



Experimental immunization with *Thalassophryne nattereri* fish venom: Striking IL-5 production and impaired of B220⁺ cells

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Abstract

Murine experimental model have been useful to understanding the toxic as well as the pharmacological properties of the *Thalassophryne nattereri* venom. However, the specific immune response to *T. nattereri* venom in mice is yet unclear. Our results showed that the venom elicited in BALB/c mice high levels of specific IgG1 and total IgE isotype with high affinity, accompanied by a striking IL-5 production, what point out to a Th2-like response. Meanwhile, the production of IFN- γ by lymphocytes pool expanded upon mitogen stimulus, suggests that the venom was also able to activate Th1 clones. Elevated number of antigen-presenting cells expressing CD11c or CD11b from day 4 to 6 supported ongoing antigen presentation process in the primary response and efficient T-cell expansion (increase of CD4⁺ cells). In contrast, decreased B220 expression was observed, suggesting that the formation of memory long lived cell compartment. In conclusion, *T. nattereri* venom stimulates an association of cytokine of both Th1 and Th2 profile, with a notable IL-5 production and specific IgG1 and total IgE isotypes secretion. Furthermore, our finding showed that *T. nattereri* venom can affect the B cell fate and induce a memory antibody response through the secretion of protective IgG subclasses. Further studies with the venom protein toxins may provide clues to molecular mechanism regulating proliferation and differentiation of antibody-secreting cells in our model. A better understating of how *T. nattereri* venom can modulate immune response could be useful in therapeutic strategies.

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1. Introduction

Thalassophryne nattereri venomous fishes are members of the order Batrachoidiformes of the family Batrachoididae. In Brazil they are commonly

found in the North and Northeast coast, and they are known by gravity of accidents that provokes in fishermen and bathers (Lopes-Ferreira et al., 1998; Haddad et al., 2003; Faco et al., 2005).

Recently Magalhães et al. (2006) identified groups of toxins in the *T. nattereri* venom: the Natterins family, constituted by 5 toxins, Natterins 1, 2, 3, 4, and P, that present homology amongst themselves but not with existent proteins in databases, and

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Nattectin that present homology with C-type lectins. In addition, Natterins was described as kininogenases, responsible for induction of the nociception and the edema (Magalhães et al., 2005). The clinical features of *T. nattereri* envenomation are pain, local swelling, erythema followed by intense necrosis (Fonseca and Lopes-Ferreira, 2000).

Experimental studies performed with the *T. nattereri* venom allowed us to determine the presence of substances with toxic activities. Low doses of the venom (starting from 0.3 µg/animal) induced local effects as the pain and edema, similar to that described in humans (Fonseca and Lopes-Ferreira, 2000), independently of the presence of hemorrhagic, phospholipasic or coagulant activities (Lopes-Ferreira et al., 1998). It was verified that the nociception and the edema caused by the venom could be mediated by the kalicein–kininogen–kinin system (Lopes-Ferreira et al., 2004). The histological analysis of the lesion provoked by the venom in the gastrocnemius muscle evidenced acute myonecrosis, presence of thrombi, a scarce infiltrate of polymorphonuclear leukocytes and macrophages, and the skeletal muscle regeneration was partially impaired (Lopes-Ferreira et al., 2001).

Recently, cytokines production and cellular influx kinetic in local acute inflammatory response induced by the venom were characterized (Lima et al., 2003). TNF- α , IL-1 β and IL-6 were detected on footpad homogenates after intraplantar injection, followed by a poor infiltration of leukocytes, mainly of mononuclear cells. Additionally, a cytotoxic effect of the venom on mononuclear cell was also observed.

Development of a specific immune response to protein antigen is triggered by interactions of CD4⁺ T lymphocyte with antigen-presenting cells and cytokines present in the microenvironment (Abbas et al., 1996). Determination of the Th1 or Th2 response profile has been important in the understanding of immunomodulatory effects induced by protein antigens. Th1 cells secrete IL-2, IFN- γ and TNF- α , and are important in the activation of phagocytic cells and on the switching to opsonizant IgG isotype. On the other hand, Th2 cells secrete IL-4, IL-5, IL-13, IL-10 and are involved with eosinophil, mast cell and B cell activation, beyond the IgE and IgG1 antibodies production.

Murine experimental model have been useful to understanding the toxic as well as the pharmacological properties of the *T. nattereri* venom. However,

the specific immune response to *T. nattereri* venom is yet unclear. Then, in this work, we investigated the adaptive immunity stimulated for this potent venom in mice. Specific antibody isotypes and cytokine profile were evaluated. In addition, we also determined the kinetic of activation of the leukocytes after *T. nattereri* venom immunization.

2. Material and methods

2.1. Animals

Seven to 8-week-old male BALB/c mice were obtained from a colony at the Butantan Institute, São Paulo, Brazil. The animals were maintained at the animal house facilities under specific pathogen-free conditions. All the procedures involving animals were in accordance to the guidelines provided by the Brazilian College of Animal Experimentation.

2.2. Extraction of venom and protein determination

Venom was obtained from wild specimens of *T. nattereri* fish collected in Mundau Lagoon, State of Alagoas, Brazil. Venom was extracted from the openings at the tip of the spines by applying pressure at their bases. For this study, venom was extracted from 50 specimens collected during different seasons, pooled, centrifuged and maintained to -20°C . Protein content was determined by the Bradford (1976) method using bovine serum albumin (BSA) (Sigma Chemical Co. St. Louis, Mo, USA) as standard protein.

2.3. Immunization of the animals with the venom of *T. nattereri*

In order to characterize the immune responses to *T. nattereri* venom, mice ($n = 5/\text{group}$) were intraperitoneally (i.p.) injected two times with venom (10 µg at 7-day intervals) with 1.6 mg Al(OH)₃ as adjuvant. Seven and 14 days after the first immunization mice were bled and the humoral immune responses were monitored. Seven days after the last sensitization spleens were taken. Blood samples from the mice were obtained by retroorbital bleeding. Six months after the immunization protocol, mice were challenge with *T. nattereri* venom (10 µg/animal, i.p.), and 7 days after, they are bled for determination of antibody production. Blood was centrifuged and the plasma was collected and stocked in the dilution 1/10,240 to -20°C .

2.4. Titration of antibodies by ELISA

Plasma were tested for IgM, IgG, IgG1 or IgG2a antibodies using *T. nattereri* venom-coated 96-well plates and peroxidase goat anti-mouse IgM and IgG or biotinylated goat anti-mouse IgG1 and IgG2a antiserum. The reactions were developed with streptavidin–horseradish peroxidase complex (Sigma Chemical Co. St. Louis, Mo, USA), O-phenylenediamine (OPD) and H₂O₂ and the plates were read at 490 nm on an automated ELISA reader (Spectramax, Molecular Devices). The results were expressed as the mean $\pm$ SEM absorbance. An IgE-specific ELISA was used to quantitate total IgE Ab levels in plasma using matched Ab pairs (553413 Pharmingen and 1130-08 Southern Biotech.), according to the manufacturer's instructions. Samples were quantified by comparison with a standard curve of IgE (0313ID, Pharmingen).

2.5. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to the method of Laemmli (1970). The venom samples were loaded onto a 12% acrylamide gel under non-reducing conditions. Prior to electrophoresis, venom samples (10 μ g of protein) were mixed 1:1 (v/v) with sample buffer. Phosphorylase B (97,000), Albumin (68,000), Ovalbumin (43,000), Anidrase carbonic (29,000) and β -lactoglobulin (18,400) (Sigma Chemical Company, St. Louis, MO, USA) were used as molecular weight markers. The gel was stained with the Comanssie Blue staining method.

2.6. Western blotting

The venom of *T. nattereri* was submitted to the eletrophorese (SDS-PAGE) according to Laemmli (1970) and transferred under constant current of 380 mA for 2 h for a nitrocelulose membrane using the system Trans Blot Pharmacia (Uppsala, Sweden), in agreement with the technique described by Towbin et al. (1979). The proteins present in the nitrocelulose membrane were submitted to the immunoenzimatic characterization. For that, the membrane was incubated by 18 h to 4 °C with phosphate buffer saline (PBS) plus tween (PBST) + 3% BSA. After the blockage, the membrane was washed (Tris/NaCl pH 7.5) and incubated by 3 h at room temperature with the plasma (dilution of 1/40) obtained 7 and 14 days after the

immunization with *T. nattereri* venom or plasma of non-immunized mice, all in PBST + 1% BSA. Afterwards a new cycle of washes was accomplished and the membrane was incubated for 2 h with the second antibody (mouse anti-IgG marked with peroxidase) in the dilution 1/1,500 in PBST + 1% BSA. The excess was removed by a new cycle of washes and the components were revealed by the addition of the cromogen 4-chlorine-1-naftol 0.05% (p/v) in methanol 15% (v/v) in the presence of 0.03% of H₂O₂ (v/v). The reaction was interrupted by successive washes with running water. The patterns of molecular mass used were—galactose 116 kDa, serum albumin bovine 66.2 kDa, ovalbumina 45 kDa, lactato desidronegase 35 kDa, 25 kDa for restriction endonuclease Bsp98I, lactoglobulina 18.4 kDa and lisosyma 14.4 (Molecular Protein Weight Marker—Ferraments Life Sciences).

2.7. Flow cytometric analysis

Spleen from venom- or control-mice collected 4, 5, 6, and 14 days after the first immunization were cut with scissors and left in a plate by 1 h to 37 °C in the presence of collagenase (1 mg/mL, Sigma Chemical Co., St. Louis, MO, USA). The cell suspension obtained was washed in RPMI 1640 supplemented with 10% fetal calf serum (FCS) both from Gibco-Invitrogen, 0.1 mM L-glutamine, 0.5 mM 2-mercaptoethanol, and 1% penicillin and streptomycin. The cells were incubated with the following antibodies (0.5 μ g/10⁶ cells): RPE anti-mouse CD4, FITC anti-mouse CD8, FITC anti-mouse CD11c, RPE anti-mouse CD11b, RPE anti-mouse CD45/B220 all from BD-Pharmingen, San Diego, CA, USA, to 4 °C for 45 min. To reduce nonspecific Ab binding, anti-Fc γ R II Ab (2.4G2; American Type Culture Collection, Manassas, VA) was included in cell surface staining. After that, the cells were washed (3 $\times$) with PBS containing azide (0.1%) and fetal bovine serum (3%). After centrifugation, the cell pellet was resuspended in PBS plus paraphormaldehid (0.5%) and maintained at 4 °C until the moment of the acquisition of the data in a FACSCalibur (BD-Pharmingen, San Diego, CA, USA) equipped with cellquest software (on 10,000 cells).

2.8. Stimulation of spleen cells in vitro

Spleen cells were isolated and cultured in 24-well tissue culture plates at a final concentration of 10 or

6×10^6 cells/mL in RPMI (Gibco-Invitrogen Co., CA, USA) supplemented with 10% fetal bovine serum, 1% L-glutamine (Sigma Chemical Co. St. Louis, Mo, USA) and 1% penicillin/streptomycin. The cells were stimulated with *T. nattereri* venom (20 μ g/mL) or concanavalin A (Con A) (10 μ g/mL). Levels of cytokines were assessed in cell culture supernatants harvested after 24 or 48 h (cultures of 10×10^6 cell) or 72 or 96 h (cultures of 6×10^6 cells).

2.9. Quantification of cytokines

Cytokines were measured in the supernatants of spleen cell cultures 14 days after immunization with venom by a specific two-site sandwich ELISA, using the following OpTEIA ELISA kits: for IL-2, 555148; for IL-4, 555232; for IL-5, 555236; for IFN- γ , 555138; for IL-10, 555252, all from BD-Pharmingen, San Diego, CA, USA. Binding of biotinylated monoclonal antibodies was detected using streptavidin-horseradish peroxidase complex (Amersham Int., Amersham, UK) and 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (Sigma Chemical Co. St. Louis, Mo, USA) in 0.1 M citrate buffer containing hydrogen peroxide. Samples were quantified by comparison with standard curves of recombinant mouse cytokines. Detection limits were 1.56 pg/mL for IL-2, 3.9 pg/mL for IL-4, 7.8 pg/mL for IL-5, 15.63 pg/mL for IL-10 and for IFN- γ .

2.10. Statistical analysis

All values were expressed as mean $\pm$ SEM. Parametric data were evaluated using analysis of variance, followed by the Tukey test for multiple comparisons. Non-parametric data were assessed using the Mann-Whitney test. Differences were considered statistically significant at $p < 0.05$. The SPSS statistical package (Release 8.0, Standard Version, 1997) was employed.

3. Results

3.1. Antibodies production elicited by *T. nattereri* venom

Mice were bled 1 week after each immunization with *T. nattereri* venom and the plasma content of anti-venom antibodies was also determined by ELISA. *T. nattereri* venom induced a moderate

antigen-specific IgM and IgG primary response (Fig. 1). The levels of venom-specific IgM remained moderate after booster, while high levels of anti-venom IgG production was observed (Fig. 2). It can be observed in Fig. 3 that the secondary response induced by the venom was characterized by high levels of IgG1 and moderate levels of IgG2a. In Fig. 3C, the total amount of IgE is shown in immune plasma from mice after booster ($230,8 \pm 24,32$ ng/mL compared to $34,3 \pm 1,16$ ng/mL from control group). In those mice, venom-specific memory IgG antibodies were detected several months after priming (Fig. 4).

To determine the specificity of the antibodies to venom components, we performed western blotting analysis with plasma from mice immunized with

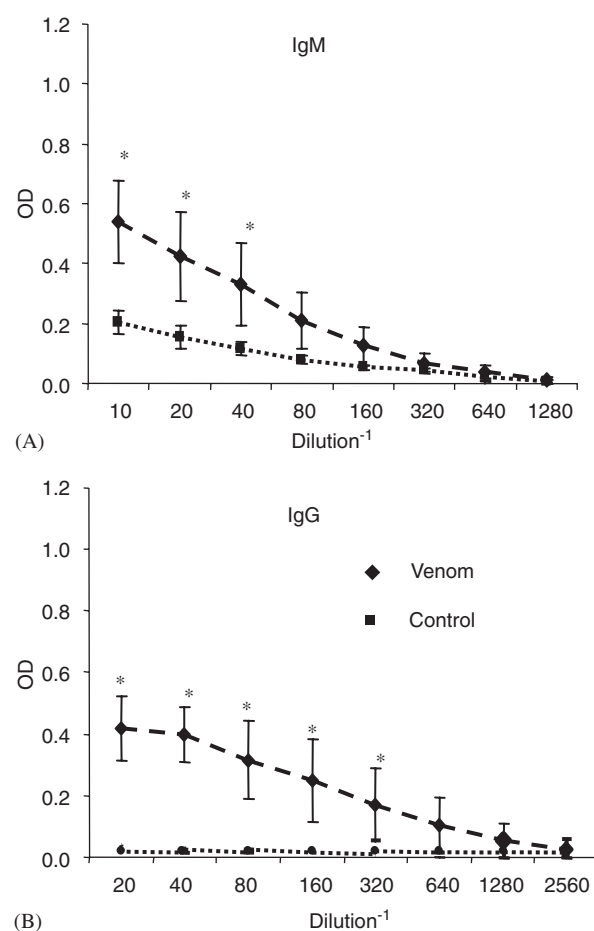


Fig. 1. Effect of *T. nattereri* venom on specific IgM and IgG primary response. BALB/c mice were immunized with *T. nattereri* venom adsorbed in 1.6 mg Alum on day 0. Seven days after immunization, mice were bled for IgM (A) and IgG (B) determination by ELISA. The results represent the mean $\pm$ SEM of 6 animals/group. * $p < 0.05$ compared with control group.

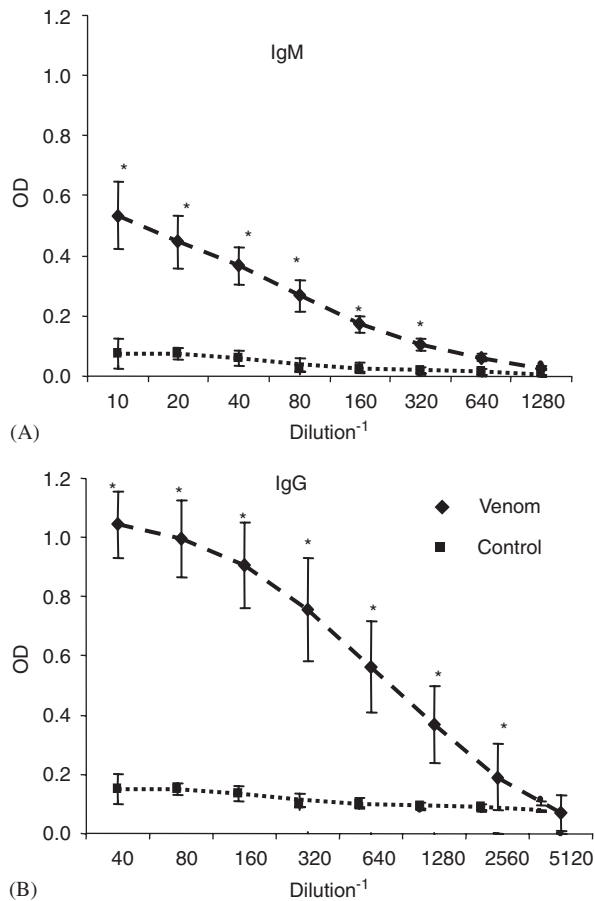


Fig. 2. Effect of *T. nattereri* venom on specific IgM and IgG secondary response. BALB/c mice were immunized with *T. nattereri* venom adsorbed in 1.6mg Alum on days 0 and 7. Seven days after the last immunization, mice were bled for IgM (A) and IgG (B) determination by ELISA. The results represent the mean $\pm$ SEM of 6 animals/group. * $p < 0.05$ compared with control group.

venom. The results depicted in Fig. 5 reveal several immunoglobulin-binding bands in different regions. Samples from mice bled 7 days after the second immunization showed increased intensity of immunoglobulin-binding bands, more pronounced in 100kDa and between 44 and 35kDa regions, and in 15kDa. Plasma from primary response also recognized all components of venom, but less intense when compared with from secondary response.

3.2. Cytokine pattern induced by *T. nattereri* venom

Cytokine levels were measured in spleen cell cultures of groups of mice immunized with *T. nattereri* venom. In Fig 6, it can be observed that the venom stimulated high levels of IL-5, but

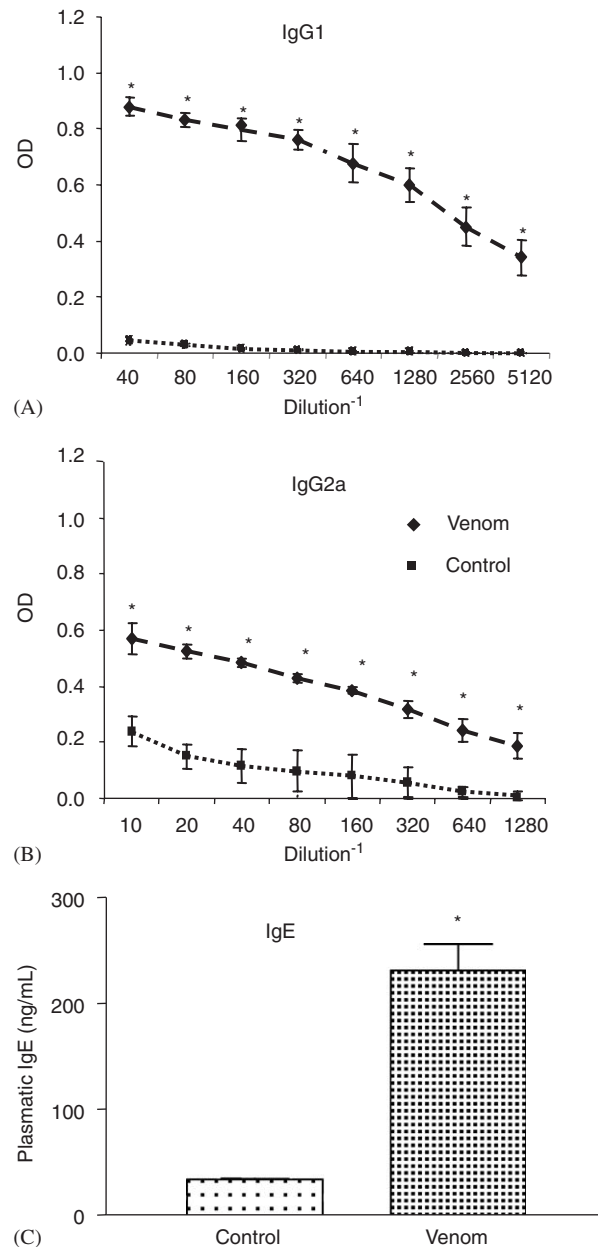


Fig. 3. Effect of *T. nattereri* venom on venom-specific IgG1, IgG2a and total IgE levels in serum. BALB/c mice were immunized with *T. nattereri* venom, and 14 after the first immunization they were bled for IgG1 (A) and IgG2a (B) determination by ELISA. The results represent the mean $\pm$ SEM of 6 animals/group. * $p < 0.05$ compared with control group. Total IgE was measured by sandwich ELISA (C). Each bar represents the mean $\pm$ SEM of 6 animals/group. * $p < 0.001$ compared with control group.

low IL-4 secretion. Cells from venom immunized-mice did not produce IL-2, IL-10, or IFN- γ after venom re-stimulation.

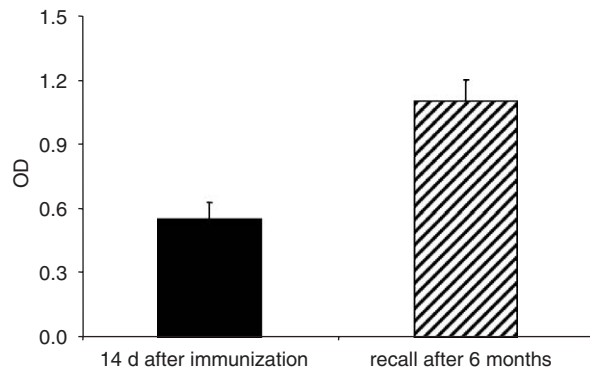


Fig. 4. Memory antibody response induced by challenge with *T. nattereri* venom. Six months after the immunization protocol, BALB/c mice were challenge with *T. nattereri* venom (10 µg/animal, i.p.), and 7 days after they are bled for determination of antibody production. Blood was centrifuged and the plasma was collected and diluted at 1/10,240 for IgG determination by ELISA. Each bar represents the mean ± SEM of 6 animals/group. * $p < 0.001$ compared with control group.

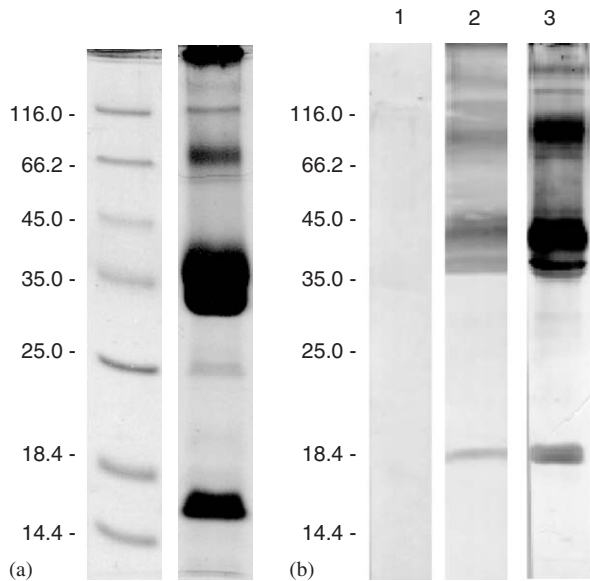


Fig. 5. SDS-PAGE and Western blotting of *Thalassophryne nattereri* venom. *T. nattereri* venom was fractionated by SDS-PAGE using polyacrylamide resolution gels (12,5%) under non-reducing conditions, and revealed by comassie blue (a) or transferred to nitrocellulose membranes by Western blotting (b) using normal mice serum (1), plasma (dilution of 1/40) obtained 7 (2) and 14 (3) days after the immunization with *T. nattereri* venom to detect the antigens. Number at left corresponds to position of M_w markers.

Upon Con A stimulation, IFN- γ and IL-4 synthesis by cells from venom immunized mice were observed. Although the venom induced the synthesis of these cytokines, they were significantly lower

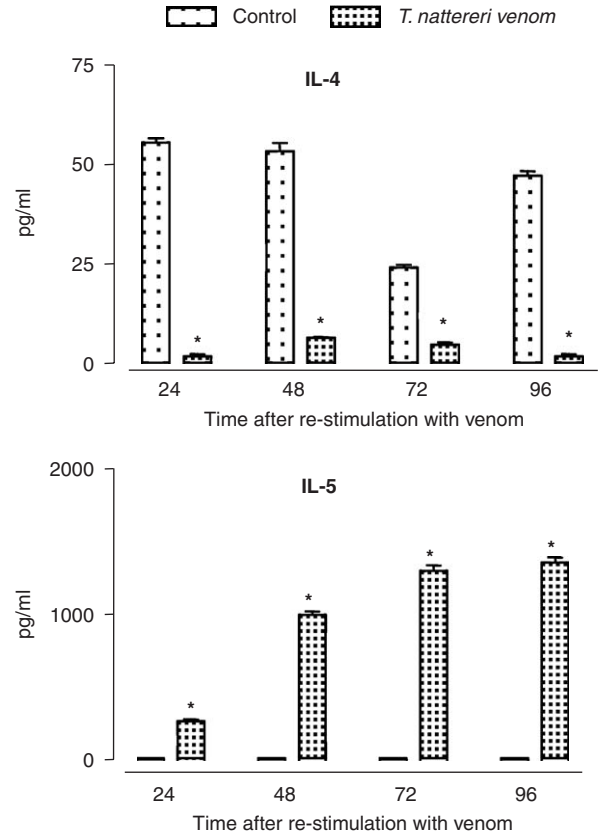


Fig. 6. Synthesis of cytokines by spleen cells from venom or control mice collected 14 days after the first immunization. The cells were stimulated with *T. nattereri* venom (20 µg/mL) or Con A (10 µg/mL). Levels of cytokines were assessed in cell culture supernatants harvested after 24 or 48 h (cultures of 10×10^6 cell) or 72 or 96 h (cultures of 6×10^6 cells). Detection limits were 3.9 pg/mL for IL-4 and 7.8 pg/mL for IL-5. The results were expressed as mean ± SD of duplicate cultures ($n = 6$). * $p < 0.05$ compared with control group.

in comparison to those obtained in the cell culture from mice injected with adjuvant alone. High IL-5 levels were detected throughout the time analyzed and a progressive increase could be noted. Venom immunized mice did not produce IL-2 or IL-10 after Con-A re-stimulation (data not shown).

3.3. Profile of spleen cells activated during the immune response to *T. nattereri*

T. nattereri venom induces peculiar events on the innate immune response in mouse. Then, we wonder about the kinetic of cellular activation elicited by venom during adaptive immune response. For this, FACS analyzes of membrane marker expression (CD11b, CD11c, MHC class II,

CD4, CD8 and B220) in spleen cell suspension was conducted on days 4, 5, 6, and 14 after the first venom immunization (Fig. 7).

When compared to control group, the percentage of cells expressing CD11b, CD11c and CD4 molecules markers was increased from day 4 to 6

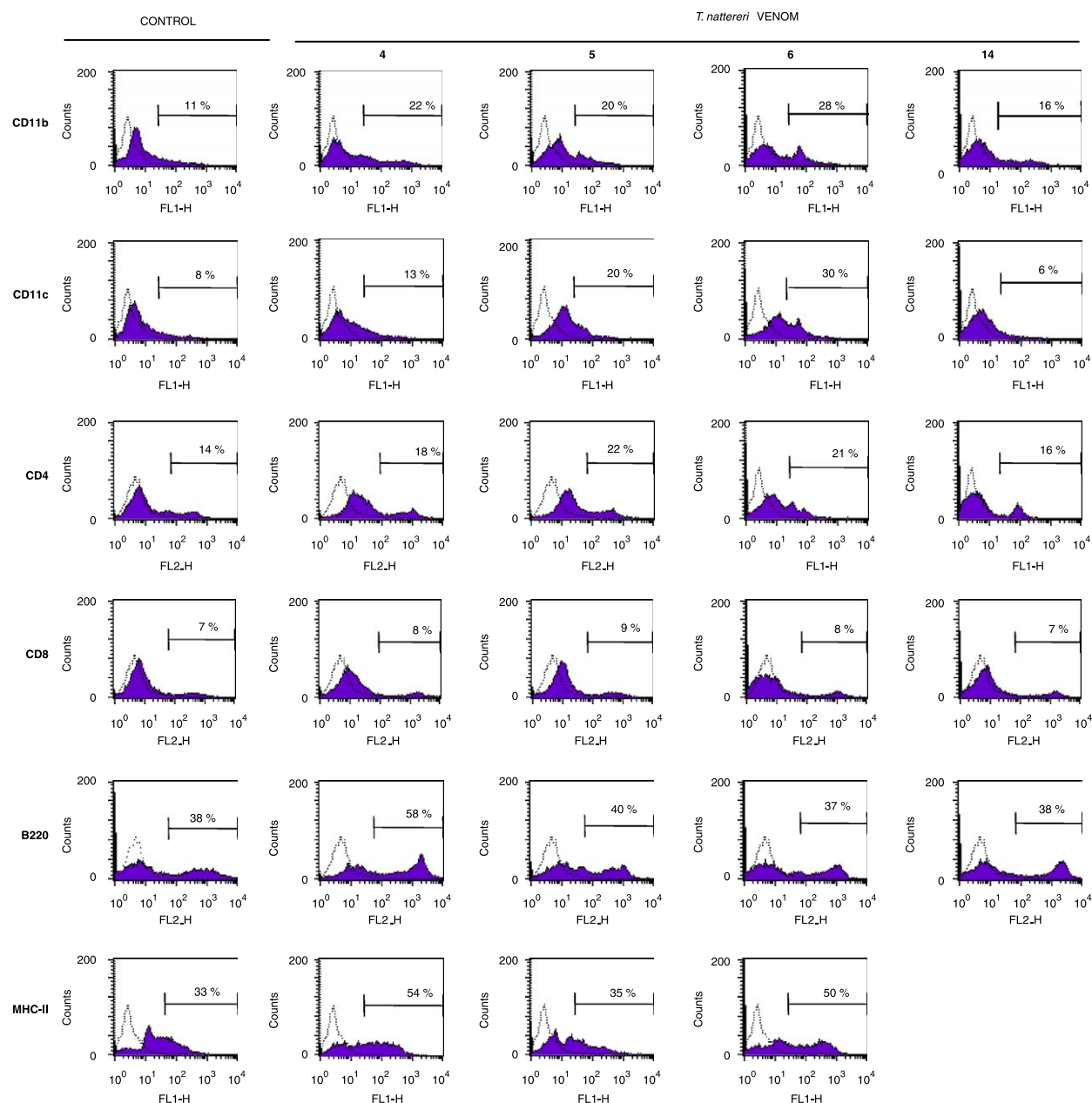


Fig. 7. Spleen cell populations obtained in mice immunized with *T. nattereri* venom in different days. BALB/c mice were immunized with the venom (10 µg/animal) plus Alum (1.6 mg/animal) via intraperitoneal. Non-immunized mice were used as control group. After 4, 5, 6, and 14 days of the immunization with venom, mice were sacrificed and its spleens were collected to prepare the cell suspensions. 1×10^6 cells were stained with anti-CD11b-FITC, anti-CD11c-FITC, anti-CD4-PE, anti-CD8-PE, anti-B220-PE or anti-MHC class II-FITC monoclonal antibodies, for 30 min and analyzed by flow cytometry. Samples of cells were also incubated with isotypic control monoclonal antibody stained with PE or FITC. All samples were made in duplicate and the results expressed in percentage of positive cells to each molecule evaluated. The full histograms represent the analyses of the cells incubated with the different antibodies and the dotted histograms, the results obtained with the cells incubated with the control antibodies. The data shown in this figure are representative of two or three separate experiments.

after immunization, declined to control levels on day 14. In contrast, an increase of cells expressing B220 was only observed at day 4. MHC class II molecules were detected in cells at days 4 and 6 after immunization. The venom did not induce the augment in the percentage of cells expressing CD8 marker.

4. Discussion

In this work, we investigated the cell-mediated and humoral immune response to *T. nattereri* venom in mice. Our results showed that the venom elicited high levels of specific IgG1 and total IgE isotype with high affinity, accompanied by a striking IL-5 production, what point out to a Th2-like response. Meanwhile, in vitro venom induced IFN- γ production when the lymphocytes pool was widely expanded, upon mitogen stimulus, suggesting that the venom was also able to activate Th1 clones. Therefore, the *T. nattereri* venom stimulated both Th1 and Th2 immune response.

In mouse model, the toxic effects of *T. nattereri* envenenation has been closely resembling human (Fonseca and Lopes-Ferreira, 2000). As clearly demonstrated by Lopes-Ferreira et al. (1998), *T. nattereri* venom possess immunogenic property by the induction of specific anti-serum in mice, able to neutralize venom toxic activities. Here, venom mixed to alum for a optimal conditions of T-cell activation, induced significantly higher anti-venom antibody responses in BALB/c mice, compared with the control group of mice immunized only with the adjuvant. Anti-venom antibodies were predominantly venom-specific IgG1 and total IgE isotypes accompanied by lower levels of specific IgG2a. In these mice, memory antibody response was detectable several months after priming and this correlated with the efficiency of the T-cell response observed after antigen recall. These results show that immunization with *T. nattereri* venom induces not only a strong antigen-specific CD4⁺ T-cell response but also a long-lasting T-cell memory against venom antigens.

Currently, CD4⁺ T-cells produce various cytokine mixtures, IL-2, IL-4, IL-5 and IFN- γ (Th0 pattern) after recognized antigen. For development of Th1 or Th2 polarized response particular conditions are required. According to this, the nature of the antigen and stimulus persistence (in chronic infections or immunological hypersensitivity) can provide a preferential production of IL-12

that shift to Th1 response or IL-4 to Th2 response (Feili-Hariri et al., 2005). In our model, venom protein content is vast (Magalhães et al., 2006) and each one of them could elicit one of the Th1 or Th2 cytokines. Moreover, our immunization protocol did not fit in these conditions of chronic stimulation.

IL-2 and IL-4 function as autocrine growth factors for lymphocytes, acting soon after T-cell activation process (Seder and Paul, 1994). On account of this, the detection of these cytokines could be difficult to done in culture supernatant under antigenic stimulation. This fact could explain the absence of IL-2 and the slight production of IL-4 after venom re-stimulus. Accordingly, Ouyang et al. (2000) show that GATA-3 specifies the Th2 fate independently of IL-4/STAT-6. It is a convincing demonstration that cytokines may not be essential to instruction differentiation. In addition, in mice model the Th2 response signature have been IL-5 and IL-13 instead of the IL-4 or STAT 6 (Zheng and Flavell, 1997; Muraille and Leo, 1998). In our model, the venom elicited a remarkable IL-5 production and significant levels of total IgE antibodies, suggesting the induction of Th2 response, once the IgE production is dependent of Th2 cytokines IL-4, and IL-13 (Finkelman et al., 1988).

IL-5 is mainly produced by Th2 lymphocytes and mast cell and acts in the development and activation of eosinophils and B cell subpopulations (Takatsu et al., 1988; Mita et al., 1989). As regard the B cell subpopulations, B-1 cells are predominant in peritoneal cavity and express IL-5 receptor alpha constitutively (Kantor and Herzenberg, 1993; Takatsu et al., 1994). Otherwise, IL-5 receptor alpha is expressed on small proportion (2–4%) of resting B-2 cells in the spleen (Kantor and Herzenberg, 1993). IL-5 enhances IgA and IgG1 synthesis by isotype-committed B-2 cells (Matsumoto et al., 1989). In our assays, the high levels of IL-5 detected indicate that venom behaves a strong stimulus for IL-5 synthesis. Additionally, ongoing IL-5 production could be consequence of great IL-5 amounts in relation to concentration of target cells—B-2 cells—for consuming it. Venom-specific IgG1 levels risen on the secondary response were in agreement with the enhancing effect IL-5 in IgM to IgG1 switching initiated by IL-4 on B cells (Takatsu et al., 1994).

Furthermore, IL-5 has been involved in B-1 and B-2 cells to induce proliferation and differentiation into antibody-secreting cells (ASC) (Messika et al.,

1998; Knodel et al., 2001) and is dependent on blimp-1 expression, which can be induced by IL-5 (Messika et al., 1998). ASC, different of memory cells, do not express CD19, MHC class II or B220 molecules (Slifka et al., 1995; Smith et al., 1996; Manz et al., 1997). Moreover, ASC B220 negative are committed with the maintenance of high affinity antibody levels in secondary response. This cell subset migrate to bone marrow and to marginal B cell zones in spleen (Manz et al., 1998; Rodig et al., 2005).

Analyses of the kinetic of cellular activation, after venom immunization, showed that the *T. nattereri* venom could induce the CD4⁺ T-cell mediated response in mice, accompanied by diminished frequency of these cells in spleen 14 days after immunization, probable due the differentiation in effector T-cell that not remain in lymph organ (Itano and Jenkins, 2003). Besides, the elevated number of antigen-presenting cells expressing CD11c from day 4 to 6 supported ongoing antigen presentation process in the primary response. In contrast, decreased B220 expression suggests the formation of memory long-lived cell compartment. Potent IL-5 production induced by the venom could explain this last finding. In our model, IL-5 could induce preferentially a fast generation of ASC that do not express the B cell isoform B220 and contributes to the rapid appearance and sustained production of high-affinity antibodies (Driver et al., 2001).

In a recent work, Cassese et al. (2003), confirmed the involvement of cytokines in the microenvironment like IL-6, TNF- α and IL-5 in the regulation of the plasma cell longevity. This may underscore the importance of the inflammatory cytokines induced by *T. nattereri* venom (Lima et al., 2003) and the high levels of IL-5 produced in the differentiation and maintenance of antibody-secreting cells in our model.

In conclusion, *T. nattereri* venom content stimulates an association of cytokine of both Th1 and Th2 profile, with a notable IL-5 production and specific IgG1 and total IgE isotypes secretion. Furthermore, our finding showed that *T. nattereri* venom can affect the B cell fate and induce a memory antibody response through the secretion of protective IgG subclasses. Further studies with the venom toxins may provide clues to molecular mechanism regulating proliferation and differentiation of ASC in our model. A better understating of how *T. nattereri* venom can modulate immune response could be useful in therapeutic strategies.

Acknowledgments

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