EP4 antagonism (179). IL-1β and IL-1R are potential therapeutic targets for multiple inflammatory diseases, including scorpion envenomation (180). As such, inhibition of the COX-2/PGE_2/EP4 pathway has shown a cardiopulmonary protective effect in envenomed mice (97, 170).

The eicosanoid leukotriene B4 (LTB_4) is also upregulated in cell culture and animal plasma following treatment with whole TsV or purified toxins (178). A study by Zoccal et al. demonstrated that activation of the class B scavenger receptor, CD36, directs eicosanoid metabolism towards LTB_4 *via* a 5-lipoxygenase (5-LOX)/peroxisome proliferator-activated receptor gamma (PPAR-γ) pathway, opposing the events of TLR and CD14 receptor signalling (97). CD14 and TLR4 appear to be critical for TsV-induced cytokine and eicosanoid secretion (180). Further work by Zoccal and colleagues showed that $CD14^{-/-}$ mice fail to produce significant levels of PGE_2 or IL-1β post-TsV envenomation (180). In addition, $CD36^{obl/obl}$ mice secrete increased levels of PGE_2 and IL-1β post-TsV envenomation but do not produce LTB_4 (180). Critically, LTB_4 synthesis suppresses IL-1β maturation and secretion and the associated animal mortality (178). CD36, therefore, represents a novel therapeutic target for severe scorpion envenomation (180).

Mouse models of TsV envenomation produce autonomic dysfunction that is similar to clinically observed symptoms (170). A lethal inoculation of TsV induces sweating, ocular and nasal secretions, lethargy, and convulsions in mice, preceding cardiovascular disturbances and death (170). Observed hyperglycemia and neutrophilia are also consistent with sting patients (170). The neurotransmitters adrenaline and ACh, responsible for sympathetic and parasympathetic symptoms, respectively, are elevated in peripheral blood as well as in cardiac tissue in response to TsV (170). Treatment with atropine, a muscarinic receptor antagonist, but not propranolol, prevented venom-induced cardiovascular alterations, which are a leading cause of death in severe scorpionism (170). Curiously, despite showing systemic elevation of adrenaline, the study did not investigate the effect of an alpha-adrenergic blocking agent, such as prazosin (170).

In parallel to excessive ACh, lethal TsV envenomation stimulates the systemic and cardiac secretion of PGE_2 and IL-1β. Reis et al. have recently proposed IL-1R as a neuro-immune link responsible for innate heart inflammation and TsV-induced heart failure (170). Research by their group demonstrated that

TsV co-administered with PGE_2 enhanced IL-1β and ACh release from cardio fibroblasts, an effect which was blocked by an EP receptor antagonist. In contrast, IL-1R silencing repressed PGE_2, IL-1β and ACh levels and rescued mice from fatal TsV administration (170). As such, the study determined that PGE_2 amplifies IL-1β release, which upon binding IL-1R potentiates upregulation of PGE_2 and PGE_2-dependent ACh release post TsV envenomation (170).

Existing scorpion sting management comprises specific antiserum and symptomatic treatment, such as pain and low dose anti-inflammatory medications (6). Grade III stings and stings in children younger than 15 require intensive care (6). Polymorphism within scorpion venom-derived proteins is geographically varied, impeding the manufacture of a standardised antivenom (6). Variability in toxin immunogenicity further limits the usefulness and cost-effectiveness of antivenom production (6). Accordingly, some experts challenge the use of antiserum therapy due to insufficient neutralising capacity and the additional shock risk associated with poorly purified serum (6, 181).

Recently, success has been reported using novel immune-based therapies in animal models of TsV envenomation. In 2019, Zoccal et al. showed that the experimental peptide, EP80317 (a CD36 ligand), protected C57BL/6 mice against a lethal dose of TsV (97). Indeed, the therapeutic administration of EP80317 at 0.5 h and 2 h post-envenomation provided complete protection against a lethal dose of venom. Lymphocytes and neutrophils in the bronchoalveolar lavage fluid were significantly lower in the treatment group than venom alone. Accordingly, cAMP concentrations and proinflammatory cytokines (IL-1β, IL-6, TNF, and CCL3) were also considerably decreased (97).

Although promising, the estimated time and cost of developing a new drug and bringing it to market is 10 – 15 years and hundreds of millions of dollars (182). Conversely, drug repurposing circumvents the requirement for lengthy and expensive preclinical development. A recent *in vivo* study from the same group found that therapeutic administration with high dose dexamethasone (DEX) (5 mg/kg) improved TsV-induced cardiac dysfunction and reduced mortality after a fatal venom dose (170). The study showed that early treatment (15 min and 1 h post-inoculation) strongly suppressed PGE_2 and IL-1β release in tissues, abrogating systemic ACh and IL1R-mediated/ACh-induced cardiac dysfunction (170).

In reviewing the effects of jellyfish venom on the immune system, Tibballs et al. highlighted similarities between the clinical features of Irukandji syndrome and scorpion envenomation (149). While the mechanisms underpinning the pathology of Irukandji syndrome have remained unresolved for decades, they may likewise involve both autonomic and inflammatory pathways. If so, immune-based therapies may also prove beneficial in severe box jellyfish envenomation and warrant further investigation.

Irukandji Syndrome

While most jellyfish stings do not require medical attention, several species found in tropical waters constitute a public health threat (141). In Australia, the box jellyfish *Chironex fleckeri* and *Carukia barnesi* are of particular medical relevance. A high *C. fleckeri* venom dose can cause rapid and fatal cardiac arrest (183). In contrast, the smaller box jellyfish, *C. barnesi*, induces an extremely painful systemic pathology known as Irukandji syndrome (IS) (184–186).

C. barnesi was the first confirmed causative agent of IS after its namesake, Dr Jack Barnes, famously subjected himself, his nine-year-old son, and a local lifeguard to intentional envenoming in 1961 (187). Yet, due in part to the elusive nature of this highly venomous jellyfish, research over the years has failed to unravel the mechanisms behind the distinctive syndromic illness (184, 188). *C. barnesi* are small and transparent, with the medusal bell measuring ~20 mm wide (**Figure 1**) (184, 189). As with other carybdeids, *C. barnesi* have a single tentacle per pedalium (184, 189). Both bell and tentacles are covered with nematocysts, comprising distinct venom composition (190).

A retrospective case study of 128 marine sting presentations to Cairns Base Hospital revealed a wide variation of symptom severity among individuals (186). Of the 39 patients with skin scrapings consistent with *C. barnesi* nematocysts, some experienced only minor symptoms, while in others, envenomation proved fatal (186). Typically, an IS presentation includes a mild local reaction followed by a characteristic incubation period of five to 60 min before the onset of systemic effects (**Figure 2**) (191, 192). Pain in the abdomen, chest, lower back, limbs, and joints, is severe, often intractable to opioids and accompanied by extreme distress and agitation (193, 194). In parallel, the manifestation of tachycardia, hypertension, diaphoresis, dyspnea, and in some instances, priapism may occur (193, 194). In severe cases, life-threatening complications, such as cardiomyopathy and cardiogenic shock, can arise (193). Due to the significant cardiac dysfunction associated with *C. barnesi* envenomation, cardiogenic pulmonary oedema may develop (195, 196). Tragically, venom-induced intracerebral hemorrhage resulted in the death of two individuals in 2002 (192, 197).

An "Irukandji" antivenom is unavailable, and, as *C. barnesi* is only one of several causative species, an antivenom is unlikely to be produced (198). Therefore, treatment of severe envenomation is heavily reliant upon opioid-based pain management and symptomatic supportive care, with a mean expected hospital stay of 1.6 days (186). The clinical manifestations of IS have been attributed to excessive catecholamine release, such as seen in pheochromocytoma, scorpionism, or funnel-web spider envenomation (149, 198, 199). As such, IS has been described as "a painful hypercatecholaminergic condition" (193). Accordingly, individuals at particular risk of fatal outcomes are those with pre-existing cardiovascular pathologies, potentially making the use of alpha/beta-adrenergic blocking agents prohibitive (200).

Supporting this hypothesis, adrenaline and noradrenaline have been transiently detected in the plasma of *C. barnesi* experimentally envenomed piglets (200). Peak catecholamine release was observed 10 min post intravenous (IV) venom administration, coinciding with the onset of systemic and pulmonary hypertension and tachycardia (200). Plasma

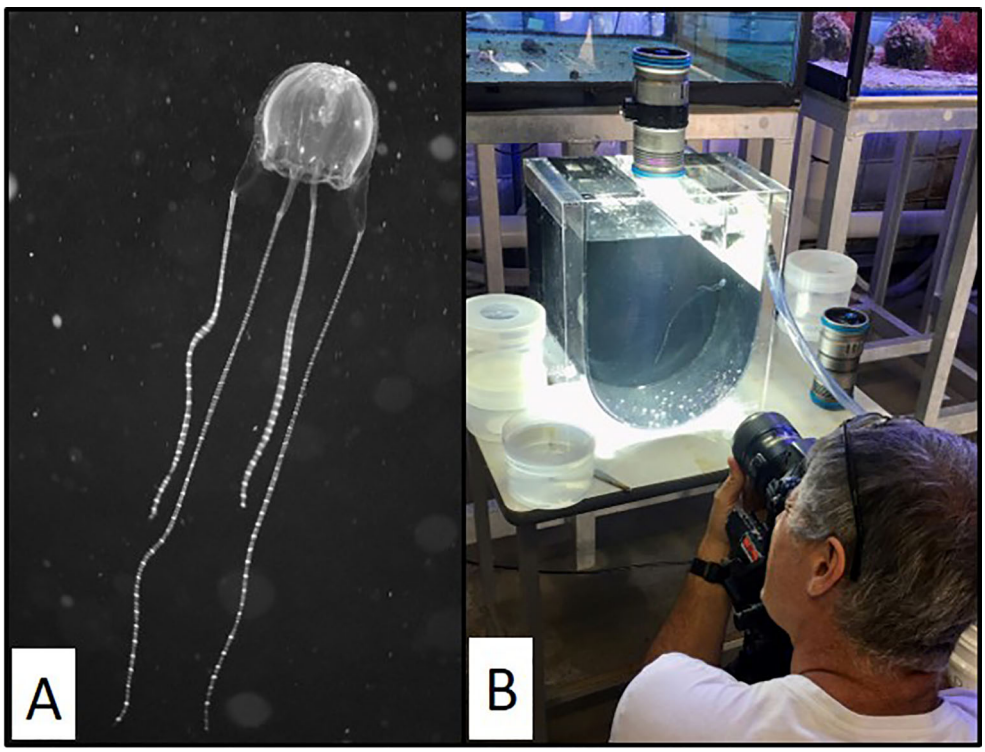

FIGURE 1 | Image of Carukia barnesi jellyfish. Images showing **(A)** close up and **(B)** relative size of adult *C. barnesi* jellyfish. Prof Jamie Seymour pictured. Photos were taken by **(A)** Jamie Seymour (JCU, Cairns, Australia) and **(B)** Rachael Ryan (JCU, Cairns, Australia).

catecholamines remained elevated in envenomed animals until 60 min but declined to non-significant levels within 2 h (200). Pretreatment with 1 μmol/L tetrodotoxin (TTX) attenuated tachycardia responses from rat and guinea-pig isolated right atria but did not significantly alter venom-induced contraction of rat mesenteric small arteries (200). These data suggest the presence of a presynaptic neuronal voltage-gated sodium channel agonist within the venom, as well as the presence of a TTX-insensitive vasoconstrictor (149, 198–200). While a physiological stress response towards IV administration of any toxin may similarly stimulate adrenaline and noradrenaline release, the authors reported the uniqueness of the reaction compared to other box jellyfish venom (200).

Research by Ramasamy et al. found pretreatment with prazosin (50 μg/kg) partially reduced tachycardia in *C. barnesi* envenomed rats, further supporting the role of endogenous catecholamines in the pathogenesis of IS (198). However, the residual pulse pressure observed in the study suggested the contribution of factors besides catecholamines (198). Furthermore, the result was not reproduced by Winkel et al. with 0.3 μM prazosin pretreatment, possibly due to dose- or time-dependent factors that were not clearly stated in either study (198, 200). Unfortunately, as both cardiovascular studies required the use of anesthetised animals, euthanised after 2 h, the critical evaluation of later time points was not possible (198). Yet, in sting victims, symptoms can remain for days, potentially requiring intensive care (186).

Regardless, in line with these findings, current clinical guidelines recommend magnesium sulphate ($MgSO_4$) therapy to attenuate pain and suppress excessive catecholamine release in severe IS (201). Its success in doing so has generated divided opinions (202). The results of a randomised trial completed in 2012, and reviewed in 2017, were unable to confirm the ability of $MgSO_4$ infusion to reduce opioid requirement (202, 203). Both studies reported varied success from the 39 patients, ultimately showing no significant benefit from $MgSO_4$ therapy (202, 203).

Akin to scorpion envenomation, the symptoms of IS cannot be wholly attributed to sympathetic hyperstimulation (10). Also akin to scorpionism, generalised IS symptoms resemble those of CRS. Interestingly, $MgSO_4$ potently suppresses MNC-mediated cytokine production following TLR stimulation (204). $MgSO_4$ increases IκBα levels in MNCs, thereby decreasing NF-κB nuclear translocation and its activity (204). Accordingly, the ability of $MgSO_4$ to inhibit pain in some sting patients could in part be due to a dampened immune response, although this theory has not been investigated.

Presently, neither catecholamines nor inflammatory mediators have been measured in *C. barnesi* sting patients. Recently, a study by Staedtke et al. proposed an intriguing link between "cytokine storm" and "catecholamine storm" in systemic inflammatory response syndrome (SIRS) and capillary leak syndrome, which may apply to venom-induced systemic inflammation (205, 206). This study showed that adrenaline

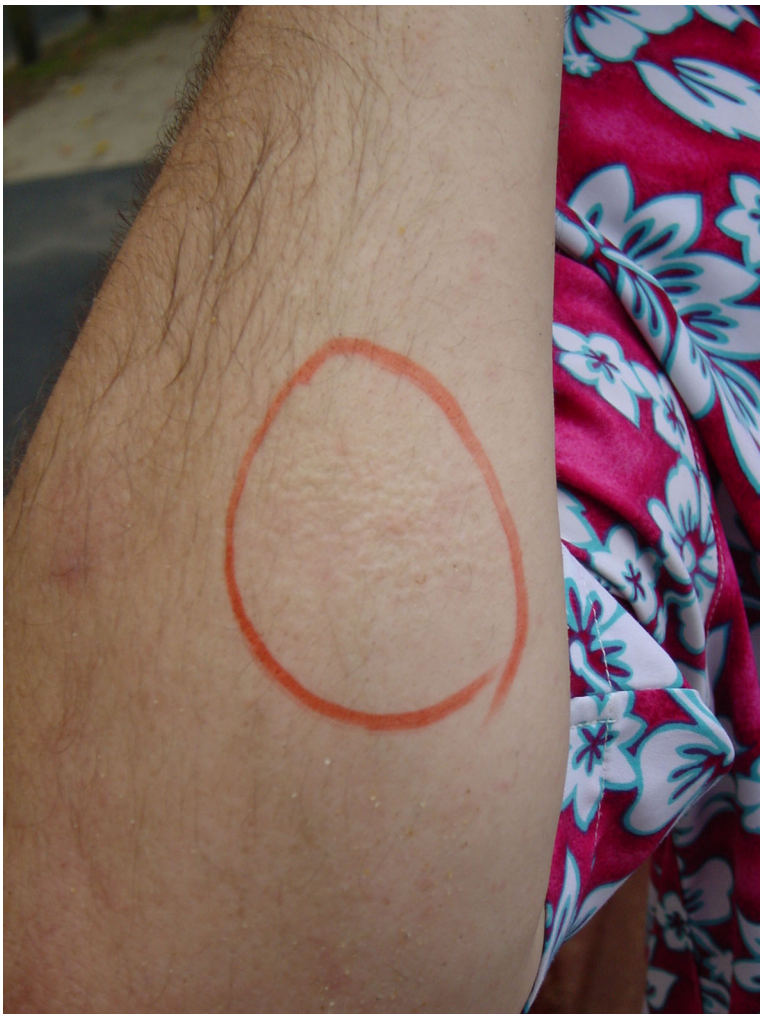

FIGURE 2 | Local response to *C. barnesi* envenomation. Image showing a typical dermal reaction on the arm following a sting from a *C. barnesi* jellyfish. The red marker indicates the sting site. Photo by Jamie Seymour (JCU, Cairns, Australia).

contributes to the positive feed-forward cytokine dysregulation seen in CRS (206). Encouragingly, the blockade of α_1-adrenergic receptors (also expressed on immune cells) or the inhibition of tyrosine hydroxylase (required for catecholamine biosynthesis) by prazosin or metyrosine hindered the self-amplifying proinflammatory loop *in vitro* and *in vivo* (206).

Specifically, CRS, induced by humanised CD19 CAR-T cells in mice engrafted with a leukemia cell line, caused excessive levels of adrenaline, noradrenaline, and myeloid-derived cytokines (IL-6, KC, MCP-1, and TNF) in the plasma of animals with high tumour burdens (206). Consequently, higher mortality was observed among these mice. In contrast, pretreatment with metyrosine or prazosin lowered circulating catecholamines and cytokines, improving survival (206). In mouse peritoneal MΦs, LPS-stimulation induced the release of catecholamines and proinflammatory cytokines, IL-6, KC, MCP-1, and TNF (206). Cytokine and catecholamine secretion was markedly enhanced in LPS/adrenaline co-cultures (206). Conversely, reduced MΦ catecholamine production significantly reduced IL-6, KC, MCP-1, and TNF levels (206). Although this model is unrelated to envenomation, it suggests that immune stimuli, such as venom-induced TLR activation, initiates a proinflammatory response that is enhanced by the presence of catecholamines. The dual stimulatory signals create a positive feed-forward loop, resulting in cytokine amplification. Collectively, these studies suggest a therapeutic potential of prazosin for severe IS, except where contraindicated, and warrant the investigation of plasma cytokines in sting patients.

However, while such research supports the therapeutic inhibition of catecholamines in envenomation, understanding their possible protective function has not been investigated. For example, it is known that catecholamine release promotes alveolar fluid clearance (174). In scorpionism, a rapid catecholamine surge following a dangerous sting is reasoned to increase alveolar fluid reabsorption, protecting the lungs from venom-induced flooding (174). Therefore, the resolution of the

surge may decrease the ability of the lungs to clear venom-induced oedema, ultimately progressing toward fatal pulmonary oedema (174). Accordingly, animal models of IS should thoroughly scrutinise both the benefits and limitations of these mediators.

Finally, given the immune system's propensity toward hyper-responsiveness and allergic reaction to jellyfish venom, the overlap in IS and CRS generalised symptoms, and the recently described link between CRS and catecholamine storm, immune involvement in the pathology is plausible. Nevertheless, to date, no *C. barnesi* immunology-based research has been published.

THE IMMUNOSUPPRESSIVE POTENTIAL OF VENOM-DERIVED MOLECULES

Despite the health burden of human envenomation, venom-immune interactions have been exploited in traditional medicine for centuries (207). More recently, research groups throughout the world have demonstrated the *in vitro* and *in vivo* efficacy of whole venom and venom-derived compounds in ameliorating a wide range of autoimmune symptoms (112, 208–211).

Presently, the most promising drug leads belong to the class of ion channel modulators. Ion channels, particularly calcium-activated and voltage-gated potassium channels, are attractive therapeutic targets for autoimmune diseases. Firstly, ion channels, such as the Shaker-related voltage-gated $K_V1.3$ and Ca^{2+}– dependent KCa3.1, regulate Ca^{2+} signalling in activated immune cells, allowing cell depolarisation and maintenance of membrane potential. Intracellular Ca^{2+} levels dictate T cell activation, proliferation, metabolism and cytokine production (212). Secondly, unique ion channel dimers are differentially expressed in various tissues, including immune cell subsets, permitting cell type and subset-specific blockade (213, 214). For example, activated effector memory T cells (T_{EM}), B cells and MΦs, known mediators in the pathogenesis of various autoimmune diseases, preferentially upregulate $K_V1.3$ (215–217). In contrast, naïve (T_n) and central memory cells (T_{CM}) express KCa3.1 ion channels, allowing for channel-specific inhibition (218). Finally, inhibition of Ca^{2+} influx *via* ion channel blockade allows targeted and reversible immune modulation, rather than complete T cell suppression, as induced by T cell Ca^{2+} modulating drugs, including calcineurin inhibitors and steroids.

Venom from snakes, spiders, scorpions, cone snails, and sea anemones comprise a diverse range of peptide and small molecule ion channel blockers that exhibit selectivity at picomolar concentrations (219). Blockade of lymphocyte ion channels using venom-derived compounds has therapeutic effects in animal models of rheumatoid arthritis (RA), asthma, multiple sclerosis (MS), delayed-type hypersensitivity, and allograft rejection (113, 208, 219–224). Notably, a selective peptide blocker, *Stichodactyla helianthus* toxin (ShK), from sea anemone venom, and anuroctoxin, a peptidyl toxin isolated from *Buthus sindicus* scorpion venom, have been shown to specifically target $K_V1.3$ channels with high affinity, preventing Ca^{2+} influx and thereby inhibiting T_{EM} activation, proliferation and cytokine production (113, 115, 225–227).

Structural studies centred on the selectivity of peptide ion channel blockers have revealed that specificity is due to single amino acid effects rather than *en bloc* backbone structure (228). Thus, venom-derived peptides may act as promising drug scaffolds, notably because disulphide bonds encode robust biological stability (229). This has important implications for drug development, as synthetic manipulation may improve drug activity or remove toxicity from the natural peptide blueprint. For example, ShK(L5), a synthetic analog of ShK, contains an N-terminal L-phosphotyrosine extension and shows higher selectivity than the native peptide for $K_V1.3$ channels over the neuronal ion channel $K_V1.1$ (113).

Aside from ion channel blockade, venom-derived components have demonstrated potent *in vitro* and *in vivo* anti-inflammatory activity through the cholinergic anti-inflammatory pathway *via* an alpha7 nicotinic acetylcholine receptor antagonist (114). Venom-derived peptides, such as the α-neurotoxin from the Thailand cobra, are potent nicotinic receptor antagonists (218). In a rodent RA model, Cobratoxin-treatment reduced expression of the pro-inflammatory cytokines IL-1β, IL-2, and TNFα, resulting in decreased paw sensitivity and joint destruction (230).

Other neurotoxins, such as the principal toxin (NTX) from *Naja atra* venom (NNAV), have shown therapeutic effects in animal models of adjunctive arthritis, RA, Systemic Lupus Erythematosus (SLE), and nephropathy (209, 211). Additionally, NTX-treatment prolonged skin allograft survival in rats and inhibited cell-mediated immune responses in a dose-dependent manner through decreased Th1-type cytokines (IL-2 and IFN-γ). Although low NTX concentrations were cytotoxic, heat-treatment reduced NTX toxicity without reducing its immunosuppressive activity (211). In another study, orally administered NTX suppressed murine T cell proliferation, specifically Th17 and $CD8^+$ T cell activity, increasing NK cell and B cell proliferation in a dose-dependent manner (209).

Venom from the honey bee has been used for centuries in traditional medicine to treat chronic inflammatory diseases due to its reported anti-inflammatory activity (207). Investigations into the mechanism of action of honey bee venom and its major components, melittin and phospholipase A2, have confirmed a protective effect in animal models of asthma and RA (207, 231). The polarisation of T cells towards a Th2 phenotype is associated with allergies and chronic inflammatory diseases (232). An essential driving factor in lineage determination is cytokine expression. It has been shown that melittin inhibits LPS-induced inflammation by binding to the C- terminus of the NF-κB p50 subunit, thus preventing translocation into the nucleus and transcription of pro-inflammatory cytokines, including TNF (233–235). Moreover, treatment with whole honey bee venom polarised T cells towards a Th1 phenotype by inducing T-bet and IFN-γ in $CD4^+$ T cells (210). Conversely, PLA_2, an enzyme found within the venom of multiple species, including the western honey bee, can hydrolyze membrane

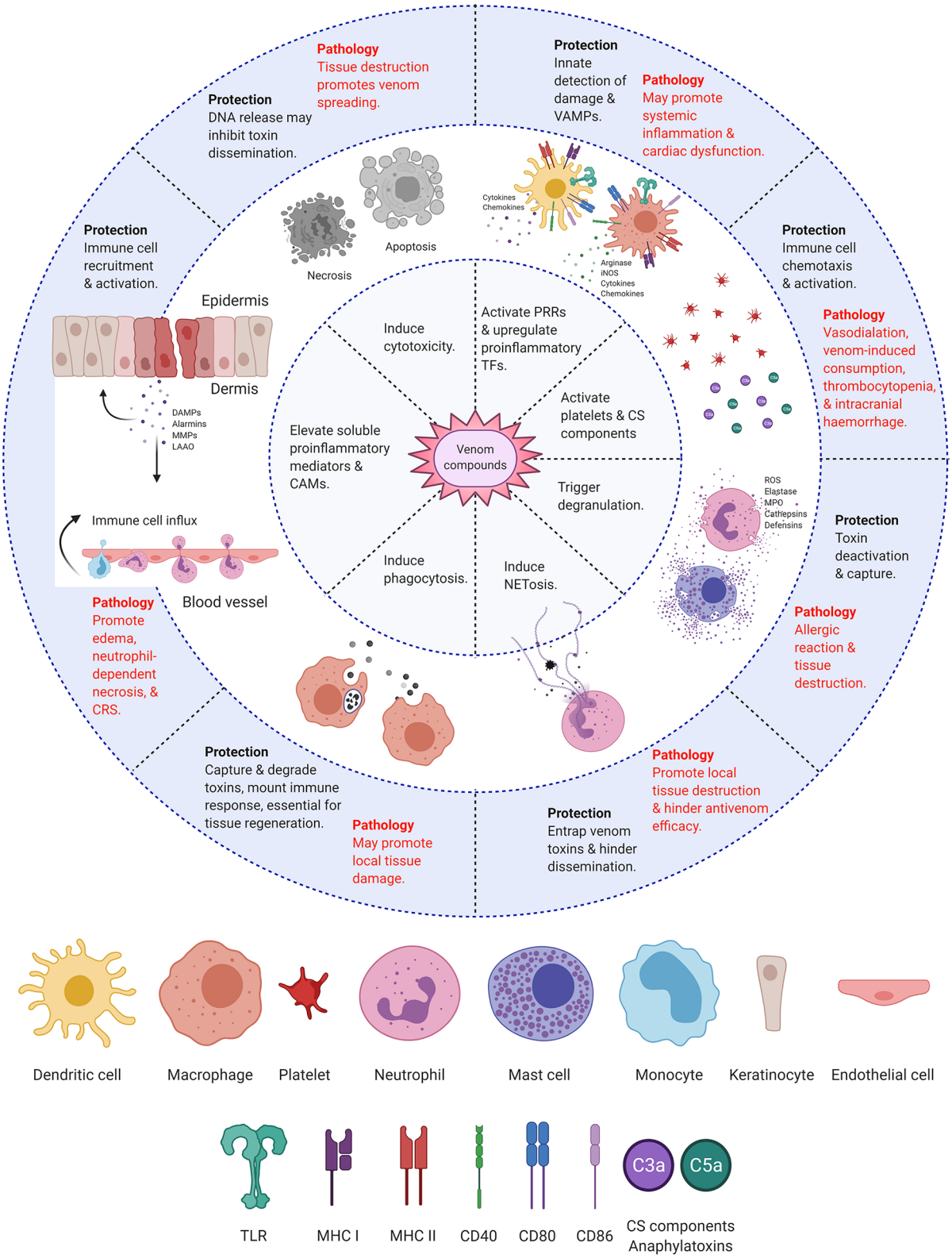

FIGURE 3 | Immunological responses to envenomation. Diagram summarizing the protective and pathological responses of the host's immune system towards venom compounds. Created with BioRender.com.

phospholipids and induce Th2 cytokine responses through the activation of ST2, a component of the IL-33 receptor on innate immune cells (236).

Other known venom immune modulators include tick salivary protein (Salp) 15 from *Ixodes scapularis* and spermine. Salp15 binds the CD4 co-receptor, MHC-II, inhibiting TCR ligation and T cell activation by misaligning CD4 with the TCR complex (237). Spermine, an acylpolyamine found in snake and spider venom, suppresses mitogen-induced activation and proliferation of PBMCs by inhibiting LAF-1 protein expression, involved in RNA remodelling (238).

Collectively, these studies highlight the potential of venom-derived molecules to modulate immune cells as unmodified venom-derived compounds or as scaffolds for drug development. Venom-derived compounds induce immune suppression using diverse modes of action. Thus, screening venom for its immunosuppressive and immune-activating potential may result in new immunomodulatory drugs and the discovery of new biological pathways.

CONCLUSION

Conferring protection against venom's potentially lethal action requires rapid immune recognition and response. Extensive research focuses on the degree to which immune responses themselves contribute to the severity of envenomation (**Figure 3**). However, there is disagreement regarding whether the body's defensive reactions are helpful or harmful. Perhaps the most significant cause of division lies in the difficulty of distinguishing the actual venom-induced symptoms from immune-induced pathology. The classic inflammation markers (heat, pain, redness, swelling, and loss of function) are typical biological responses to envenomation across many species. Therefore, determining which symptoms are treatable using immunological approaches requires further research.

Nevertheless, venom's ability to modulate immune activity has two therapeutic implications. Firstly, continued research could inform improved treatment strategies for fatal bites and stings. Secondly, as venom is a rich source of specific and potent biomodulators, exploring venom-immune interactions may lead to discovering novel pathways/receptors or the development of venom-derived immunomodulatory drugs.

AUTHOR CONTRIBUTIONS

Writing—original draft preparation, RR. Writing—review and editing, RR, JM, MI, JS, AL, and JL. Supervision, JM, MI, and JL. Funding acquisition, JM. All authors contributed to the article and approved the submitted version.

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