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The dynamics of cytokine d nitric oxide secretion in mice injected with *Tityus serrulatus* scorpion venom

Vera L. Petricevich[1,CA] and Carlos F. Peña[+2]

[1]Laboratório de Imunoquímica, Instituto Butantan, São Paulo, Brazil; [2]Instituto de Biotecnología, UNAM Cuernavaca, México

[CA]Corresponding author:
Tel: 005511 3726-7222; ext 2231
Fax: 005511 3726-1505
E-mail: velupetri@hotmail.com

***Aims*: The effects of *Tityus serrulatus* venom (TSV) were analysed with respect to the susceptibility of four isogenic mouse, the symptoms following injection of venom and the inflammatory mediators in an experimental model of severe envenomation induced in mice.**

***Methods*: The susceptibility was analysed by lethal dose (LD50) determination, including the symptoms observed during envenomating and glucose levels. The detection of cytokines in serum from mice were analysed using enzyme-linked immunosorbent assay, and nitric oxide (NO) was analysed using nitrite determination.**

***Results*: The estimated LD_{50} values were in micrograms per 100 microliters, and the susceptibility of mice to TSV varies with: (a) mouse strain and route of injection (A/J < BALB/c < C57Bl/6 = DBA); (b) mouse strain and sex (A/J female and male < BALB/c female and male); and (c) body weight (all groups of A/J < BALB/c groups).**

Among the mouse strains studied, BALB/c mice presented moderate sensibility to TSV, with changes in specific signs and serum levels of glucose, several cytokines and NO, when injected intraperitoneally (i.p.) with 1 LD50 of venom. Sweating, salivation and tremor were the specific signs that preceded death. The maximum levels of glucose in sera from mice injected i.p. with 1 LD50 of TSV were observed 60–90 min post-injection. Significant differences were observed in the time-course of cytokine levels, and the venom induced marked elevations of interleukin (IL)-1α, IL-1β, IL-6, IL-10 and interferon gamma (IFN-γ). The maximum levels of IL-1α and IL-1β were observed 2 h post-injection. The more pronounced levels of IL-6 were observed 4 h post-injection. There was an early increase in IFN-γ followed by an even higher level after 4 h. IL-10 levels peaked between 6 and 8 h, and this cytokine probably modulates the secretion of IFN-γ. Tumor necrosis factor release was not detected in BALB/c mice injected with TSV. NO levels attained maximal release after 2 h, following venom injection, while a second peak for NO was at 6 h.

***Conclusions*: These findings indicate that the susceptibility to the systemic effects of the venom varies among mice of different haplotypes, and that the cytokines such as IL-1, IL-6, IFN-γ and NO are strongly involved in the pathogenesis caused by this venom and are correlated with the severity of envenomation.**

Key words: *Tityus serrulatus* venom, Symptoms, Cytokines, Nitric oxide

Introduction

Scorpion stings are important not only because of their incidence, but also for their potential ability to induce severe, and often fatal, clinical manifestations. In Brazil, two scorpion species of medical importance occur in the state of São Paulo, the brown scorpion *Tityus bahiensis* and the toxic yellow scorpion *Tityus serrulatus*.

Patients with scorpion envenomation present signs such as fever, vomiting, prostration, sweating, cardiac failure, psychomotor agitation, pulmonary edema and shock.[1] The severity of symptoms presented in these patients depends on the intensity of the

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envenomation. Specific signs are directly related to the venom components; patients stung by scorpions may develop a systemic inflammatory response syndrome, and the cytokine released may also play a major role in the pathogenesis.[2,3]

Experimental studies in induced models have the advantage over those in spontaneous models in that the onset and progression of pathology can be controlled. On the contrary, genetics plays a key role in susceptibility and determining the expression of sex hormones and neuroendocrine factors. In an individual with a susceptible genotype, exposure to environmental factors such as infectious agents or toxins can act to initiate an immunizing process. Various factors together affect the immune response to one's own and foreign antigens through the modulation of cytokine production and effects on cell function. To address these issues, basic information obtained in a representative animal model using adequate venom samples as inflammatory inducers is necessary. Previous studies have shown that the different susceptibility to venom varies according to the strain of mice used.[4] However, some coincidences or discrepancies were also observed in venom composition among several snakes and scorpions according to the age, sex, nutritional state and geographic regions where the animals were captured.[5,6] To minimize the experimental bias, mice strains were used to of *Tityus serrulatus* venom (TSV) action and a mixture of venom obtained from 40 adult specimens from the same geographic region were used throughout all experiments.

Cytokines are important mediators in cellular interactions in a variety of immunological and inflammatory occurrences. In cases where septic shock demonstrated high levels of cytokines,[7] experimental studies have shown the participation of cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, IL-10 and interferon gamma (IFN-γ) in the shock originating from various etiologies.[8–12] The cytokines play an important role as mediators of systemic inflammatory response syndrome and may have prognostic value.[8]

Nitric oxide (NO) is known to be involved in multiple reactions[13] of biological importance, such as being an endogenous mediator in the control of vascular tone due to its powerful vasodilating activity.[14] NO also has been implicated in the genesis of a variety of pathological conditions such as inflammation, arteriosclerosis and hypertension vascular lesions in which the infiltration of inflammatory cells occurs.[15–17]

The pathogenesis of systemic effects in scorpion envenomation is complex, involving both the direct action of venom components on the tissues and the release of various endogenous mediators. However, the role of cytokines and NO in this pathology has been the subject of few studies. It has been suggested that cytokines are involved in envenomations by different scorpions.[3,18]

The aims of the present study are: (1) to analyze the influence of genetic background on the susceptibility of some isogenic mouse strains to the toxic effects of the venom; (2) to investigate the symptoms following injection of whole TSV; and (3) to evaluate the increment of inflammatory mediators in an experimental model of severe envenomation induced in mice by the scorpion venom.

Materials and methods

Chemical, reagents and buffers

Actinomycin D, orthophenyldiamine (OPD), sodium nitrate (NO), *N*-naphthylethylenediamine, sulfanilamida, and NO reductase were purchased from Sigma (St Louis, MO, USA); fetal calf serum (FCS) and RPMI-1640 medium were purchased from Cutilab (Campinas, SP, Brazil); murine anti-IL-6 (clones MP5–20F3 and MP5–32C11), recombinant IL-6, anti-IL-10 (clones JES5–2A5 and SXC-1), recombinant IL-10, and anti-IFN-γ (clones XGM1.2 and AN18), recombinant IFN-γ, were purchased from Pharmingen (Torreyana, San Diego, CA, USA); recombinant TNF was purchased from Boehringer Mannheim (Mannheim, Germany); and IL-1α and IL-1β were purchased from Genzyme (Cambridge, MA, USA).

Venom

T. serrulatus scorpions were provided by Laboratório de Artrópodos, Instituto Butantan (SP, Brazil). The venom was obtained by electrostimulation.[19] Briefly, 15–20 V of electrical stimuli were repeatedly applied to the scorpion and the venoms were collected with a micropipette, lyophilized and stored at −20°C. Venom was pooled from more than 40 adult specimens and is referred to as TSV.

Animals

Different mouse strains (A/J, BALB/c, C57Bl/6 and DBA/2), female or male, of different age and weight, obtained from Instituto Butantan, were used throughout the study. The animals were maintained and used under strict ethical conditions according to international recommendations for animal welfare (Committee Members, International Society in Toxicology, 1992).[20]

Lethality

The lethal dose (LD_{50}) was estimated by injecting intraperitoneally (i.p.) and/or subcutaneously (s.c.) with increasing doses of venom into groups of 20 female and 20 male mice (with different ages and weights). Deaths occurring during 24 h were recorded and LD_{50} was calculated by probit.

Table 1. Susceptibility to TSV of different strains of isogenic mice

Strain	Haplotype	LD_{50} (μg/100 μl)	
		i.p.	s.c.
A/J	a	22.5 ± 4.5[a]	27.5 ± 5.5[e]
BALB/c	a	37.5 ± 5.5[b]	42.5 ± 4.5[f]
C57Bl/6	b	47.5 ± 6.5[c]	52.5 ± 6.5[g]
DBA/2	d	47.5 ± 6.5[d]	55.0 ± 4.0[h]

Groups of female mice from different strains, 16–20 g of body weight, were injected i.p. or s.c. with different amounts of TSV. The LD_{50} value was calculated by probit analysis of death occuring within 24 h of venom injection.
[a,b] $p > 0.05$, [a,c,d] $p < 0.001$, [b,c,d] not significant. [e,f] $p > 0.05$, [e,g,h] $p < 0.001$, [f,g,h] not significant.

Effect of TSV on cytokine levels

Groups of mice were injected i.p. with 1 LD_{50} of TSV, dissolved in 0.1 ml of saline solution. Control mice received 0.1 ml of saline solution. Since mortality was of a fraction of the injected animals, the number of mice per experimental group ranged between 5 and 15 to obtain blood samples from at least five mice for each time interval. Mice were bled at 2, 4, 6, 12, 18 and 24 h, and sera were separated and stored at –20°C until use.

Cytokine determination

The levels of cytokines IL-1α, IL-1β, IL-6, IL-10 and IFN-γ in mice sera were determined by two-site sandwich enzyme-linked immunosorbent assay (ELISA).[21] In brief, ELISA plates were coated with 100 μl (1 μg/ml) of the monoclonal antibodies anti-IL-1α, anti-IL-1β, anti-IL-6, anti-IL-10 or anti-IFN-γ in 0.1 M sodium carbonate buffer (pH 8.2), and incubated for 6 h at room temperature. The wells were then washed with 0.1% phosphate-buffered saline (PBS/Tween-20) and blocked with 100 μl of 10% FCS in PBS for 2 h at room temperature. After washing, duplicate sera samples of 50 μl were added to each well. After 18 h of incubation at 4°C, the wells were washed and incubated with 100 μl (2 μg/ml) of the biotinylated monoclonal antibodies anti-IL-1α, anti-IL-1β, anti-IL-6, anti-IL-10 or anti-IFN-γ as second antibodies for 45 min at room temperature. After a final wash, the reaction was developed by the addition of OPD to each well. Optical densities were measured at 405 nm in a microplate reader. The cytokine content of each sample was read from a standard curve established with the appropriate recombinant cytokine (expressed in ng/ml). The minimum levels of each cytokine detectable in the conditions of the assays were 0.20 ng/ml for IL-6, 0.10 ng/ml for IL-10, 0.3 ng/ml for IFN-γ, and 0.09 ng/ml for IL-1α and IL-1β.

To measure the cytotoxicity of TNF present in the sera mice, a standard assay with L-929 cells, a fibroblast continuous cell line, was used as described previously.[22]

Nitrite determination

The nitrite levels in mice sera as an indication of NO production were determined as previously described.[23] Briefly, 40 μl of each mice sera sample were incubated in a 96-well, flat-bottomed plate with 40 μl of the reduction solution (NADPH, 1.25 ng/ml; FAD, 10.4 ng/ml; KH_2PO_4, 0.125 M) containing 0.5 U of NO_2^- reductase for 2 h at 37°C. After this time, 80 μl of Griess reagent (0.1% naphthylenediamine hydrochloride, 1% sulphonylamide, 3% H_3PO_4) were added to each well. The optical densities were measured at 540 nm in a microplate reader. NO_2^- concentrations were determined using a standard curve of $NaNO_3$ ranging from 1.25 to 270 nm (expressed as nmol/ml).

Glucose determination

The glucose levels present in blood from mice injected with TSV were measured using a standard colorimetric assay (expressed as mg/dl).

Statistical analyses

Data are expressed as the mean ± standard deviations (SD). Statistical analyses were performed by Student's *t*-test, and the level of significant was set at $p < 0.05$.

Results

Effect of TSV on different animal parameters

Mouse strain and route of injection. To verify whether the mouse strain and route of injection showed an effect on mortality, TSV was injected i.p. or s.c., at different concentrations, in female A/J, BALB/c, C57Bl/6 or DBA/2 strain mice to determine the LD_{50}. Different sensibilities for various amounts of venom were observed in all groups of animals injected i.p. or s.c. (Table 1). After injecting i.p., the A/J mice presented more susceptibility when compared with other strains of mice (Table 1). The moderate levels of susceptibility were observed for

Table 2. Effects of scorpion venom toxicity according to mouse strain and sex

Strain	LD_{50} value (μg/100 μl)	
	Female	Male
A/J	22.5 ± 4.5[a,c]	30.0 ± 4.0[a,d]
BALB/c	37.5 ± 5.5[b,c]	37.5 ± 5.5[b,d]

Groups of female and male from A/J and BALB/c strains, 16–20 g of body weight, were injected i.p. with different amounts of TSV. Deaths occurring during 24 h were recorded and the LD_{50} value was calculated.
[a] Not significant, [b] not significant, [c] $p > 0.05$, [d] not significant.

BALB/c mice (Table 1). Similar levels of susceptibility were obtained for C57Bl/6 and DBA/2 mice (Table 1). After injecting s.c., the LD_{50} for all mouse strains presented an increase of the TSV concentration (Table 1). The levels of susceptibility with respect to mouse strain were similar to those obtained for injecting i.p.Mouse strain and sex. To determine the sensibility of mice strain and sex, different concentrations of TSV were i.p. injected either in female and male A/J or BALB/c mice, respectively. The intraperitoneal LD_{50} mean values of venom and their differences with respect to the sex and the mouse strain are presented in Table 2. There was a modest difference with the LD_{50} value of females and males from A/J and BALB/c strains (Table 2). However, significant LD_{50}value differences were observed between female and male mice from the A/J and BALB/c strain (Table 2), respectively.Body weight. To verify whether body weight showed an effect on mortality, female mice from A/J and BALB/c strains were injected i.p. with different TSV concentrations. These animals were distributed in four groups, with different body weights. As presented in Table 3, females from A/J and BALB/c strains presented different susceptibility to TSV. The highest susceptibility was observed for female groups from strain A/J at different body weights. As body weight increased, it was possible to observe a decrease in susceptibility for both strains (Table 3). When mice received an intraperitoneal injection of 1 LD_{50} of TSV, the time-course of mortality did not differ between the groups studied. In all groups, the majority of deaths occurred within first 2 and 6 h.Effect of TSV on symptomsSymptoms. When mice received an intraperitoneal injection of 1 LD_{50} of the venom, a fraction of the injected animals died. The majority of deaths occurred within the first 6 h. No deaths were observed in mice injected with saline solution (results not shown). Death was usually preceded by certain signals or symptoms, such as sweating, salivation and tremor. Groups of BALB/c female mice of 16–20 g of body weight were injected i.p. with 1 LD_{50} of TSV, and at different intervals of time specific signs were observed (Fig. 1). The number of animals with sweating and tremor started to appear 15 min post-injection. The maximum number of animals that presented these symptoms was observed at 120 and 90 min post-injection, respectively (Fig. 1). Regarding salivation, this symptom appears at 30 min and progressed until 120 min post-injection (Fig. 1). Similar symptoms were observed for A/J, C57Bl/6 and DBA/2 strains when injected in the same conditions (data not shown). The time-course of specific signs did not differ between the groups analyzed (data not showed).Glucose levels. The metabolism of glucose could be responsible for the pathogenesis of the variety of clinical manifestations, including scorpion envenomation. To determine the glucose levels, groups of BALB/c female mice with 16–20 g of body weight were injected i.p. with 1 $LD_{50}$of TSV. These animals were divided into two groups: one that showed symptoms, and the other that did not show symptoms. Figure 2 shows that the TSV also induced an increase in glucose levels. The maximum levels of glucose in injected sera mice that showed symptoms of envenomation could be observed 60–90 min after injection, decaying thereafter (Fig. 2). In contrast, in groups of animals that did not show specific signals of envenomation,

Table 3. Effects of scorpion venom according to body weight (BW)

Strain	LD_{50} value (μg/100 μl)			
	10–12 g BW	12–15 g BW	16–20 g BW	21–26 g BW
A/J	17.5 ± 5.5[a]	20.0 ± 3.0[c]	22.5 ± 4.5[e]	27.5 ± 5.6[g]
BALB/c	22.5 ± 5.5[b]	28.0 ± 4.0[d]	37.5 ± 5.5[f]	40.0 ± 4.0[h]

Groups of female mice from A/J and BALB/c strains, with different body weights, were injected i.p. with different amounts of TSV. Deaths occurring during 24 h were recorded and the LD_{50} value was calculated.
[a,b] Not significant, [c,d] $p < 0.1$, [e,f] $p > 0.05$, [g,h] $p < 0.1$.

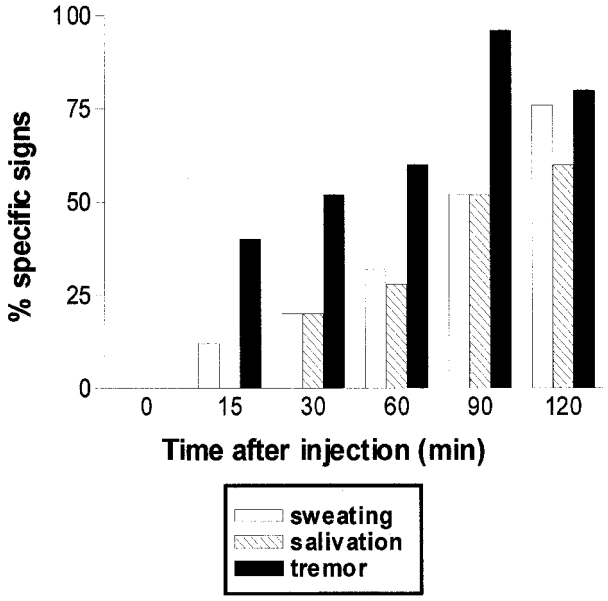

FIG. 1. Symptoms. Groups of 25 female mice from the BALB/c strain, 16–20 g of body weight, were injected i.p. with 1 LD_{50} of TSV. At different time intervals, the specific signs were observed. Each point represents the per cent animal number with symptoms.

no differences in glucose levels were observed (Fig. 2). The levels of glucose in groups of mice with symptoms were significantly higher ($p > 0.001$) when compared with those obtained in groups without symptoms (Fig. 2). Similar results were observed for A/J, C57Bl/6 and DBA/2 strains when injected in the same conditions (data not shown).Effect of TSV on cytokine and NO production*Cytokine levels in serum*. To determine cytokine secretion and NO production, groups of BALB/c female mice with 16–20 g of body weight were injected i.p. with 1 LD_{50} of TSV and bled after different time intervals. Mice injected with saline solution had undetectable levels of all cytokines assayed in the serum. TSV induced a marked increase in IL-1α serum levels, with an early increase occurring within the first 2 h, decaying thereafter (Fig. 3). The highest levels of IL-1β after injection of TSV were observed with one peak at 2 h and a second one at 18 h (Fig. 3). The IL-6 levels increased gradually, reaching its highest at 4 h post-injection. The highest levels of IFN-γ after injection of TSV were observed at 4 h post-injection (Fig. 3). TSV was also able to induce an increase in the serum levels of IL-10, with the highest values occurring 6–8 h post-injection (Fig. 3).NO levels in serum. Figure 4 shows NO levels, which were assessed by determining the concentration of NO_2^-. At all time intervals, envenomed mice presented elevated levels when compared with those observed in group mice injected only with saline solution ($p > 0.001$) (Fig. 4). The highest levels of NO_2^- after TSV injection were observed with one peak at 2 h and a second peak at 6 h, decaying thereafter. Similar kinetics of cytokine secretion and NO production were observed for A/J, C57Bl/6 and DBA/2 strains injected in the same conditions (data not shown).

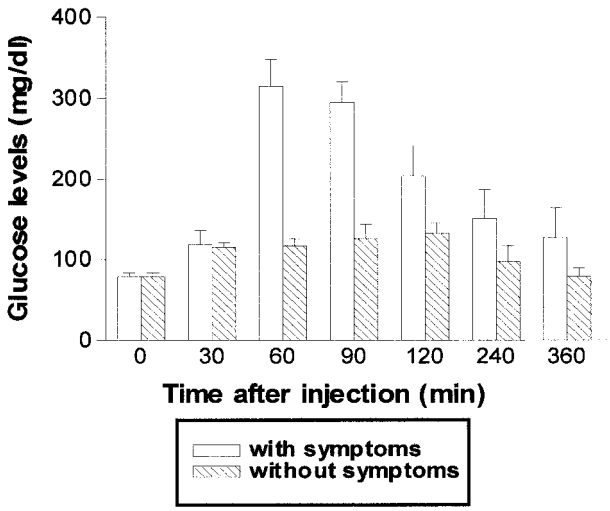

FIG. 2. Glucose determination. Groups of female mice from the BALB/c strain, 16–20 g of body weight, were injected i.p. with 1 LD_{50} of TSV. The groups of animals were then divided into two groups: one showing symptoms, and the other not showing symptoms. The glucose levels were observed at different times. Each point represents the values of samples from two experiments ± SD in different groups of five to 15 mice.

Discussion

Scorpion venom contains a complex mixture of several toxins that exhibit various biological activities. Various factors can contribute to the presence of specific signs and symptoms following stings with respect the scorpion venom toxicity variations.[6] However, it has been demonstrated that other factors also may also contribute to clinical signs, such as age or size of the victims (so that children are normally more severely affected), as well as the site of injection and the vulnerability of the victim to the venom.[1,2]

The present study was designed to simulate accidental envenomation in humans; wherein several injection application strategies were tested. With respect to the route of TSV administration, the time elapsed between the injection and specific signals, the dose administered, the severity of envenomation, and the cytokine and NO production were studied and discussed.

Different animal models of the same envenomation state have unique values: some models highlight genetic factors, whereas others emphasize effects in their mechanisms. The experimental models studied should involve genetically homogeneous animals with differing susceptibility to the venom toxic effects. This was achieved in the present study by determining the LD_{50} value of the venom in four isogenic strains of mice. Among the analyzed strains, A/J was significantly more susceptible to the TSV lethal effects

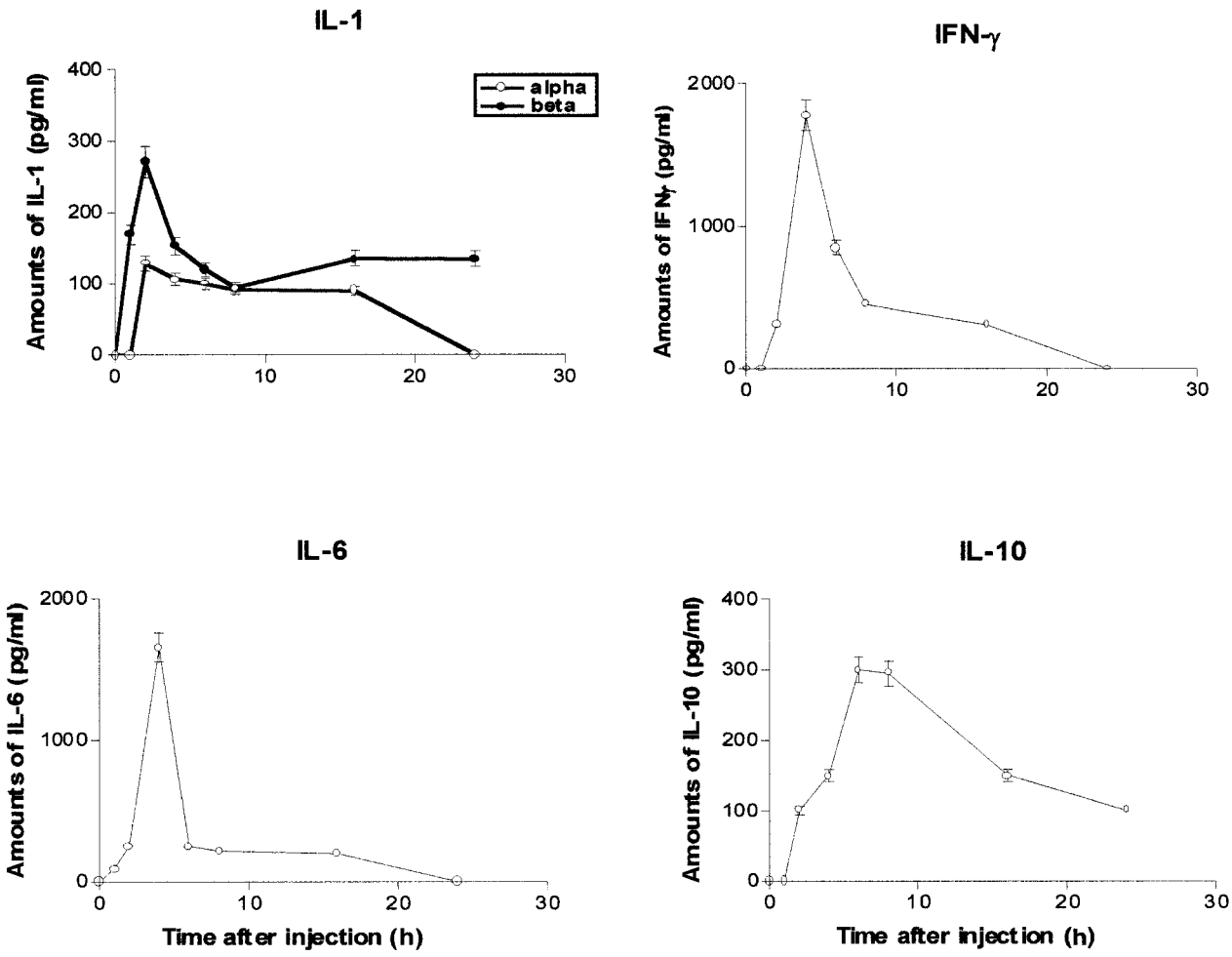

FIG. 3. Cytokine secretion. Groups of female mice from the BALB/c strain, 16–20 g of body weight, were injected i.p. with 1 LD_{50} of TSV. After different times, the animals were bled and the levels of cytokines present in the serum were determined as described in the text. Each point represents the values of samples from two experiments ± SD in different groups of five to 15 mice.

than the other strains studied herein. BALB/c mice were found to be a moderately susceptible strain. C57Bl/6 and DBA/2 mice were mildly resistant to the venom's toxic effects. These results were in accordance with earlier studies.[8,12]

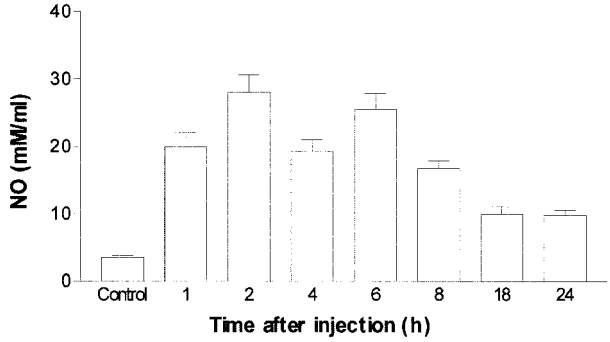

FIG. 4. NO production. Groups of female mice injected as described in the text. The levels of NO present in serum were determined as previously described. Each point represents the values of samples from two experiments ± SD in different groups of five to 15 mice.

The experimental optimization of TSV was also studied to evaluate the efficacy of the injection route. This study compared the effect of injecting TSV i.p. and s.c. A different distribution of susceptibility was observed among analyzed strains. Referring to an increase in age and body weight, a modest decrease in susceptibility to TSV injection was observed among A/J and BALB/c strains.

The rapid absorption and distribution of scorpion venom toxins indicate that scorpion envenomation is an extreme emergency case. Specific signs and symptoms were started immediately after envenomation, developing systemic inflammatory manifestations and organ failure. Scorpion envenomation also presents an elevation of catecholamines, angiotensin II, glucagon, and cortisol accompanied by changes in insulin secretion.[24] The insulin and glucose metabolism alterations could be responsible for the pathogenesis of a variety of clinical manifestations, including scorpion envenomation. The present study showed elevated levels of glucose in blood from mice injected with TSV. These results are in line with

previous reports showing that glucose levels are high in dogs injected with scorpion venom.[24] The absence of clinical signs in group mice injected with TSV was corroborated by a significant reduction of glucose levels. These alterations result not only from the direct toxic action of venom components, but also from the prominent inflammatory reaction associated with this envenomation.

Severe envenomation induced in mice by the injection of venoms was associated with the elevation in serum levels of various cytokines and NO. Cytokines are a group of regulatory and immunomodulatory proteins involved in a number of physiological processes. The inflammatory responses were investigated in the mouse model, as part of the characteristic reaction of the host to TSV. Pro-inflammatory cytokines induce local and systemic inflammatory manifestations. The local effects include the activation of vascular endothelium, an increase in vascular permeability, and the access of leukocytes to the affected tissue, as well as their activation and local tissue destruction. The systemic manifestations include fever, the acute-phase response, and induction of a systemic shock in severe inflammatory processes. The pro-inflammatory cytokines such as IL-1, TNF and IL-6 are endogenous pyrogens, raising the body temperature, which is believed to help eliminate infections.[25,26] IL-1 mediates inflammation and fever, and also induces the expression of adhesion molecules,[27] and the release of leukocyte chemotatic mediators.[28] Thus, IL-1 probably contributes to the systemic inflammatory response in envenomed mice. In this study, TSV induced elevations of IL-1α and IL-1β. Since IL-1α is active in its secreted form, its effects on inflammatory responses are more widespread and prominent than those of IL-1β.[28] Biological actions of IL-1α appear to contribute to inducing shock.[28]

The present study showed that the levels of IL-6 increased until 4 h after TSV injection. IL-6 is a potent pro-inflammatory cytokine,[29] and its biological actions appear to contribute in sepsis and septic shock.[30] The present study showed that TSV induced a severe systemic envenomation in groups of mice injected i.p. with 1 LD_{50}. These results suggest that the severe envenomation caused by this venom is probably associated with marked elevations of IL-1 and IL-6 levels, and that these cytokines may act synergistically to induce shock and other systemic alterations leading to death. IL-6 has been shown to inhibit significantly the TNF production caused by lipopolysaccharide, providing evidence that IL-6 may represent an endogenous negative feedback mechanism of endotoxin-initiated cytokine-mediated acute inflammation.[31] The effects of IL-1 and IL-6 on the production of TNF caused by the scorpion venom suggests a role, as a positive correlation exists between high levels of IL-6 and undetectable levels of TNF in this model.

IFN-γ may originate from a variety of cell types and probably plays a role in the early stages of host response to venoms. IFN-γ is a key cytokine in host defenses against intracellular organisms and enhances the ability in various aspects of macrophage activation.[26,32] In the present study, an early increase in IFN-γ is followed by a marked high at 4 h post-injection.

IL-10 is capable of mediating the suppression of activated macrophages and monocytes leading to striking down-regulating multiple aspects of inflammatory monokines as TNF-α, IL-1, IL-6 and IL-8.[33] In the case of TSV, the high levels of IL-10 were associated with low levels of IFN-γ. These experimental results were in accordance with previous studies, which correlated with low levels of IFN-γ.[11,12] Thus, the anti-inflammatory function of IL-10 may exert a protective effect in venom-induced shock, in accordance with earlier studies that described experimental models of shock in mice.[34]

NO is known to be involved in multiple biologically important reactions.[13] Significant elevation in serum NO levels has been described in mice envenomation.[8,11,12] High NO_2^- serum levels were also observed after injection with TSV venom. Cytokines are powerful modulators of murine macrophage reactive nitrogen intermediate synthesis. While TNF and IFN-γ are potent activators of inducible nitric oxide synthase (iNOS), IL-4 and IL-10 suppress it.[32] Thus, elevated levels of these cytokines in TSV envenomations may be at least partially responsible for NO synthesis. Cellular iNOS activity can generate micromolar concentrations of NO, which might contribute to the hypotension characteristic of this envenomation due to its strong vasodilating action seen during septic shock. These results agree with previous reports, which correlated the cytokine therapy with increased serum NO levels.[16] The precise role played by NO in TSV envenomation needs to be further investigated, but it is probably important in venom-induced systemic alterations.

The mechanisms of TSV inducing cytokine and NO production need to be further investigated. Cytokines are a group of regulatory and immunomodulatory proteins involved in a number of physiological processes. Various disease states are involved in an alteration of normal cytokine activity. Several studies suggest that β cells are very sensitive to the action of pro-inflammatory cytokines such as IFN-γ, TNF-α and IL-1.[35,36] IL-1 inhibits secretion of insulin,[37] which would further exacerbate the hyperglycemic state and lead to increased islet destruction.[36] Treatment of rat islets with IL-1 results in a time-dependent inhibition of insulin secretion that is associated with a similar time-dependent production of NO.[37] The combination of IL-1α and IFN-γ also results in inhibition of insulin secretion and islet destruction that is prevented by the iNOS inhibitor.[36] Glucose-induced insulin secretion is also inhibited by the

cytokines IL-1α, IL-6, TNF[38] and IFN-γ.[36] Interestingly, the present study described a positive correlation that was found between blood glucose levels and IL-1α, IL-1β and NO serum levels. Some venom toxins may act directly on endothelial cells, macrophages and circulating leukocytes to stimulate cytokine production in *Bothrops* envenomation.[8,11,39] Previous studies provided evidence that metalloproteinases from *Bothrops jararaca* and *Echis pyramidium leakey* venoms can cleave pro-TNF-α into its mature form *in vitro*.[40] Other studies have shown that the myotoxin II, a Lys-49 phospholipase A2 homologue from *Bothrops asper*, induces increments of IL-6 in mice.[41] On the contrary, cytokine secretion may be a consequence of venom-induced symptoms and specific signals, which are characteristic of scorpion envenomations[3,18] and the spider *Loxosceles intermedia*.[12]

In conclusion, the results obtained in this study provide experimental evidence that the activation and release of cytokines and NO levels may play an important role in the pathophysiology of envenomation with lethal doses of TSV, and that they may be responsible for some systemic inflammatory manifestations and organ failure.

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