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ORIGINAL ARTICLE

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Resiniferatoxin modulates the Th1 immune response and protects the host during intestinal nematode infection

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Summary

In the early stage of the intestinal phase of *Trichinella spiralis* infection, the host triggers a Th1-type immune response with the aim of eliminating the parasite. However, this response damages the host which favours the survival of the parasite. In the search for novel pharmacological strategies that inhibit the Th1 immune response and assist the host against *T. spiralis* infection, a recent study showed that resiniferatoxin had anti-inflammatory activity contributed to the host in *T. spiralis* infection. In this study, we evaluated whether RTX modulates the host immune response through the inhibition of Th1 cytokines in the intestinal phase. In addition, it was determined whether the treatment with RTX affects the infectivity of *T. spiralis*-L1 and the development of the *T. spiralis* life cycle. Our results show that RTX decreased serum levels of IL-12, INF-γ, IL-1β, TNF-α and parasite burden on muscle tissue. It was observed that *T. spiralis*-L1 treated with RTX decreased their infectivity affecting the development of the *T. spiralis* life cycle in mouse. These results demonstrate that RTX is able to inhibit the production of Th1 cytokines, contributing to the defence against *T. spiralis*, which places it as a potential drug modulator of the immune response.

KEYWORDS
immune response, resiniferatoxin, Th1 cytokines, *Trichinella spiralis*

1 | INTRODUCTION

About one-third of the world's population is infected with helminth parasites, which makes them one of the most prevalent infectious agents in the world, responsible for many diseases.[1] In both human and animal hosts, helminths establish chronic infections associated with a significant downregulation of the immune response.[2,3] Trichinellosis is a zoonotic-cosmopolitan parasitic disease caused by nematode parasites of the genus *Trichiella*.[4,5] In humans, Trichinellosis is acquired by the ingestion of infective larvae (L1) of *Trichinella spiralis*.[6] These L1 invade the enterocytes of the host's small intestine, where they mature to male and female adult (AD) worms to then produce newborn larvae (NBL).[7] Subsequently, these NBL invade the muscle cells and mature to an infecting stage (L1) and thus complete their life cycle.[8] Because the entire life cycle of *T. spiralis* is completed in a single host, infection by *T. spiralis* represents a major challenge for the immune system, and as the host is under the influence of the antigenic components of the parasite in each stage of its life cycle,[9] this creates an environment that favours its survival, through the modulation of immune responses of the host.[10,11]

Trichinella spiralis infection is characterized by the induction of an early immune response of T helper type 1 (Th1) at the start of the intestinal phase, with subsequent predominance of a Th2-type immune response, resulting in a mixture of both Th1/Th2 immunes responses,[12,13] dependents on $CD4^+$ T cells.[14] During the early stage of infection, *T. spiralis* induces numerous cellular changes in the small intestine with significant increases in the number of goblet cells, mast cells and eosinophils,[15] which together with an increased synthesis of

†Deceased.

Th1 (pro-inflammatory) cytokines[16,17] favours the development of intestinal pathology, damaging the host.[18,19]

Glucocorticoids (GCs) are potent anti-inflammatory drugs of the steroid type,[20] which also act as immunosuppressant, as they regulate the function of various inflammatory cells,[21] in addition to inhibiting the expression of inflammatory genes by suppressing transcription factors, such as nuclear factor κB (NF-κB)[22] and the activator protein-1 (AP-1)[23] through the protein-protein interaction,[24] inhibiting the synthesis of Th1 cytokines.[25] For this reason, GCs have been used as pharmacological therapy in Trichinellosis, in order to inhibit the Th1-type immune response. However, its poor pharmacological efficacy against the parasite has limited its therapeutic use, as it reduces the effectiveness of the host's immune system against *T. spiralis*, increasing the invasion of the parasite in muscle tissue, ensuring its survival.[26,27]

Based on the limited pharmacological efficacy of GCs against *T. spiralis* infection, it is necessary to investigate new pharmacological strategies based on the suppression of the Th1-type immune response, which will help the host against *T. spiralis* infection. A molecule with therapeutic potential for the suppression of the Th1 response during *T. spiralis* infection could be resiniferatoxin (RTX). RTX is a vanilloid derived from a cactuslike plant named *Euphorbia resinifera*, an agonist of the transient receptor potential vanilloid 1 (TRPV1),[28,29] which activates and then desensitizes the TRPV1 receptor, producing analgesia.[30,31] Because several studies have shown that RTX exhibits anti-inflammatory activity in both in vitro[32,33] and in vivo[34,35] models, our research group recently published an investigation, where treatment with RTX in the intestinal phase of infection by *T. spiralis* decreased rat serum levels of pro-inflammatory mediators such as nitric oxide (NO), prostaglandin E_2 (PGE_2) and tumour necrosis factor α (TNF-α), associated with a diminished intestinal pathology and parasite burden in muscle tissue.[36] Based on these findings, the purpose of this research was to evaluate whether RTX is capable to inhibiting the Th1-type immune response, by decreasing the levels of Th1 cytokines such as interleukin (IL)-12, interferon gamma (INF-γ), IL-1β and TNF-α, involved in the immune response against *T. spiralis*. In addition, we evaluated the effect of treatment on the muscle phase of the infection. Finally, infection and reproduction of the life cycle of *T. spiralis*-L1 treated with RTX (in the intestinal phase) in another host (mouse) were determined.

2 | MATERIALS AND METHODS

2.1 | Experimental animal model

2.1.1 | Rat model

Female Long-Evans rats with a body weight between 250 and 300 grams were used. For the intestinal phase, groups of six rats were each formed as follows: a healthy control group (HC); a healthy control group treated with RTX (HC-RTX); four control groups infected with *T. spiralis* (CITsp-1, CITsp-2, CITsp-3 and CITsp-4) sacrificed on days 1, 3, 5 and 7 post-infection (p.i.) respectively; four groups infected with *T. spiralis* treated with (DEX) dexamethasone (Tsp-DEX-1_{IP}, Tsp-DEX-2_{IP}, Tsp-DEX-3_{IP} and Tsp-DEX-4_{IP}) sacrificed on days 1, 3, 5 and 7 p.i., respectively; four groups infected with *T. spiralis* treated with RTX (Tsp-RTX-1_{IP}, Tsp-RTX-2_{IP}, Tsp-RTX-3_{IP} and Tsp-RTX-4_{IP}) sacrificed on days 1, 3, 5 and 7 p.i. respectively. For the muscle phase, the following groups of six rats each were formed: a control group infected with *T. spiralis* (CITsp-5) sacrificed on day 28 p.i.; four control groups infected with *T. spiralis* treated with DEX (Tsp-DEX-1_{MP}, Tsp-DEX-2_{MP}, Tsp-DEX-3_{MP} and Tsp-DEX-4_{MP}) sacrificed on day 28 p.i.; four groups infected with *T. spiralis* treated with RTX (Tsp-RTX-1_{MP}, Tsp-RTX-2_{MP}, Tsp-RTX-3_{MP} and Tsp-RTX-4_{MP}) were sacrificed on day 28 p.i.

2.1.2 | Mouse model

Female BALB/c mice with body weight between 20 and 25 grams were used. Groups of 3 mice were each formed as follows: an infected control group (rCITsp) sacrificed on day 28 p.i.; four control groups infected with *T. spiralis*-L1 treated with DEX (rTsp-DEX-1, rTsp-DEX-2, rTsp-DEX-3 and rTsp-DEX-4), sacrificed on day 28 p.i.; four groups infected with *T. spiralis*-L1 treated with RTX (rTsp-RTX-1, rTsp-RTX-2, rTsp-RTX-3 and rTsp-RTX-4), sacrificed on day 28 p.i. This study was reviewed and approved by the Bioethics Committee and the Academic Council of the Academic Unit of Biological Sciences (UACB) of the Autonomous University of Zacatecas (UAZ), in accordance with the Official Mexican Norm (NOM-062-ZOO-1999), published by the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA) in the official Gazette of the Federation (México) on 28 June 2001.

2.2 | Experimental infection

2.2.1 | Rat model

Rats from all control and treated with DEX and RTX groups were infected orally with 500 *T. spiralis*-L1 from *T. spiralis*-infected canned rats.

2.2.2 | Mouse model

Mice were infected with *T. spiralis*-L1 from groups of rats as follows: the control group rCITsp was infected with 150 *T. spiralis*-L1 from the control group CITsp-5. Control groups infected with *T. spiralis*-L1 from control groups treated with DEX: rTsp-DEX-1 infected with 150 *T. spiralis*-L1 from Tsp-DEX-1_{MP} group; rTsp-DEX-2 infected with 150 *T. spiralis*-L1 from Tsp-DEX-2_{MP} group; rTsp-DEX-3 infected with 150 *T. spiralis*-L1 from Tsp-DEX-3_{MP} group and rTsp-DEX-4 infected with 150 *T. spiralis*-L1 from Tsp-DEX-4_{MP} group. Control groups infected with *T. spiralis*-L1 from control groups treated with RTX: rTsp-RTX-1 infected with 150 *T. spiralis*-L1 from Tsp-RTX-1_{MP} group, rTsp-RTX-2 infected with 150 *T. spiralis*-L1 from Tsp-RTX-2_{MP}, rTsp-RTX-3 infected with 150 *T. spiralis*-L1 from Tsp-RTX-3_{MP} group and rTsp-RTX-4 infected with 150 *T. spiralis*-L1 from Tsp-RTX-4_{MP} group. The parasite (Mexican strain) was identified by PhD Edoardo Pozio, in *Istituto Superiore di Sanita* in Rome, Italy, and has

been maintained by serial passage in mice and rats since 1986 at the Laboratory of Cell Biology and Microbiology at the Academic Unit of Biological Sciences (UACB) from the Autonomous University of Zacatecas. Zacatecas (UAZ), México. All animals were obtained from the bioterror of the UACB-UAZ, which were maintained in temperature-controlled rooms and fed with rodent balanced food.

2.3 | Pharmacotherapy

Commercial dexamethasone sodium phosphate (dose: 1 mg/kg)[37] administered intraperitoneally. The Tsp-DEX-1_{IP} and Tsp-DEX-1_{MP} control groups were treated with a dose of DEX on day 1 p.i. The Tsp-DEX-2_{IP} and Tsp-DEX-2_{MP} groups were treated with two doses of DEX on days 1 and 3 p.i. Tsp-DEX-3_{IP} and Tsp-DEX-3_{MP} groups were treated with three doses of DEX on days 1, 3 and 5 p.i. The Tsp-DEX-4_{IP} and Tsp-DEX-4_{MP} groups were treated with three doses of DEX on days 3, 5 and 7 p.i.

Resiniferatoxin (Sigma-Aldrich, 3050 Spruce St., Saint Louis, MO, USA, 63103, dose: 20 μg/kg)[34] The Tsp-RTX-1_{IP} and Tsp-RTX-1_{MP} groups were treated with a dose of RTX on day 1 p.i. The Tsp-RTX-2_{IP} and Tsp-RTX-2_{MP} groups were treated with two doses of RTX on days 1 and 3 p.i. Tsp-RTX-3_{IP} and Tsp-RTX-3_{MP} groups were treated with three doses of RTX on days 1, 3 and 5 p.i. Tsp-RTX-4_{IP} and Tsp-RTX-4_{MP} groups were treated with three doses of RTX on days 3, 5 and 7 p.i.

2.4 | Determination of serum Th1 cytokines

The concentrations in rat serum of Th1 cytokines were determined quantitatively in the CITsp-1, -2, -3 and -4 groups; and 90 minutes after treatment administration in all groups treated with DEX (Tsp-DEX-1_{IP}, -2_{IP}, -3_{IP} and 4_{IP}) and RTX (Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and 4_{IP}), on days 1, 3, 5 and 7 p.i., using ELISA kit (Life Technologies Corporation, Frederick, MD, USA) for IL-12 and (PEPREOTECH, Rocky Hill, NJ, USA) for INF-γ, IL-1β and TNF-α.

2.5 | Determination of the percentage of eosinophils in the blood

The blood smears were performed from the CS and CS-RTX groups. From the CITsp-1, -2, -3 and -4 groups, a blood smear was performed on days 1, 3, 5 and 7 p.i. Of the groups treated with DEX: Tsp-DEX-1_{IP}, -2_{IP}, -3_{IP} and 4_{IP}, a blood smear was performed on days 1, 3, 5 and 7 p.i., respectively. Of the groups treated with RTX: Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and 4_{IP}, a blood smear was performed on days 1, 3, 5 and 7 p.i., respectively. Subsequently, the Wright's stain method (Wright's Kit, GOLDEN BELL Reagents 82300) was performed to determine the percentage of eosinophils in peripheral blood. The number of eosinophils present in the sample was observed under an optical light microscope (Carl Zeiss Primo Star, model 3708; Carl Zeiss Microscopy GmgH, Göttingen, Germany) using a 100× immersion objective. The average number of eosinophils per 100 white cells was determined from three different counts (300 white cells in total).[36]

2.6 | Determination of implanted *Trichinella spiralis*-L1 in muscle tissue

Samples of muscle tissue such as masseter, tongue, leg and diaphragm[38,39] were obtained from the control group (CITsp-5) and from the groups treated with DEX (Tsp-DEX-1_{IP}, -2_{IP}, -3_{IP} and 4_{IP}) and RTX (Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and 4_{IP}), sacrificed on day 28 p.i. The samples were placed between two compression plates composed of two microscope slides and were observed after compression under an optical light microscope (Carl ZEISS Primo Star, model 3708; Carl Zeiss Microscopy GmgH) using 4×, 10× and 40× objectives in order to assess the presence of nurse cells. The average number of implanted *T. spiralis*-L1 in muscle tissue was determined for each sample by averaging the total count of *T. spiralis*-L1 from three fields observed using a 4× objective.[36,40]

2.7 | Determination of the parasite burden

Muscle tissue was obtained from rats and mice of all groups sacrificed on day 28 p.i. Subsequently, the muscle tissue was thoroughly grinded (using an Oster® processor model 3212). Portions of 30 g of grinded muscle tissue were taken and placed in a tulle sleeve bag inside a separation funnel containing artificial digestive solution of 0.3% pepsin (1:10 000), 7% HCl (37%, 0.2 mol/L) and 90% distilled water. Samples were incubated at 37°C for 24 hours in an incubator (Thelco, Model 4; Precision Scientific Co, Chicago, IL, USA). Larvae packages that resulted of digestion separated and settled at the bottom of the separation funnel were retrieved in conical tubes. After three PBS washes (pH 7.3), the parasite burden in samples of 30 g was determined.[36,41]

2.8 | Viability determination of the *Trichinella spiralis*-L1

To evaluate the viability of *T. spiralis*-LI, 15 μL of *T. spiralis*-L1 from the control groups and treated with DEX and RTX (from Long-Evans rats) was taken and placed in an Eppendorf tube of 200 μL, and then, trypan blue 2% (trypan blue dye IC 23850, 2 g, 100 mL distilled H_2O) was added and left to stand for 15 minutes. Subsequently, the samples were observed in an optical light microscope (Carl ZEISS Primo Star, model 3708; Carl Zeiss Microscopy GmgH) to the 4×, 10× and 40× objectives to observe the viability of the *T. spiralis*-L1. The criterion used was staining by exclusion, staining only dead *T. spiralis*-L1 (nonviable), because live *T. spiralis*-L1 (viable) do not allow the passage of the dye into them.

2.9 | *Trichinella spiralis*-L1 infectivity: reproduction of *Trichinella spiralis* life cycle in BALB/c mice

To evaluate the infectivity of *T. spiralis*-L1 treated with DEX and RTX, the *T. spiralis* life cycle in BALB/c mice was reproduced (see Section 2.2.2). After all groups were infected and the *T. spiralis* life cycle was completed (on day 28 p.i.), all animals were sacrificed and parasite burden was determined.[36]

2.10 | Statistical analysis

Results are presented as mean±standard deviation (SD). Significance was determined by a one-way analysis of variance (ANOVA) to test for overall differences between group means. Student's *t* test for paired samples was used to compare means of paired samples. A *P* value<.05 was considered statistically significant. Statistical analyses were performed in GraphPad PRISM for Windows version 6 (GraphPad Software, San Diego, CA, USA).

3 | RESULTS

3.1 | Production of Th1 cytokines during the intestinal phase of *Trichinella spiralis* infection

The early immune response against *T. spiralis* at the intestinal level is characterized by an initial predominance of a Th1-type cellular response, through the synthesis of pro-inflammatory cytokines.[13-17] In this study, serum levels of Th1 cytokines were analysed during the intestinal phase of *T. spiralis* infection, and it was observed that at 24 hours p.i. the serum levels of IL-12 (813±36 pg/mL), INF-γ (5390±151 pg/mL), IL-1β (2310±143 pg/mL) and TNF-α (1667±252 pg/mL) increased significantly (*P<.05), compared with the HC group. On day 3 p.i., the serum levels of IL-12 (1068±110 pg/mL), INF-γ (7086±310 pg/mL), IL-1β (2408±128 pg/mL) and TNF-α (1719±167 pg/mL) remained significantly increased (*P<.05) compared to HC group. On day 5 p.i., serum levels of IL-12 (987±126 pg/mL) were slightly decreased, but similar to serum levels at day 3 p.i., while the serum levels of INF-γ (7111±306 pg/mL), IL-1β (2484±142 pg/mL) and TNF-α (1715±226 pg/mL) remained significantly increased (*P<.05) compared to the HC group. Finally, on day 7 p.i., it was observed that serum levels of INF-γ (5753±188 pg/mL), IL-1β (2088±67 pg/mL) and TNF-α (1650±212 pg/mL) were slightly decreased, but serum levels of IL-12 (1096±124 pg/mL) remained significantly increased (*P<.05), compared to HC group (Figure 1A-E).

3.2 | Effect of resiniferatoxin treatment on the Th1 cytokines production during the intestinal phase of *Trichinella spiralis* infection

3.2.1 | IL-12

Figure 1 shows how serum levels of IL-12 increased significantly (*P<.05) during the intestinal phase of *T. spiralis* infection (compared to the HC group). When Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and 4_{IP} groups were treated with RTX, it was observed that the serum levels of IL-12 decreased significantly (*P<.05) on days 1 p.i. (41.9±7 pg/mL), 3 p.i (91.8±14.4 pg/mL), 5 p.i. (558±49.8 pg/mL) and 7 p.i. (485±69.8 pg/mL), compared to the CITsp-1, -2, -3 and -4 groups, respectively. When DEX was administered, it was observed that serum levels of IL-12 were similar to treatment with RTX on days 1 p.i. (47.6±6.5 pg/mL) and 3 p.i. (164±67.8 pg/mL), whereas on days 5 p.i. (159±22.4 pg/mL) and 7 p.i. (167±51.4 pg/mL), it was observed that treatment with DEX decreased significantly (*P<.05) the serum levels of IL-12 compared to treatment with RTX (Figure 2A-D).

3.2.2 | INF-γ

On day 1 p.i., it was observed that INF-γ serum levels increased significantly (*P<.05) with respect to the HC group (Figure 1C). When a dose of RTX was administered to the Tsp-RTX-1_{IP} group on day 1 p.i., the serum levels of INF-γ (5318±174 pg/mL) were similar to those in the CITsp-1_{IP} group (Figure 3A). However, in the Tsp-RTX-2_{IP}, -3_{IP} and -4_{IP} groups treated with RTX, it was observed that the serum levels of INF-γ were decreased significantly (*P<.05) on days 3 p.i. (4316±719 pg/mL), 5 p.i. (5761±432 pg/mL) and 7 p.i. (5016±204 pg/mL), similar to the groups treated with DEX, compared to the CITsp-2, -3 and -4 groups, respectively (Figure 3B-D).

3.2.3 | IL-1β

It was observed that serum levels of IL-1β significantly increased (*P<.05, compared to the HC group) during the intestinal phase of *T. spiralis* infection (Figure 1). When Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and 4_{IP} groups were treated with RTX, it was observed that the serum levels of IL-1β decreased significantly (*P<.05) on days 1 p.i. (2050±104 pg/mL), 3 p.i (1970±57.7 pg/mL), 5 p.i. (1923±186 pg/mL) and 7 p.i. (1863±42.5 pg/mL), compared to the CITsp-1, -2, -3, and -4 groups, respectively. When DEX was administered, it was observed that serum levels of IL-1β decreased significantly (*P<.05) on days 1 p.i. (1310±97.7 pg/mL), 3 p.i. (1138±234 pg/mL), 5 p.i. (1060±163 pg/mL) and 7 p.i. (1002±50.1 pg/mL), compared to treatment with RTX (Figure 4A-D).

3.2.4 | TNF-α

Serum levels of TNF-α increased significantly (*P<.05, compared with the HC group) during the intestinal phase of *T. spiralis* infection (Figure 1). When Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and 4_{IP} groups were treated with RTX, it was observed that the serum levels of TNF-α decreased significantly (*P<.05) on days 1 p.i. (1387±103 pg/mL), 3 p.i (1409±106 pg/mL), 5 p.i. (1229±72.4 pg/mL) and 7 p.i. (1120±70.1 pg/mL), compared to the CITsp-1, -2, -3, and -4 groups, respectively. When DEX was administered, it was observed that serum levels of IL-1β decreased significantly (*P<.05) on days 1 p.i. (732±38.4 pg/mL), 3 p.i. (780±60.1 pg/mL), 5 p.i. (686±23 pg/mL) and 7 p.i. (814±40.1 pg/mL), compared to treatment with RTX (Figure 5A-D).

3.3 | Effect of resiniferatoxin treatment on percentage of eosinophils in the blood during the intestinal phase of *Trichinella spiralis* infection

Figure 6B shows that during the intestinal phase of *T. spiralis* infection the percentage of eosinophils in the blood increased

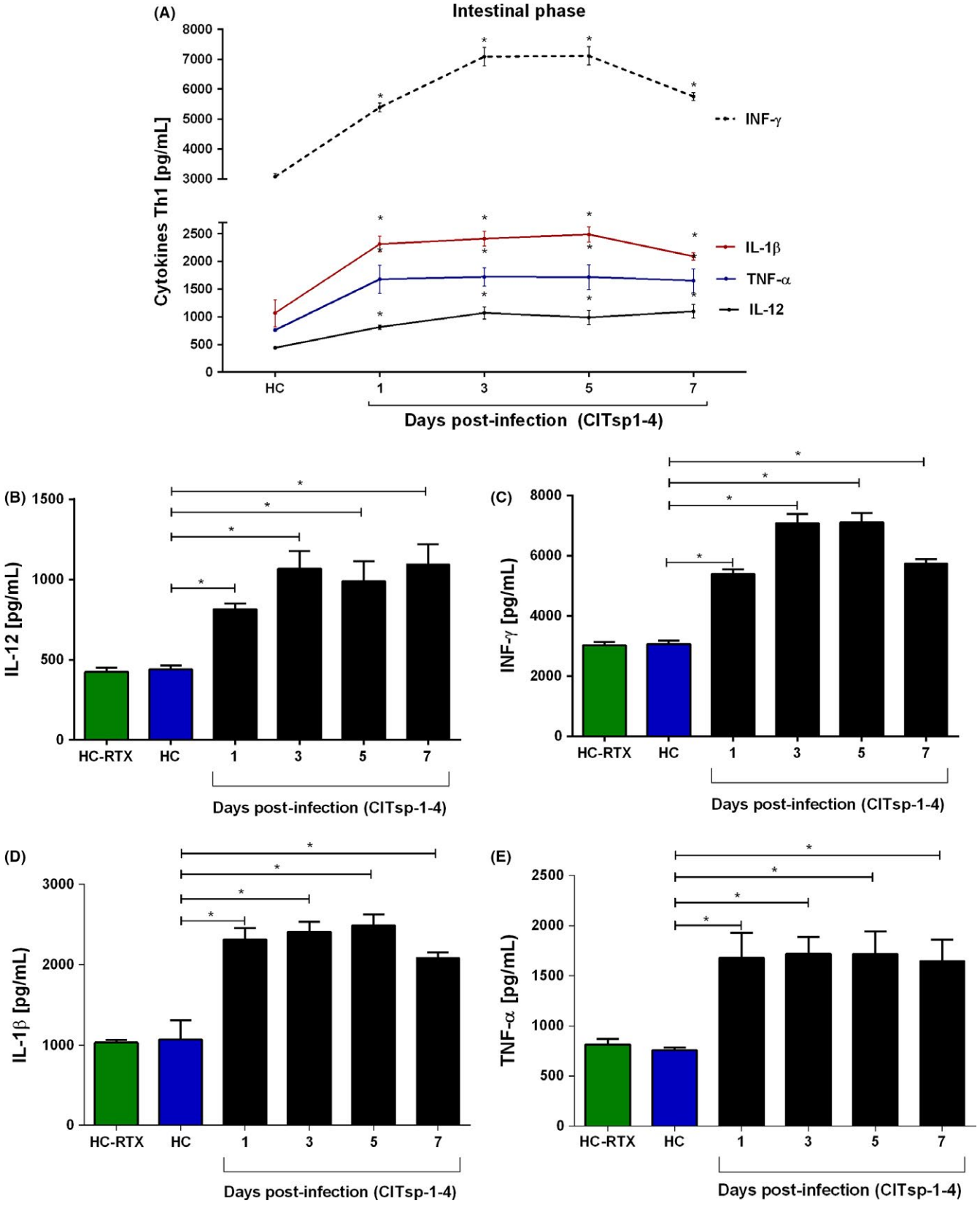

FIGURE 1 Production of Th1 cytokines during the intestinal phase of *Trichinella spiralis* infection. (A) The course of the release of IL-12 (black line), INF-γ (dotted line), IL-1β (red line) and TNF-α (blue line) during the intestinal phase of *T. spiralis* infection. The levels of IL-12 (B), INF-γ (C), IL-1β (D) and TNF-α (E) in serum of rats on days 1, 3, 5 and 7 of *T. spiralis* infection were determined quantitatively by ELISA. Values are presented as group means±SD, indicating the level of significance (*P<.05)

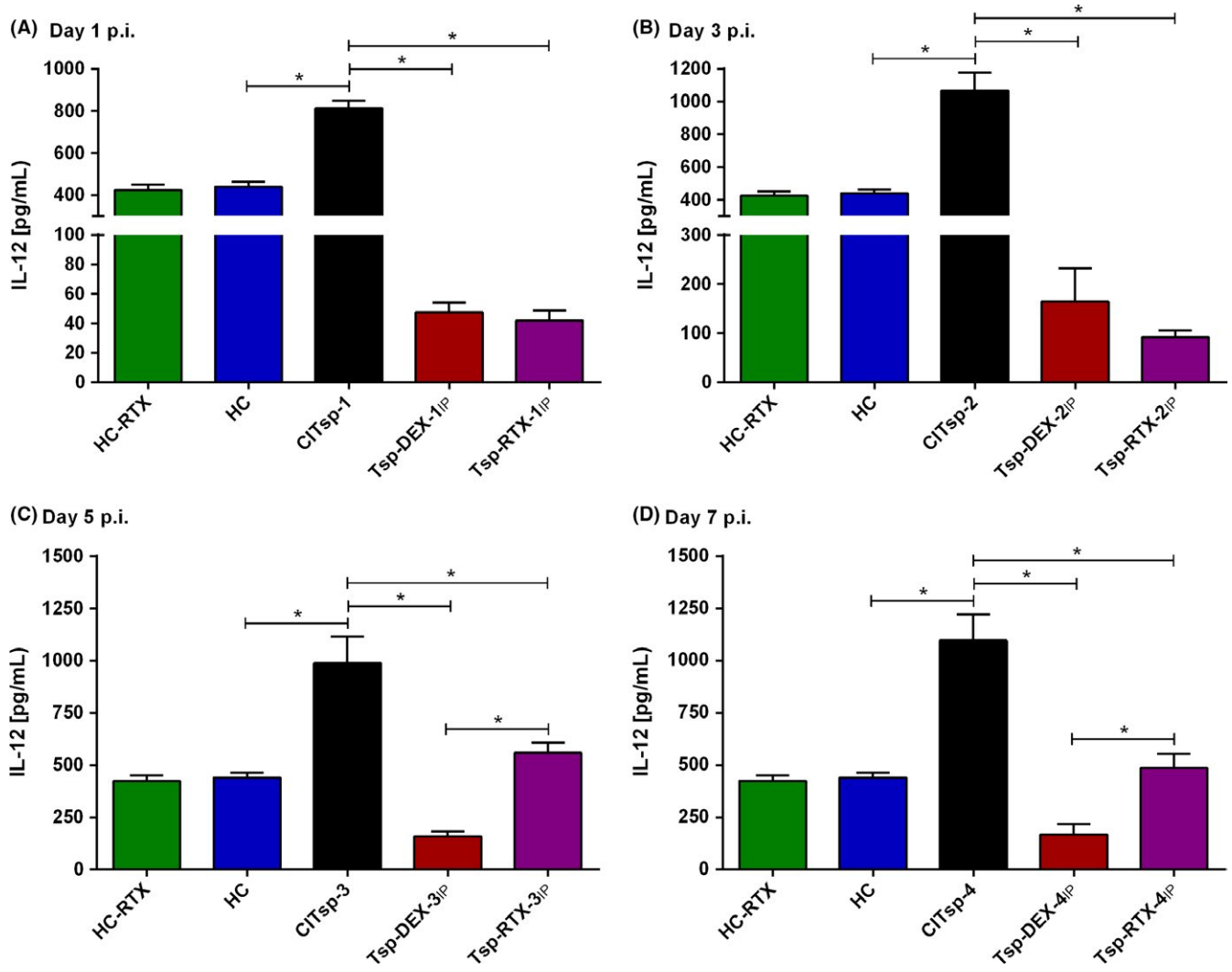

FIGURE 2 Serum levels of IL-12 in rats treated with DEX and RTX during the intestinal phase of *Trichinella spiralis* infection. Treatment with (A) one dose of DEX and RTX on day 1 p.i.; (B) two doses of DEX and RTX on days 1 and 3 p.i.; (C) three doses of DEX and RTX on days 1, 3 and 5 p.i.; and (D) three doses on days 3, 5 and 7 p.i. Serum levels of IL-12 from HC-RTX group (green bars), HC group (blue bars), CITsp-1, -2, -3 and -4 groups (black bars), Tsp-DEX-1_{IP}, -2_{IP}, -3_{IP} and -4_{IP} groups (red bars) and Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and -4_{IP} groups (purple bars) were determined quantitatively by ELISA. Values are presented as group means±SD, indicating the level of significance (*P<.05)

significantly (*P<.05) on day 1 p.i. (5±1%Eos, group CITsp-1), day 3 p.i. (7±1%Eos, group CITsp-2), day 5 p.i. (8±1.4%Eos, group CITsp-3) and day 7 p.i. (12±1.6%Eos, group CITsp-4), whereas the percentage of eosinophils in the HC-RTX group (0.8±0.2%Eos) decreased significantly (*P<.05), compared with the HC group (2±0.2%Eos). When RTX was administered to Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP}, and -4_{IP} groups, the percentage of eosinophils in the blood was significantly decreased (*P<.05) on day 1 p.i. (2±0.7%Eos), day 3 p.i. (0.9±0.3%Eos), day 5 p.i. (1±0.6%Eos) and day 7 p.i. (0.9±0.5%Eos), compared with the CITsp-1, -2, -3 and -4 groups. When DEX was administered, it was observed that the percentage of eosinophils in the blood was similar to treatment with RTX on days 1 p.i. (1.5±0.9%Eos) and 5 p.i. (2±1%Eos), whereas on days 3 p.i. (2.2±1.2%Eos) and 7 p.i. (3.7±0.5%Eos), it was observed that treatment with RTX decreased significantly (*P<.05) the percentage of eosinophils compared to treatment with DEX (Figure 6C-F).

3.4 | Effect of resiniferatoxin treatment on muscle phase of *Trichinella spiralis* infection

We evaluated the effect of RTX treatment on the muscle phase of *T. spiralis* infection. First, the effect of RTX treatment on the implantation of *T. spiralis*-L1 was evaluated. Figure 7A shows that the treatment with a dose of RTX (on day 1 p.i.) significantly decreased (*P<.05) the number of *T. spiralis*-L1 on day 28 p.i. in the diaphragm (67±12 L1), tongue (44±13 L1), masseter (31±7 L1) and leg (26±9), compared with the CITsp-5 group (157±17 L1, 96±21 L1, 116±12 L1 and 68±8 L1, respectively). In contrast, treatment with a dose of DEX (on day 1 p.i.) significantly increased (*P<.05) the number of *T. spiralis*-L1 on day 28 p.i. in leg (96±10 L1), whereas the number of *T. spiralis*-L1 in diaphragm (184±29 L1), tongue (101±12 L1) and masseter (89±3 L1, *P<.05) was similar to the CITsp-5 group. Treatment with two doses of RTX (on days 1 and 3 p.i.) showed the same effect, as treatment

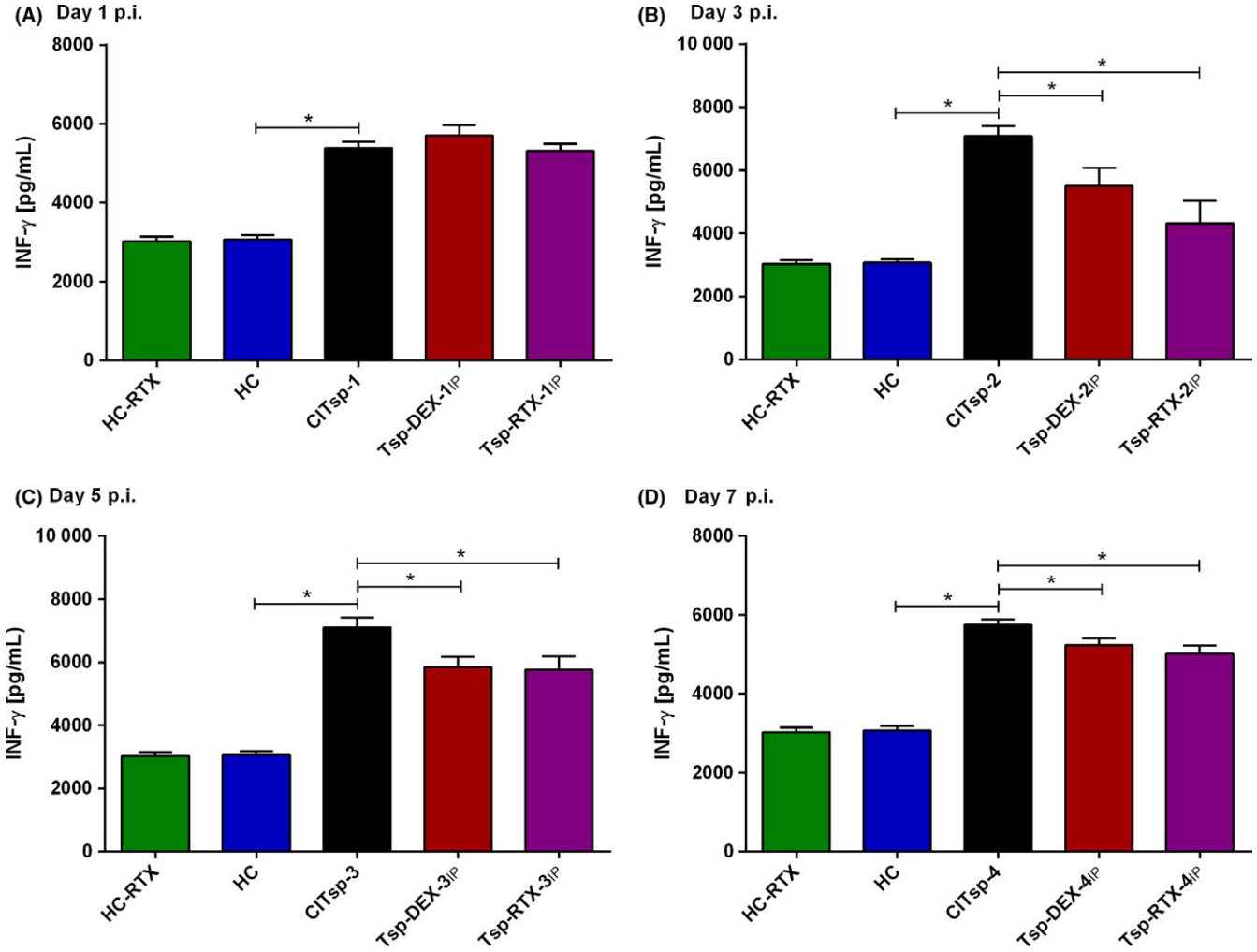

FIGURE 3 Serum levels of INF-γ in rats treated with DEX and RTX during the intestinal phase of *Trichinella spiralis* infection. Treatment with (A) one dose of DEX and RTX on day 1 p.i.; (B) two doses of DEX and RTX on days 1 and 3 p.i.; (C) three doses of DEX and RTX on days 1, 3 and 5 p.i.; and (D) three doses on days 3, 5 and 7 p.i. Serum levels of INF-γ from the HC-RTX group (green bars), HC group (blue bars), CITsp-1, -2, -3 and -4 groups (black bars), Tsp-DEX-1_{IP}, -2_{IP}, -3_{IP} and -4_{IP} groups (red bars) and Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and -4_{IP} groups (purple bars) were determined quantitatively by ELISA. Values are presented as group means±SD, indicating the level of significance (*P<.05)

with RTX significantly decreased (*P<.05) the number of *T. spiralis*-L1 implanted on day 28 p.i. in diaphragm (65±13 L1), tongue (47±13 L1), masseter (36±10 L1) and leg (20±8 L1). Whereas the treatment with two doses of DEX (on days 1 and 3 p.i.) significantly increased (*P<.05) the number of *T. spiralis*-L1 on day 28 p.i. in diaphragm (224±21 L1) and leg (112±11 L1), compared with the CITsp-5 group (Figure 7B). When three doses of RTX were given (on days 1, 3 and 5 p.i.), it was observed a significant decrease (*P<.05) in the number of *T. spiralis*-L1 on day 28 p.i. in diaphragm (49±16 L1), tongue (41±17 L1), masseter (23±6 L1) and leg (15±3 L1). Whereas the treatment with three doses of DEX (on days 1, 3 and 5 p.i.) increased significantly (*P<.05) on day 28 p.i., the number of *T. spiralis*-L1 in diaphragm (225±34 L1), tongue (132±9 L1) and leg (116±14 L1), compared with the CITsp-5 group (Figure 7C). Finally, Figure 7D shows as treatment with three doses of RTX (on days 3, 5 and 7 p.i.) significantly decreased (*P<.05) the number of *T. spiralis*-L1 on day 28 p.i. in diaphragm (53±12 L1), tongue (25±11 L1), masseter (34±15 L1) and leg (19±8 L1). Whereas in the treatment with three doses of DEX (on days 3, 5 and 7 p.i.), no significant difference was observed compared with the CITsp-5 group.

On the other hand, the effect of RTX treatment on the parasite burden in the muscle phase of *T. spiralis* infection was determined. Figure 8 shows how the Tsp-RTX-1_{MP}, -2_{MP}, -3_{MP} and -4_{MP} groups treated with RTX significantly decreased (*P<.05) the parasite burden on day 28 p.i. (8375±1737 L1, 8438±2009 L1, 8250±2554 L1 and 7125±1352 L1, respectively), compared to the CITsp-5 group (14 500±2872 L1). The Tsp-DEX-1_{MP} and -2_{MP} groups, treated with DEX, increased the parasite burden (16 625±3807 L1 and 19 650±3686 L1, respectively); however, this increase was not significant, whereas the Tsp-DEX-3_{MP} and -4_{MP} groups treated with DEX significantly increased (*P<.05) the parasite burden on day 28 p.i. (22 125±3243 L1 and 25 000±5728 L1, respectively), compared to the CITsp-5 group (Figure 8B-E).

Subsequently, the viability of *T. spiralis*-L1 treated with RTX and DEX was determined qualitatively through trypan blue stain method. Figure 8A shows that in all groups treated with DEX, all *T. spiralis*-L1

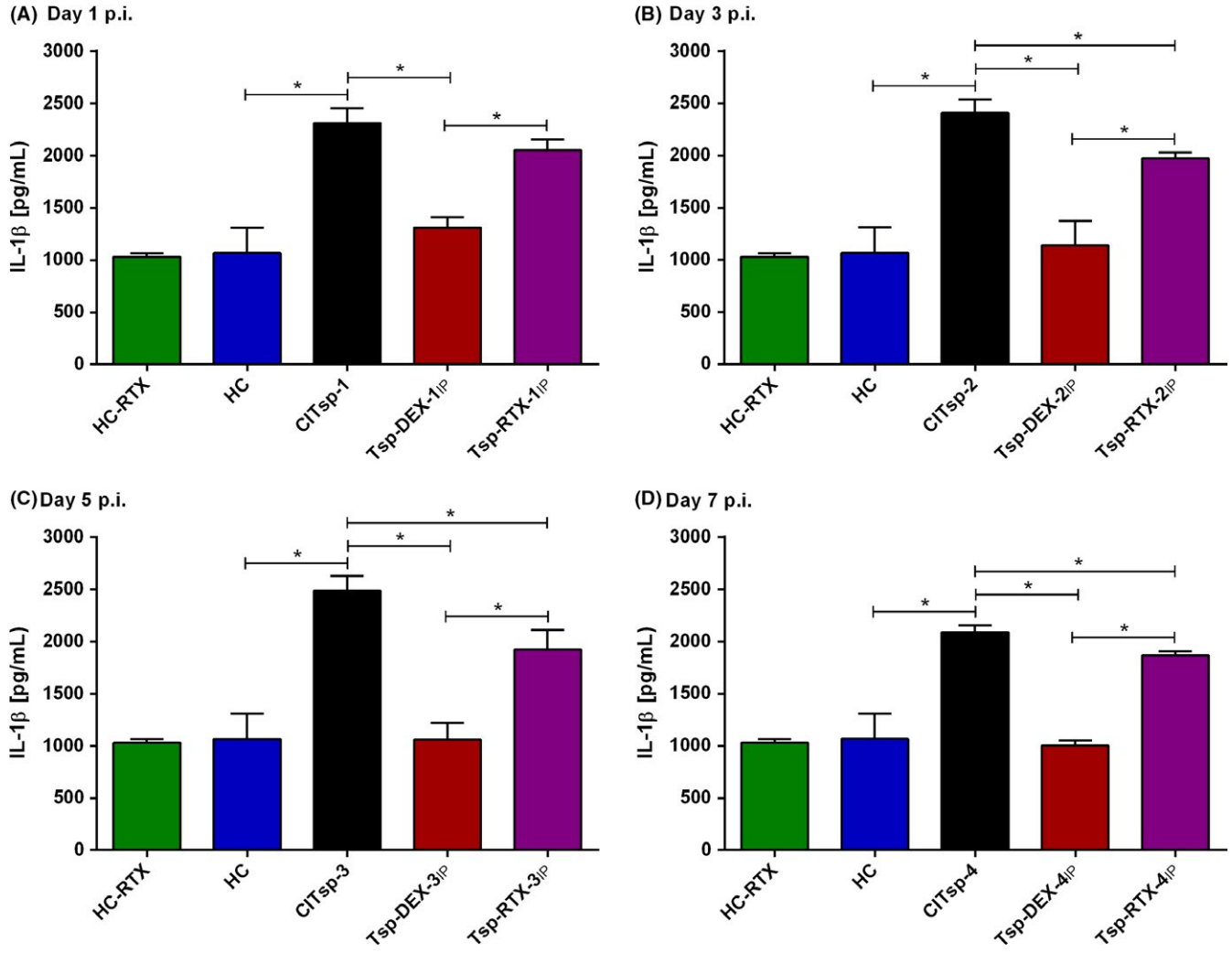

FIGURE 4 Serum levels of IL-1β in rats treated with DEX and RTX during the intestinal phase of *Trichinella spiralis* infection. Treatment with (A) one dose of DEX and RTX on day 1 p.i.; (B) two doses of DEX and RTX on days 1 and 3 p.i.; (C) three doses of DEX and RTX on days 1, 3 and 5 p.i.; and (D) three doses on days 3, 5 and 7 p.i. Serum levels of IL-1β from the HC-RTX group (green bars), HC group (blue bars), CITsp-1, -2, -3 and -4 groups (black bars), Tsp-DEX-1_{IP}, -2_{IP}, -3_{IP} and -4_{IP} groups (red bars) and Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and -4_{IP} groups (purple bars) were determined quantitatively by ELISA. Values are presented as group means±SD, indicating the level of significance (*P<.05)

were viable, because the trypan blue dye did not penetrate into the *T. spiralis*-L1, similar to the CITsp-5 group. Whereas in the groups treated with RTX, nonviable *T. spiralis*-L1 were observed, because the trypan blue dye penetrated into them.

3.5 | Effect of resiniferatoxin treatment on the infectivity of *Trichinella spiralis*-L1 in BALB/c mice

Finally, the effect of RTX treatment on the infectivity of *T. spiralis*-L1 from the CITsp-5 and DEX and RTX groups was determined. As shown in Figure 9, when the rTsp-RTX-1, -2, -3 and -4 groups of BALB/c mice were infected with 150 *T. spiralis*-L1 from the Tsp-RTX-1_{MP}, 2_{MP}, 3_{MP} and 4_{MP} groups of rats, respectively, it was observed that on day 28 p.i. the parasite burden from the rTsp-RTX-1 (8076±1990 L1), rTsp-RTX-2 (7758±1632 L1), rTsp-RTX-3 (9423±759 L1) and rTsp-RTX-4 (8428±517 L1) decreased significantly (*P<.05) compared to the rCITsp group (14 037±1807 L1). Whereas in the rTsp-DEX-1 (10 323±2872 L1), rTsp-DEX-2 (11 056±3572 L1), rTsp-DEX-3 (10 820±2432 L1) and rTsp-DEX-4 (10 622±1642 L1) groups of BALB/c mice infected with 150 *T. spiralis*-L1 from the Tsp-DEX-1_{MP}, -2_{MP}, -3_{MP} and -4_{MP} groups of rats, it was observed that there was no significant difference compared to the rCITsp group.

4 | DISCUSSION

In the intestinal phase of *T. spiralis* infection, the polarization of T cells to a Th1 immune response depends on the type of signal derived from dendritic cells (DCs). DCs represent an important link between innate and adaptive immunity, which play an important role during the immune response against parasites.[42,43] The *T. spiralis*-L1 antigens induce the maturation of DCs, which leads to the expression of the major histocompatibility complex class II (MHC II),[11,44] promoting the development to a Th1 immune response.[10] Several studies, both in

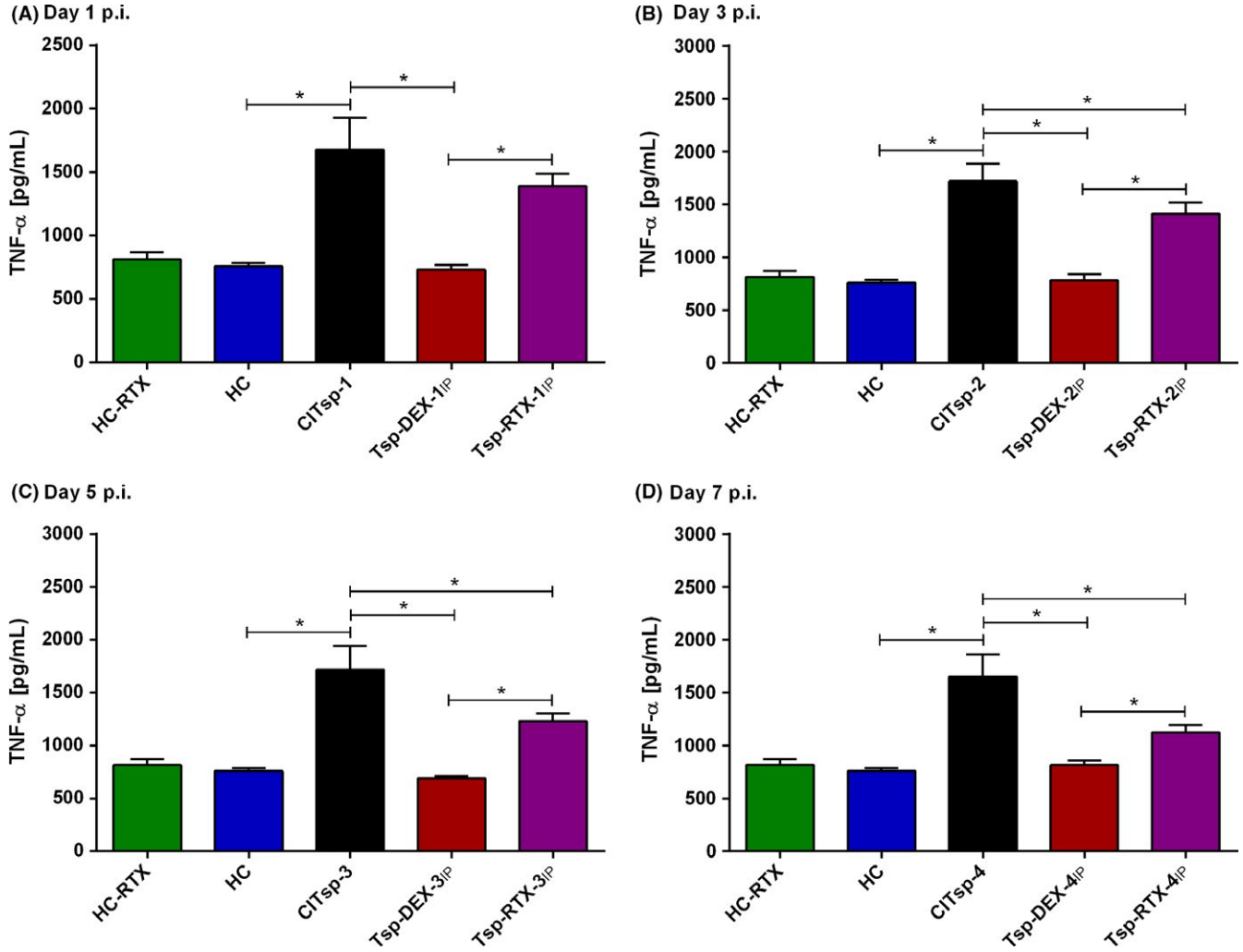

FIGURE 5 Serum levels of TNF-α in rats treated with DEX and RTX during the intestinal phase of *Trichinella spiralis* infection. Treatment with (A) one dose of DEX and RTX on day 1 p.i.; (B) two doses of DEX and RTX on days 1 and 3 p.i.; (C) three doses of DEX and RTX on days 1, 3 and 5 p.i.; and (D) three doses on days 3, 5 and 7 p.i. Serum levels of TNF-α from the HC-RTX group (green bars), HC group (blue bars), CITsp-1, -2, -3 and -4 groups (black bars), Tsp-DEX-1_{IP}, -2_{IP}, -3_{IP} and -4_{IP} groups (red bars) and Tsp-RTX-1_{IP}, -2_{IP}, -3_{IP} and -4_{IP} groups (purple bars) were determined quantitatively by ELISA. Values are presented as group means±SD, indicating the level of significance (*P<.05)

vitro and in vivo, have shown that during the early stage of intestinal infection by *T. spiralis* there is a significant increase in Th1 cytokines such as IL-12,[45,46] INF-γ,[10,45-47] IL- 1β[48] and TNF-α.[45,46] Due to the important role of these cytokines (Th1) in the host immune response against *T. spiralis* infection, in this study, the Th1 cytokine profile was first evaluated during the intestinal phase of *T. spiralis* infection, and we observed that serum levels of IL-12, INF-γ, IL-1β and TNF-α increased significantly.

Several studies have demonstrated the importance of the Th1 immune response during *T. spiralis* infection. However, these investigations suggest that this Th1 immune response favours *T. spiralis* infection. On one hand, IL-12 is a cytokine, which, in both mice and humans, is composed of two linked subunits, IL-12p40 and IL-12p35, which acts through its IL-12 receptor (IL-12R). IL-12 promotes the differentiation of *naïve* T cells to INF-γ-producing Th1 cells.[49] INF-γ (interferon type II) is a cytokine that forms an important part of both innate and adaptive immunity, which is produced by various immune cells such as $CD4^+$ T cells in response to some immune or inflammatory stimuli,[50,51] such as pathogens, specific antigens, or by activation of the T-cell receptor. INF-γ, as an immunomodulatory molecule, acts pleiotropically, which has the capacity to increase the cytotoxic and phagocytic activity of macrophages, and it also induces the expression of MHC class I and II molecules in DCs and other antigen-presenting cells (APC).[52] INF-γ increases the development and differentiation of Th1 cells, induces the expression of *inducible nitric oxide synthase* (iNOS), activates transcription factors such as NF-κB[53] and regulates the production of pro-inflammatory cytokines, such as TNF-α.[54] In the immune response against *T. spiralis* infection, IL-12, together with INF-γ, is of vital importance as they participate in the polarization of the immune response type Th1[10,45-47] (Figure 10A). However, the exogenous administration of IL-12 in *T. spiralis* infection suppresses intestinal mastocytosis, delaying the parasite expulsion and increasing the parasite burden.[55]

On one hand, TNF-α is a potent pro-inflammatory cytokine, which belongs to a superfamily of ligand/receptor proteins called the tumour

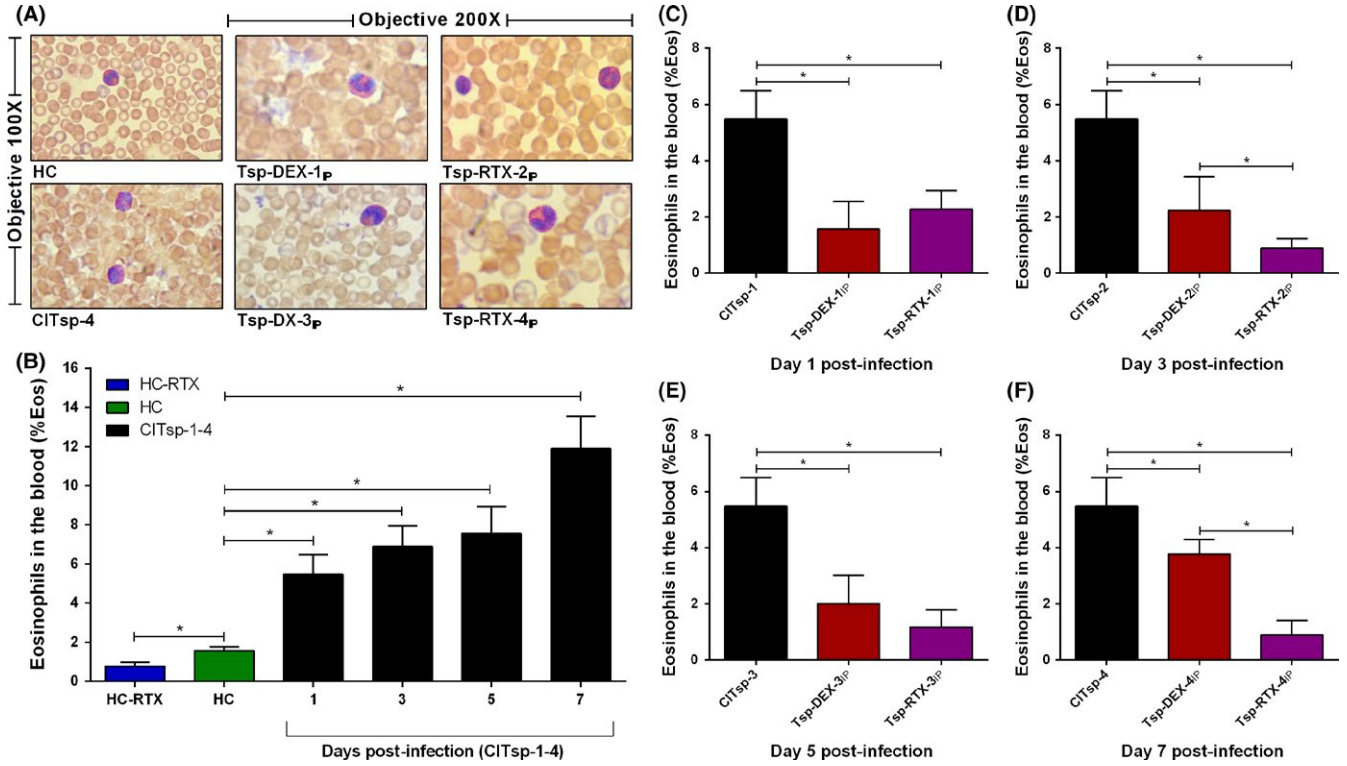

FIGURE 6 Effect of resiniferatoxin treatment on the percentage of eosinophils in the blood during the intestinal phase of *Trichinella spiralis* infection. (A) Photomicrographs of eosinophils in the blood from the HC (100×), CTIsp-4 (day 7 p.i., 100×), Tsp-DEX-1IP (day 1 p.i., 200×), Tsp-DEX-3IP (day 5 p.i., 200×), Tsp-RTX-2IP (day 3 p.i., 200×) and Tsp-RTX-4IP (day 7 p.i., 200×). (B) Percentage of eosinophils in the blood during the intestinal phase of *T. spiralis* infection. HC group (green bar), CITsp-1, -2, -3 and -4 groups (black bars) and HC-RTX group (blue bar). (C) Percentage of eosinophils in the blood on day 1 p.i. in rats treated with DEX (red bars) and RTX (purple bars) with a dose on day 1 p.i. (D) Percentage of eosinophils in the blood on day 3 p.i. in rats treated with DEX (red bars) and RTX (purple bars) with two doses on days 1 and 3 p.i. (E) Percentage of eosinophils in the blood on day 5 p.i. in rats treated with DEX (red bars) and RTX (purple bars) with three doses on days 1, 3 and 5 p.i. (F) Percentage of eosinophils in the blood on day 7 p.i. in rats treated with DEX (red bars) and RTX (purple bars) with three doses on days 3, 5 and 7 p.i. The percentage of eosinophils (%Eos) in the blood was determined by Wright's stain method. Values are presented as group means±SD, indicating the level of significance (**P*<.05).

necrosis factor/tumour necrosis factor receptor superfamily proteins (TNF/TNFR SFP).[56] TNF-α is a cytokine produced by different types of immune system cells, such as DCs, macrophages, Th1 cells, mast cells, which exerts its biological effects in a pleiotropic manner, thus having a key role in the pathogenesis of inflammatory diseases, as it participates in the activation of a cascade of pro-inflammatory cytokines, especially IL-1β, IL-6 and IL-8.[57,58] IL-1β is a pro-inflammatory and pyrogenic cytokine of excellence that regulates systemic and local responses by generating fever, activates lymphocytes and promotes the recruitment and activation of myeloid cells at the site of infection. IL-1β has been shown to participate in host defence by inducing the adaptive immune response skewed to Th17 and Th1 lymphocytes.[59,60] In Trichinellosis, the role of IL-1β is not well understood; however, it is known to participate in the intestinal inflammatory response, as *T. spiralis*-L1 are capable to increase levels of IL-1β derived from intestinal epithelial cells. With regard to TNF-α, studies have shown that TNF-α is a cytokine that is produced during the intestinal phase of *T. spiralis* infection.[45,46] However, several studies have associated TNF-α production with the development of intestinal pathology during Trichinellosis. One study showed that TNF receptor 1 (TNRF1)-deficient mice are still capable of expelling *T. spiralis*, although a reduction in intestinal pathology was observed.[18] Another study showed that TNF-α derived from mastocytes is required for mastocytosis as well as for the generation of the Th2 immune response, which are both needed for the expulsion of *T. spiralis*.[61] In addition, the soluble form of TNF-α plays a critical role in the protection against the parasite through the Th2 immune response, as the absence of soluble TNF-α in transgenic mice delayed the expulsion of *T. spiralis* significantly, along with a reduction in the intestinal pathology and mastocytosis[62] (Figure 10A). In a recent study published by our research group, we observed that increased serum levels of TNF-α were associated with the development of intestinal pathology in the intestinal phase of *T. spiralis* infection[36] (Figure 10B). These investigations suggest that the host Th1 type immune response favours *T. spiralis* infection. In this study, it was observed that the four treatments with RTX and DEX (one dose on day 1 p.i., two doses on days 1 and 3 p.i., three doses on days 1, 3 and 5 p.i. and three doses on days 3, 5 and 7 p.i.) significantly decreased serum levels of IL-12, INF-γ, IL-1β and TNF-α in the intestinal phase of *T. spiralis* infection. On the one hand, studies have shown that steroidal anti-inflammatory drugs (such as DEX) suppress the expression of

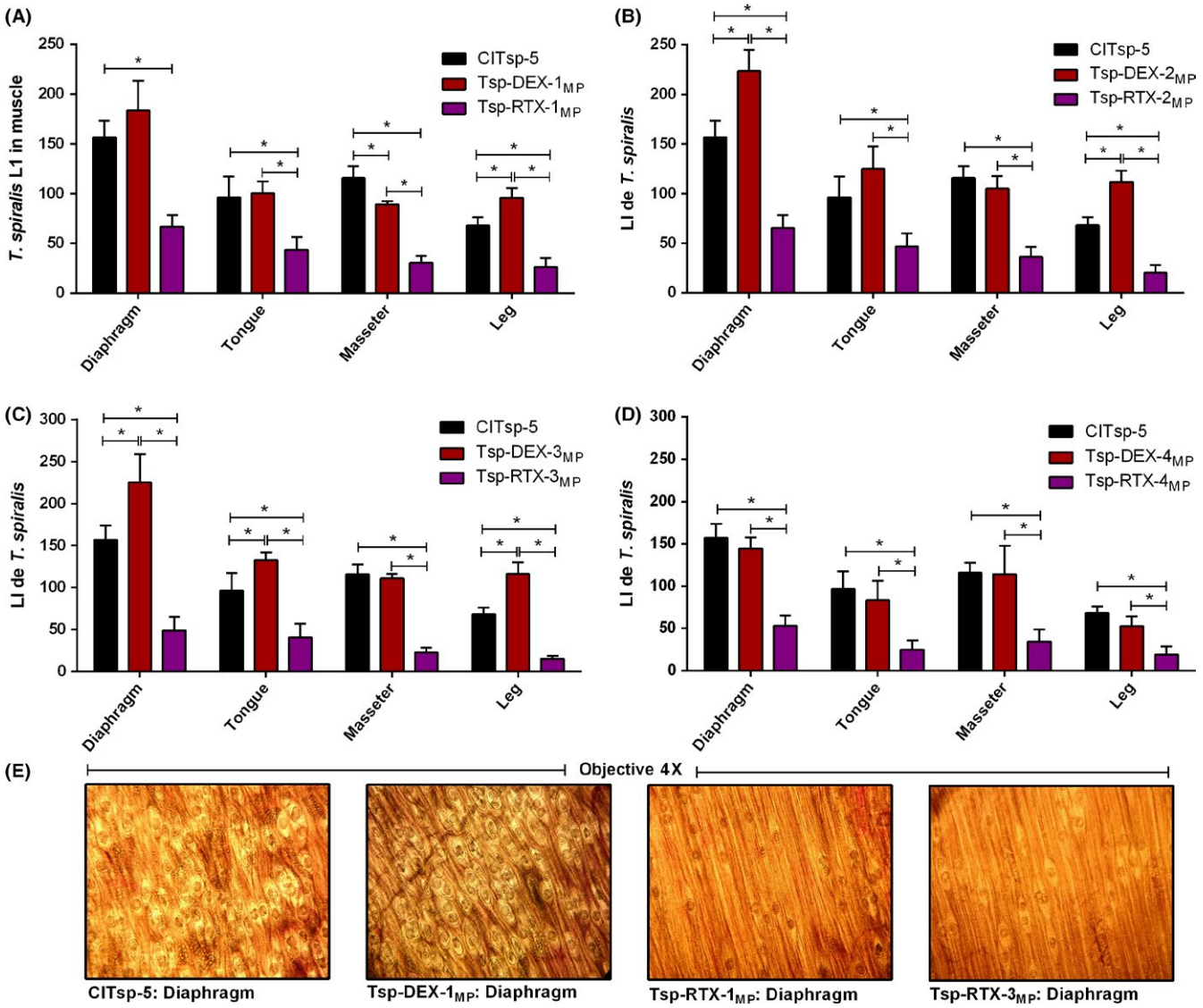

FIGURE 7 Effect of treatment with resiniferatoxin on the implantation of *Trichinella spiralis*-L1 in muscle tissue. The number of *T. spiralis*-L1 in diaphragm, tongue, masseter and leg is shown on day 28 p.i. of CITsp-5 group (black bars), Tsp-DEX-1MP, -2MP, -3MP and -4MP groups (red bars) and Tsp-RTX-1MP, -2MP, -3MP and -4MP groups (purple bars) treated with (A) one dose of DEX and RTX on day 1 p.i.; (B) two doses of DEX and RTX on days 1 and 3 p.i.; (C) three doses of DEX and RTX on days 1, 3 and 5 p.i.; and (D) with three doses on days 3, 5 and 7 p.i. (E) Photomicrographs of *T. spiralis*-L1 implanted in diaphragm on day 28 p.i. of the CITsp-5, Tsp-DEX-1_{MP}, Tsp-RTX-1_{MP} and Tsp-RTX-3_{MP} groups. Muscle tissue samples were observed under the optical light microscope with the 4× objective. Values are presented as group means±SD, indicating the level of significance (*P<.05)

pro-inflammatory genes by inhibition of transcription factors such as NF-κB,[22] AP-1[23] and *Signal transducer and activator of transcription* 4 (Stat4) preventing the transcription of inflammatory cytokines such as IL-1β, TNF-α,[25] IL-12[63] and INF-γ.[64] With respect to RTX, it is known that it performs most of its biological functions through the TRPV1 receptor,[28,29] activating and then desensitizing the TRPV1 receptor producing analgesia.[30,31] However, in vitro studies have shown that RTX has a significant anti-inflammatory effect, as it inhibits the expression of NF-κB in ML-1a cells stimulated with TNF-α in a dose-dependent manner.[32] Similarly, it was also demonstrated that RTX inhibits the expression of iNOS and cyclooxygenase-2 (COX-2) in RAW264.7 macrophages stimulated with lipopolysaccharide (LPS) and IFN-γ, thus resulting in a decrease in PGE_2 and nitric oxide (NO).[33] The same anti-inflammatory effect of RTX has been observed *in vivo* models. A study based on a model of acute renal failure (ARF) showed that RTX treatment prevented renal damage by inhibiting the inflammatory response while inducing a decrease in the expression of renal TNF-α and an increase of IL-10 in plasma.[34] Another study in LPS-stimulated BALB/c mice showed that RTX significantly decreased serum levels of PGE_2, NO and TNF-α.[35] Finally, recent research by our research group found that treatment with RTX in the intestinal phase of *T. spiralis* infection significantly decreased levels of PGE_2, NO and TNF-α in rat serum.[36] Our results agree with these studies, as RTX decreased serum levels of Th1 cytokines such as IL-12, INF-γ, IL-1β and TNF-α, as our data

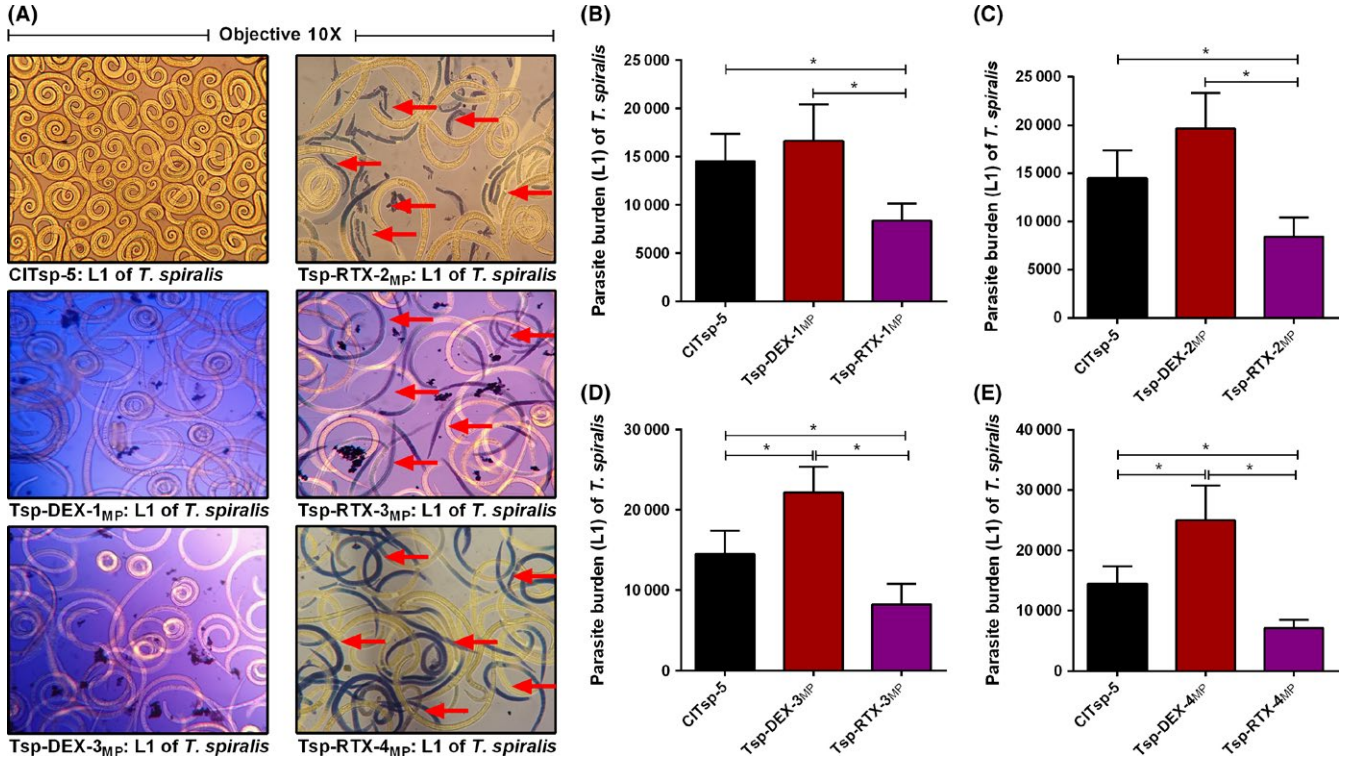

FIGURE 8 Effect of treatment with resiniferatoxin on the parasite burden of *Trichinella spiralis*. The parasite burden of *T. spiralis* is shown per 30 g of infected meat from the CITsp-5 (black bars), treated with RTX (purple bars) and DEX (red bars) groups. (A) The viability of *T. spiralis*-L1 from the groups treated with DEX and RTX, where the red arrows indicate nonviable *T. spiralis*-L1. The viability of *T. spiralis*-L1 was determined by the trypan blue stain method and was observed under optical light microscopy with the 10× objective. (B) Parasite burden on day 28 p.i. of the groups treated with a dose (on day 1 p.i.) of RTX and DEX. (C) Parasite board on day 28 p.i. of the groups treated with two doses (on days 1 and 3 p.i.) of RTX and DEX. (D) Parasite burden on day 28 p.i. of the groups treated with three doses (on days 1, 3 and 5 p.i.) of RTX and DEX. (E) Parasite burden on day 28 p.i. of the groups treated with three doses (on days 3, 5 and 7 p.i.) of RTX and DEX. Values are represented as the mean±SE is by group, indicating the level of significance (*P<.05)

indicate a similar pharmacological effect of RTX during *T. spiralis* infection (Figure 10B). It is important to note that both treatments with DEX and RTX on days 1, 3, 5 and 7 p.i. significantly decreased INF-γ serum levels, showing a fine down-regulation and not a suppression of INF-γ synthesis. Regarding IL-12, both treatments with DEX and RTX on days 1 and 3 p.i. showed suppression of production in IL-12 synthesis. However, on days 5 and 7 p.i., DEX treatment continued to suppress IL-12 synthesis, whereas RTX treatment maintained IL-12 levels similar to HC group levels. Similarly, it was observed that on days 1, 3, 5 and 7 p.i., treatment with DEX suppressed both IL-1β and TNF-α synthesis, whereas treatment with RTX maintained both IL-1β and TNF-α serum levels similar to HC group, showing an immunomodulatory effect by RTX on the Th1 immune response.

During the inflammatory response associated with helminth infection, inflammatory cells such as eosinophils are prominent.[65] In this study, we evaluated the number of eosinophils in the blood during the intestinal phase of *T. spiralis* infection. T cells are stimulated by *T. spiralis*-L1 antigens, which leads to the release of cytokines such as IL-4 and IL-5, which induce terminal differentiation and proliferation of eosinophils,[66] thus promoting the inflammatory response. In this study, a significant increase in the number of eosinophils in the blood during the intestinal phase of *T. spiralis* infection was observed. When the four treatments were administered with DEX in the intestinal phase, it was observed that the number of eosinophils in the blood decreased significantly. It is known that DEX induces apoptosis of eosinophils,[67] inhibiting their survival[25] in a dose-dependent manner,[68] which coincides with our results on the pharmacological effect observed in the treatment with DEX. On the other hand, it was observed that RTX treatment on days 1 and 5 p.i. exhibited a similar effect as treatment with DEX, whereas on day 3 and 7 p.i., it was observed that treatment with RTX decreased the number of eosinophils more than the treatment with DEX, but generally both treatments showed similar effects, as the number of eosinophils in the blood decreased significantly in the intestinal phase of *T. spiralis* infection. Previous studies have associated eosinophil survival with TNF-α production, as TNF-α-derived mast cell induces eosinophil survival by autocrine production of *Granulocyte-Macrophage Colony-Stimulating Factor* (GM-CSF),[69] and also, it is involved in adhesion of endothelial cells and induces activation, degranulation and cytokines production of eosinophils.[70] In addition, in our previous study, it was observed that treatment with RTX in the intestinal phase of *T. spiralis* infection decreases both serum levels of TNF-α and the number of eosinophils in blood.[36] In this study, it was also observed that treatment with RTX simultaneously decreased levels of TNF-α and the number of eosinophils in the blood, which,

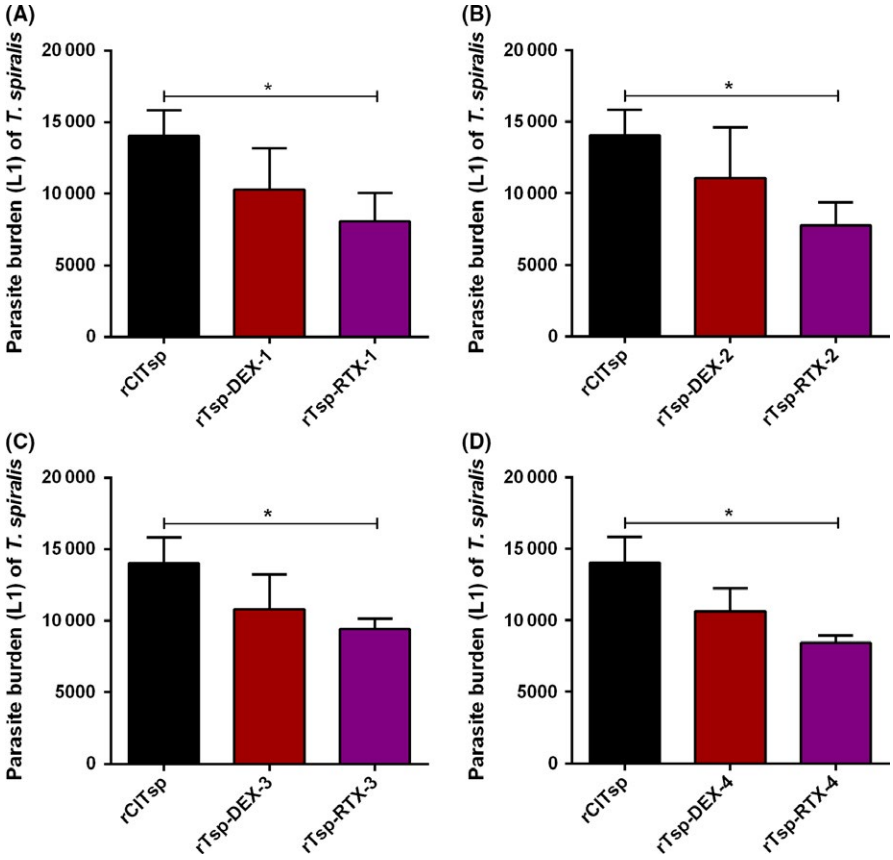

FIGURE 9 Effect of resiniferatoxin treatment on the infectivity of *Trichinella spiralis*-L1 in BALB/c mice. The parasite burden of *T. spiralis* in BALB/c mice from the rCITsp group (black bars), rTsp-RTX-1, -2, -3 and -4 groups (purple bars) and rTsp-DEX-1, -2, -3 and -4 groups (red bars). (A) Parasite burden on day 28 p.i. of the groups infected with *T. spiralis*-L1 treated with a dose (on day 1 p.i.) of RTX and DEX. (B) Parasite burden on day 28 p.i. of the groups infected with *T. spiralis*-L1 treated with two doses (on days 1 and 3 p.i.) of RTX and DEX. (C) Parasite burden on day 28 p.i. of the groups infected with *T. spiralis*-L1 treated with three doses (on days 1, 3 and 5 p.i.) of RTX and DEX. (D) Parasite burden on day 28 p.i. of the groups infected with *T. spiralis*-L1 treated with three doses (on days 3, 5 and 7 p.i.) of RTX and DEX. Values are represented as the mean±SE is by group, indicating the level of significance (*P<.05)

together with literature results, allowed us to hypothesize that the decrease in eosinophils could be associated with the effect of RTX on TNF-α synthesis, although further studies are needed to confirm this hypothesis (Figure 10B).

Currently, the GCs are used as pharmacological treatment for the inflammatory response during Trichinellosis. However, its therapeutic use is limited because previous studies have shown that GCs favour *T. spiralis* infection. One study showed that in rats treated with betamethasone were more susceptible to infection as shown by the increased parasite burden when compared to the infected control group.[71] Another study showed similar results, where it was observed that the treatment with DEX increased the proportion of apoptotic and necrotic lymphocytes, as well as the number of larvae in muscular tissue in mice treated with DEX.[27] In our previous study, it was observed that treatment with DEX in the intestinal phase of *T. spiralis* infection increased significantly the implantation of *T. spiralis*-L1 in muscle tissue, as well as parasite burden.[36] Our results agree with these investigations, as in this study it was observed that the treatment with DEX during the intestinal phase increased significantly in a dose-dependent manner both the implantation of *T. spiralis*-L1 and parasite burden of *T. spiralis*. This is due to the systemic suppression of the immune response by treatment with DEX.

With regard to RTX, our previous study showed that treatment with RTX in the intestinal phase of *T. spiralis* infection decreased significantly both *T. spiralis*-L1 implantation in muscle tissue and parasite burden of *T. spiralis*.[36] This study agrees with our results because, similarly, in this study it was observed that treatment with RTX in the intestinal phase decreased significantly both the implantation of *T. spiralis*-L1 in muscle tissue and parasite burden in a dose-dependent manner (Figure 10B).

Several studies have shown that the absence of eosinophils decreases the parasite burden in *T. spiralis* infection[72] and that eosinophils may influence the immune response in a manner that supports chronic infection and ensures survival of the parasite in the host.[73-75] Based on these studies and our results, the hypothesis emerges that the reduction in the parasite burden is associated with the treatment with RTX, as it decreases the number of eosinophils in the blood, as well as the systemic production of Th1 cytokines.

On the other hand, we showed for the first time that the treatment with RTX significantly decreased the infective capacity of *T. spiralis*-L1 in another host, as in this study it was observed that in BALB/c mice infected with *T. spiralis*-L1 previously treated with RTX showed a reduction in the parasite burden compared to the control group (infected with *T. spiralis*-L1 without treatment) and the control group infected with *T. spiralis*-L1 previously treated with DEX.

In conclusion, our current opinion according to our results is that treatment with RTX modulates the production of pro-inflammatory cytokines, while the DEX suppresses it, as serum levels of Th1 cytokines such as IL-12, INF-γ, IL-1β and TNF-α decreased significantly, as well as the number of eosinophils in blood. In addition, treatment with RTX in the intestinal phase exhibits a protective effect against *T. spiralis* infection, as RTX decreased significantly both the implantation of *T. spiralis*-L1 and parasite burden in the muscular phase of the *T. spiralis* infection. Also, the treatment with RTX affects the *T. spiralis* life cycle,

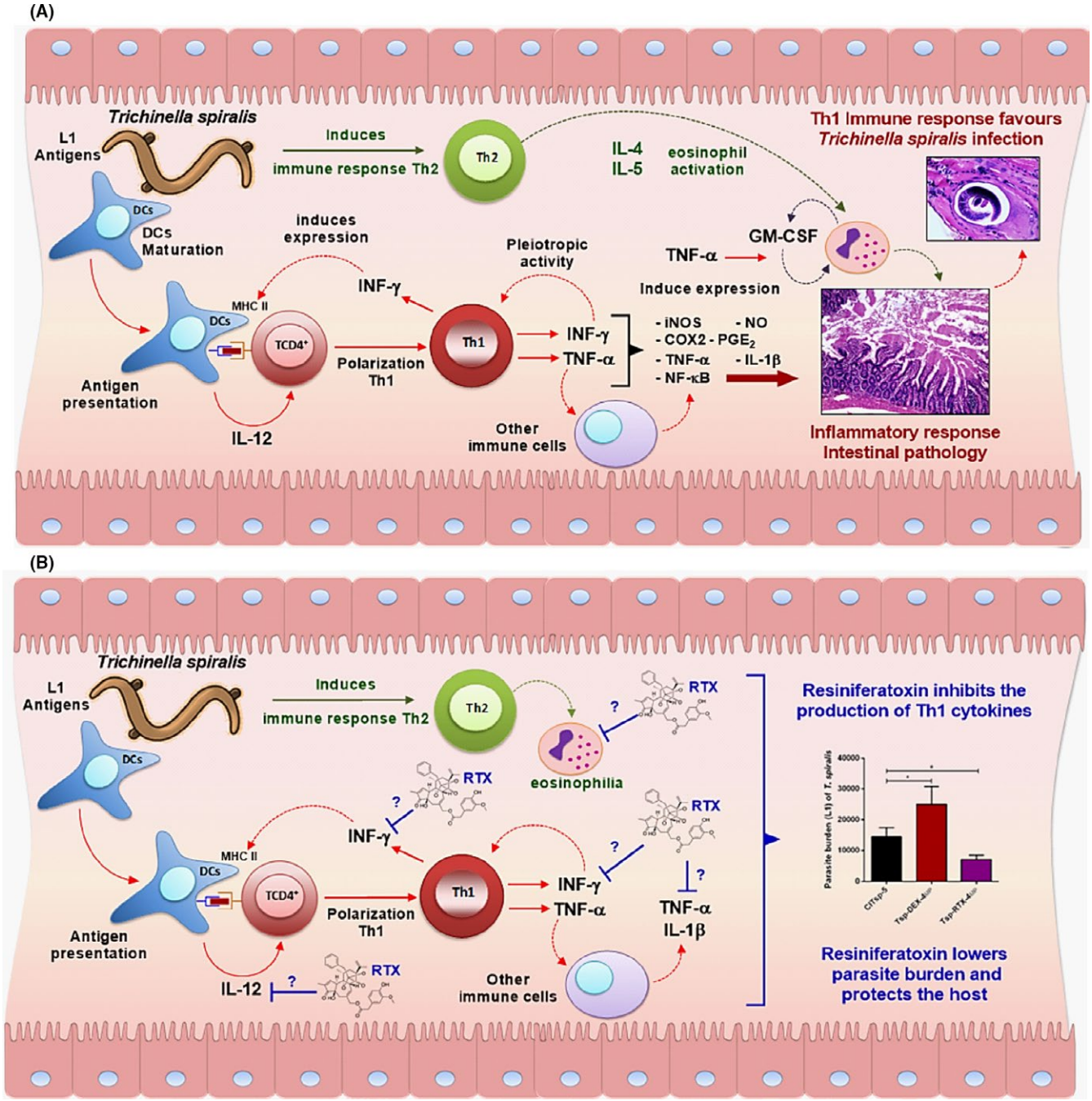

FIGURE 10 (A) Current panorama of the Th1 type immune response during the intestinal phase of *Trichinella spiralis* infection. The *T. spiralis*-L1 antigens induce the maturation of DCs, which together with the production of IL-12 and INF-γ promote and enhance the differentiation of Th1 cells, releasing pro-inflammatory cytokines such as IL-1β And TNF-α, which together with eosinophilia (derived from the Th2 response) potentiate the intestinal inflammatory response, resulting in the development of intestinal pathology, creating a favourable environment for the survival of *T. spiralis*, completing its cycle of life in the host. (B) RTX exhibits a protective effect against *T. spiralis* infection, as it modulates the Th1 type immune response, by decreasing Th1 cytokines (pro-inflammatory) such as IL-12, INF-γ, IL-1β and TNF-α, and the number of eosinophils in the blood, resulting in a decrease in intestinal pathology, in the intestinal phase of *T. spiralis* infection, along with a reduction in *T. spiralis*-L1 implantation and parasite burden in the muscle phase of *T. spiralis* infection. Models proposed by Muñoz-Carrillo JL, et al.

significantly reducing the parasite burden in the muscle phase of *T. spiralis* infection in another host (mouse). This places the RTX as a potential drug in the modulation of the immune system for the treatment of inflammatory diseases.

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