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Trichinella spiralis – induced modulation of IFN-γ and TGF-β shapes host immunity to support chronic infection

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Abstract

Trichinella spiralis is a widely prevalent foodborne helminth that establishes chronic infection through modulation of host immunity. This study investigated the temporal dynamics of interferon-γ (IFN-γ), transforming growth factor-β (TGF-β), and intestinal TGF-β receptor II expression during experimental *T. spiralis* infection and examined their relationship to parasite burden and tissue pathology. The study included 40 Swiss albino mice, orally infected with 250–300 larvae and sacrificed at 3, 7, 14 and 28 days post-infection (dpi). Adult worms and muscle larvae were quantified, serum cytokines were measured by an enzyme-linked immunosorbent assay, and intestinal TGF-β receptor II expression was evaluated immunohistochemically. Histopathological changes in intestinal and muscle tissues were assessed using hematoxylin and eosin staining. Adult worm burden peaked at 7 dpi (300.19 ± 25.8) and declined sharply thereafter, with complete clearance by 28 dpi. Muscle larvae accumulated progressively, reaching approximately 1×10^4 larvae/mouse at 28 dpi. IFN-γ levels rose significantly during the early intestinal phase, peaking at 7 dpi, and then declined toward baseline by 28 dpi. In contrast, TGF-β increased steadily throughout infection, and remained markedly elevated during the muscle phase. A significant negative correlation was observed between IFN-γ and TGF-β ($r = -0.42$, $p=0.012$). TGF-β receptor II expression in the intestine was significantly up-regulated at 3–14 dpi and declined by 28 dpi. Histopathology revealed pronounced intestinal inflammation at 7–14 dpi with near-complete mucosal restoration by 28 dpi, while skeletal muscle displayed characteristic nurse cell–larva complexes. These findings demonstrated a coordinated shift from early IFN-γ –mediated inflammation to sustained TGF-β–driven regulation, supporting parasite persistence and limiting tissue injury.

Keywords: *Trichinella spiralis*; Interferon-γ; TGF-β; Immune modulation; Immunopathology; Chronic infection.

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Introduction

Trichinella spiralis (*T. spiralis*) is a globally distributed zoonotic nematode and one of the most prevalent foodborne parasites worldwide, responsible for an estimated 10,000 cases annually.[1] Infection follows ingestion of raw or undercooked meat containing encysted larvae. After excystment, larvae mature into adults within the small intestine, where newborn larvae subsequently disseminate hematogenously to striated muscles and induce transformation of infected myocytes into "nurse cells." This biphasic intestinal–muscular cycle underlies clinical manifestations and the complex host–parasite immunological interplay.[2, 3]

The pathogenesis of trichinellosis is predominantly immune-mediated, as *T. spiralis* has evolved sophisticated strategies to evade, modulate, and redirect host immunity. The acute intestinal phase is marked by a T helper 1 (Th1)-response characterized by interferon-γ (IFN-γ), interleukin-2 (IL-2), Tumor necrosis factor alpha (TNF-α), macrophage activation, and cytotoxic T-cell engagement, which restrict early parasite establishment but also contribute to intestinal inflammation. As infection transitions to the muscle stage, immunity shifts toward a T helper 2 (Th2)-dominant and regulatory profile, with IL-4, IL-5, IL-10, and IL-13 driving eosinophilia, Ig E production, mast-cell activation, and localized tissue remodeling. These responses promote containment of encysted larvae while preventing excessive tissue injury.[4]

A defining feature of *T. spiralis* infection is its ability to modulate host immunity through excretory–secretory (ES) products, which suppress dendritic-cell maturation, induce regulatory T cells, enhance IL-10 and transforming growth factor-β (TGF-β) production, and drive alternatively activated macrophage responses that favor tissue repair over parasite elimination.[5]

TGF-β has gained particular attention as a central immunoregulatory cytokine up-regulated in intestinal and muscle tissues during infection, where it contributes to epithelial regulation, fibrosis, and immune suppression.[6-8] Produced by a wide range of immune and non-immune cells, TGF-β is secreted in a latent form and becomes activated in inflamed or remodeling tissues.[9] Evidence suggests that *T. spiralis* enhances TGF-β signaling to promote T regulatory (Treg)-mediated suppression of pro-inflammatory pathways and to facilitate collagen deposition around nurse cell–larva complexes, supporting long-term parasite persistence.[10] In contrast, IFN-γ plays a pivotal role in early host resistance. Primarily derived from Th1 cells, natural killer (NK) cells, and Cluster of Differentiation 8 ($CD8^+$) T cells this type II interferon cytokine (IFN-γ) activate macrophages and limit intestinal worm establishment. Its decline during the muscle phase reflects the natural transition to Th2/regulatory dominance, which minimizes tissue pathology while facilitating encystment.[11-13]

Despite growing understanding of these pathways, the coordinated dynamics of IFN-γ and TGF-β across both phases of *T. spiralis* infection remain insufficiently defined. The present study investigated the hypothesis that *T. spiralis* actively modulates these cytokines to evade host immunity, thereby enhancing persistence and promoting the establishment of chronic muscle infection.

Materials and Methods

The present study was conducted at the Theodor Bilharz Research Institute (TBRI), Giza, Egypt, during the period from October 2024 to July 2025.

Experimental Animals and Infection

The study included 40 specific pathogen-free female laboratory-bred Swiss albino mice, aged 3–4 weeks and weighing 20–25 g. Animals were maintained under controlled environmental conditions at 21 °C and provided with sterile water and a balanced commercial dry diet containing 14% protein. Mice were housed in wire-floored cages, with two to three animals per cage.

T. spiralis larvae were obtained from previously infected albino mice. Encysted larvae

were recovered, suspended in 100 ml of tap water, and counted using a binocular microscope to determine larval concentration.

Mice were randomly divided into two equal groups, each contained 20 mice, anon-infected control group (G1) and an infected group (G2). Prior to infection, mice were fasted for 12 h. Each mouse in the infected group was orally inoculated with 250–300 *T. spiralis* larvae using a blunt-ended tuberculin syringe.

Worm Burden Determination

Infected mice were sacrificed at 3, 7, 14, and 28 days post-infection (dpi) for subsequent analyses.[14] To determine the intestinal worm burden, the small intestine was dissected, opened longitudinally, and cut into 10-cm segments. The intestinal segments were incubated in Petri dishes containing physiological saline at 37 °C for 4 h to allow adult worms to migrate into the medium. The intestinal tissues were then discarded, and the adult worms were recovered from the sediment and counted using a stereomicroscope.

For assessment of muscle worm burden, the diaphragm from each mouse was excised and digested overnight at 37 °C in pepsin–HCl solution. Following digestion, released larvae were harvested and counted under a light microscope.[15, 16]

Cytokines Assay

Serum concentrations of IFN-γ and TGF-β were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Camarillo, CA, USA), according to the manufacturer's instructions.

Histopathological Examination

Small intestine and skeletal muscle specimens were collected from each mouse, washed three times with 0.01 M phosphate-buffered saline (PBS, pH 7.4), fixed in 10% neutral-buffered formalin, and embedded in paraffin. Serial sections were cut at a thickness of 5 µm, with an interval of 250 µm between sections to avoid re-measurement of the same lesions. Sections were stained with hematoxylin and eosin (H&E) for routine histopathological examination and examined under light microscopy at 100× and 400× magnifications.[17]

The presence of *T. spiralis* adult worms in the intestine and encysted larvae in skeletal muscles was evaluated, and the mean parasite counts were calculated for each experimental group.

Immunohistochemical Analysis

Paraffin-embedded intestinal tissue sections were deparaffinized in xylene and rehydrated through graded ethanol concentrations, followed by distilled water and PBS. Endogenous peroxidase activity was blocked by immersion in 3% hydrogen peroxide. Tissue sections were incubated with anti-TGF-β monoclonal antibody at a concentration of 5 µg/ml for one h at room temperature. Negative control sections were incubated with PBS containing normal goat serum in place of the primary antibody.

Immunoreactivity was detected using a VECTASTAIN® peroxidase kit (Vector Laboratories, Burlingame, CA, USA) and visualized with diaminobenzidine (DAB) as the chromogen. Sections were counterstained with hematoxylin and examined under a light microscope.[18]

Statistical Analysis

Data were collected, tabulated, and analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 18 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using the two-tailed Student's t-test for unpaired samples. Differences were considered statistically significant at $p < 0.05$.

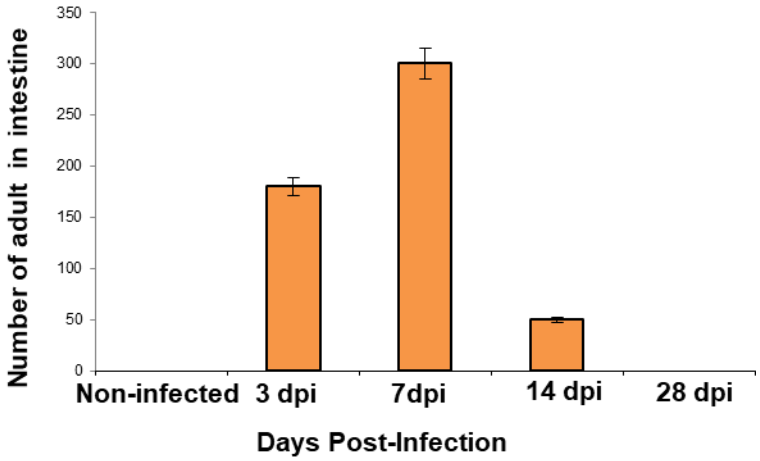
Results

Worm burden assessment

Adult *T. spiralis* worms were recovered from the small intestine during the early phase of infection, whereas encysted larvae were enumerated in muscle tissues at later stages. Quantitative evaluation of intestinal worm burden demonstrated a dynamic pattern across the examined time points. Mice sacrificed at 3,

7, 14, and 28 days post-infection exhibited mean adult worm counts of 150.9 ± 12.5, 300.19 ± 25.8, 50.14 ± 6.5, and 0, respectively. Adult worm counts differed significantly across the infected time points groups ($p < 0.001$), with the highest burden recorded at 7 dpi (Figure 1a).

For larval enumeration, diaphragmatic muscles were collected at the designated time points and subjected to overnight pepsin–HCl digestion. Larval detection commenced as early as 7 and 14 dpi, marking the onset of larval migration from the intestine to peripheral musculature. At 28 dpi, fully encysted larvae were recovered in substantial numbers, ranging from 9.74×10^3 to 10.15×10^3 larvae per mouse, with a mean burden of 1.002×10^4 larvae (Figure 1b).



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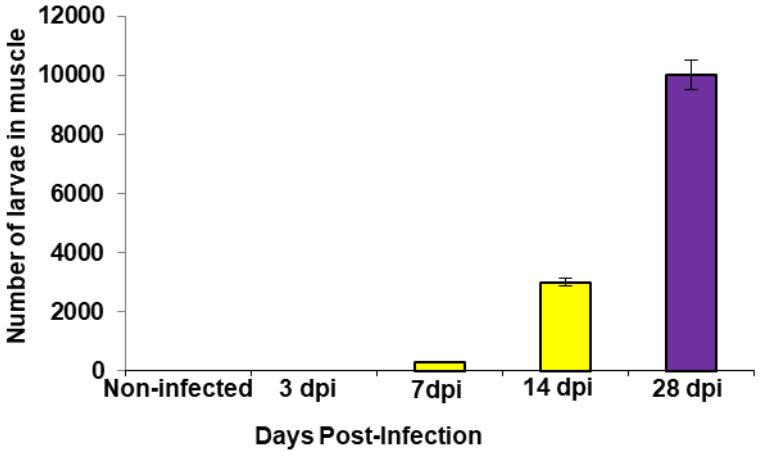

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Figure 1. Changes in *T. spiralis* worm count at different post-infection intervals. A histogram illustrates the dynamic pattern of *T. spiralis* development from the intestinal to the muscular phase. (a) Worm counts in the intestine peaked at 7 dpi, declined sharply by 14 dpi (b) they were replaced by increasing numbers of encysted larvae in skeletal muscles by 28 dpi, indicating successful transition from adult to larval stages.

Differential regulation of INF--γ and TGF--β during murine T. spiralis infection

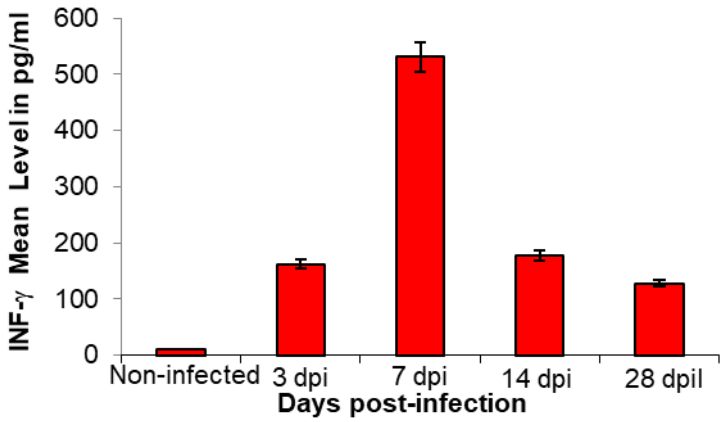
To characterize the host immune dynamics during *T. spiralis* infection, serum concentrations of IFN-γ, a key Th1 cytokine, and the regulatory cytokine TGF-β were quantified at successive time points post-infection (Figure 2; a and b). A significant variation in IFN-γ concentrations was observed across the experimental groups (Figure 2-a). The uninfected control group exhibited the lowest level (10 ± 0.7 pg/ml). In contrast, infected mice displayed significantly elevated concentrations at 3 dpi (162.4 ± 7.3 pg/ml, $p<0.05$), 7 dpi (531.5 ± 28.5 pg/ml, $p=0.001$), and 14 dpi (177.7 ± 3.1 pg/ml, $p=0.041$). The maximal IFN-γ response occurred at 7 dpi, coinciding with the acute intestinal phase, followed by a marked decline thereafter. By 28 dpi, IFN-γ levels had decreased substantially to near-baseline levels (128.5 ± 7.2 pg/ml), representing a significant reduction compared with the 7 dpi group ($p = 0.001$).

In contrast, serum TGF-β levels exhibited a progressive and sustained elevation throughout the course of infection (Figure 2 b). The lowest concentration was recorded in the negative control group (549.2 ± 52.4 pg/ml). Infected animals showed a robust and significant rise at 3 dpi (1453.4 ± 52.9 pg/ml, $p<0.001$), 7 dpi (1931.8 ± 63.7 pg/ml, $p<0.001$), and 14 dpi (2663.2 ± 130.1 pg/ml, $p<0.001$). By 28 dpi, TGF-β concentrations remained elevated (2917.4 ± 83.9 pg/ml), indicating the persistence of a pronounced regulatory response into the later phase of infection.

Together, these findings reveal a biphasic immune pattern characterized by an early, transient Th1 activation reflected by elevated IFN-γ, followed by a sustained up-regulation of TGF-β, indicative of a shift toward regulatory immune modulation during the chronic phase of *T. spiralis* infection.

Correlation between serum IFN-γ and TGF-β levels during T. spiralis infection

To investigate the immunoregulatory interactions influencing host responses during *T. spiralis* infection, the Pearson correlation analysis was conducted on $\log_{10}$-transformed serum cytokine concentrations. A significant negative correlation was observed between IFN-γ and TGF-β ($r = -0.42$, $p = 0.012$), suggesting that elevated TGF-β levels were associated with reduced IFN-γ concentrations. IFN-γ, indicative of a Th1-type immune response, reached its highest levels at 7 days post-infection before gradually declining. In contrast, TGF-β, associated with regulatory and Th2-type responses, gradually increased during the muscle phase (14–28 days post-infection), reflecting the shift toward immune regulation and tissue repair during chronic infection.



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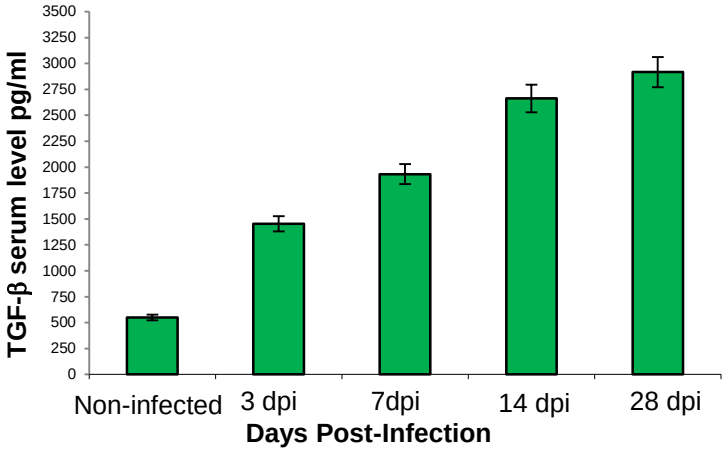

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Figure 2. Histograms showing differential regulation of interferon-γ (IFN- γ) and transforming growth factor-β (TGF-β) during *T. spiralis* infection. A histogram illustrating the changes in serum levels of IFN- γ and TGF-β throughout the course of infection. (a) IFN- γ, representing the Th1-type immune response, peaks by 7 days post-infection and declines thereafter. (b) In contrast, TGF-β, associated with regulatory and Th2-type responses, gradually increases during the muscle phase (14–28 days post-infection), reflecting the shift toward immune regulation and tissue repair during chronic infection.

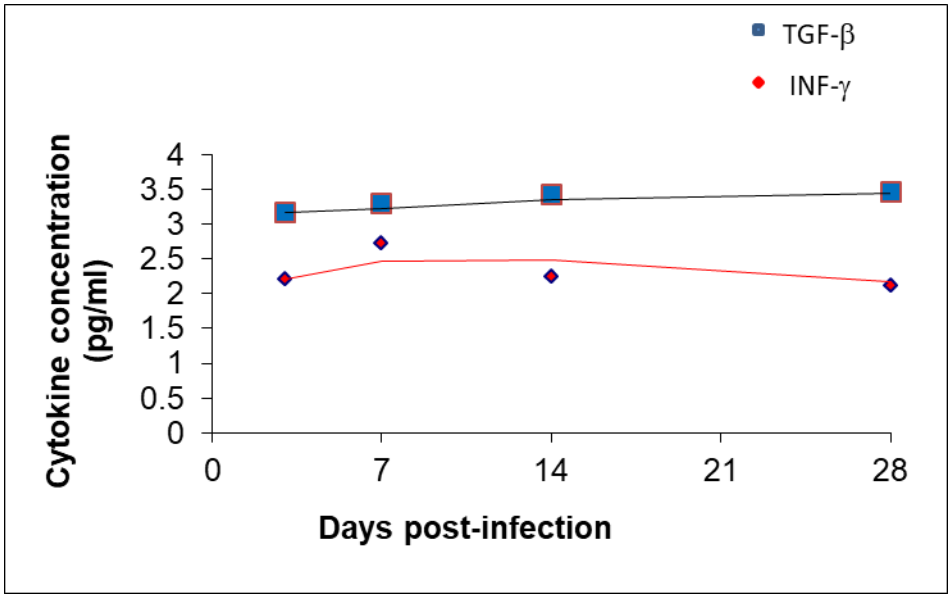

Figure 3. Dynamics of interferon-γ (IFN- γ) and transforming growth factor-β (TGF-β) regulation during *T. spiralis* infection. Line graph showing the $\log_{10}$-transformed concentrations of IFN-γ (red line) and TGF-β (blue line) throughout the course of infection. The scatter plot of individual values demonstrated a clear downward trend for IFN-γ, while TGF-β levels rise suggesting an inverse relationship between these cytokines across the studied time points. This pattern was most pronounced during the early intestinal phase of infection, reflecting active parasite-driven modulation of the host immune response from pro-inflammatory (Th1) toward regulatory profiles.

Expression of TGF-β receptors II in intestinal tissues

Immunohistochemical analysis was performed to assess the expression and distribution of TGF-β receptor II in the small intestine at various time points post-infection with *T. spiralis* (Figure 4a–f). TGF-β receptor II expression was low in intestinal tissues of the uninfected control group, with a mean immunoreactivity score of 4.4 ± 1.1. In contrast, infected mice demonstrated a marked up-regulation of TGF-β receptor II as early as 3 dpi (43.7 ± 4.5), reflecting the initiation of host immunomodulatory responses. Expression increased further at 7 dpi (62.4 ± 6.7) and reached its peak at 14 dpi (71.2 ± 3.9), corresponding to the period of maximal intestinal inflammation and tissue remodeling during the acute phase of infection.

By 28 dpi, during the late or chronic phase, intestinal TGF-β receptor II expression declined significantly (26.4 ± 3.1), approaching baseline levels observed in uninfected controls. Statistical analysis confirmed a significant difference between groups ($p < 0.001$), indicating a dynamic regulation of TGF-β receptor II in response to infection. TGF-β receptor II immunoreactivity was predominantly localized to epithelial cells lining the villi and to subepithelial inflammatory cells, suggesting that both epithelial and immune cell populations contribute to the cytokine-mediated modulation of intestinal immunity.

These findings demonstrated that TGF-β receptor II expression is transiently up-regulated during the early acute intestinal phase of *T. spiralis* infection and decreases as inflammation resolves, supporting its role in regulating local immune responses and tissue repair.

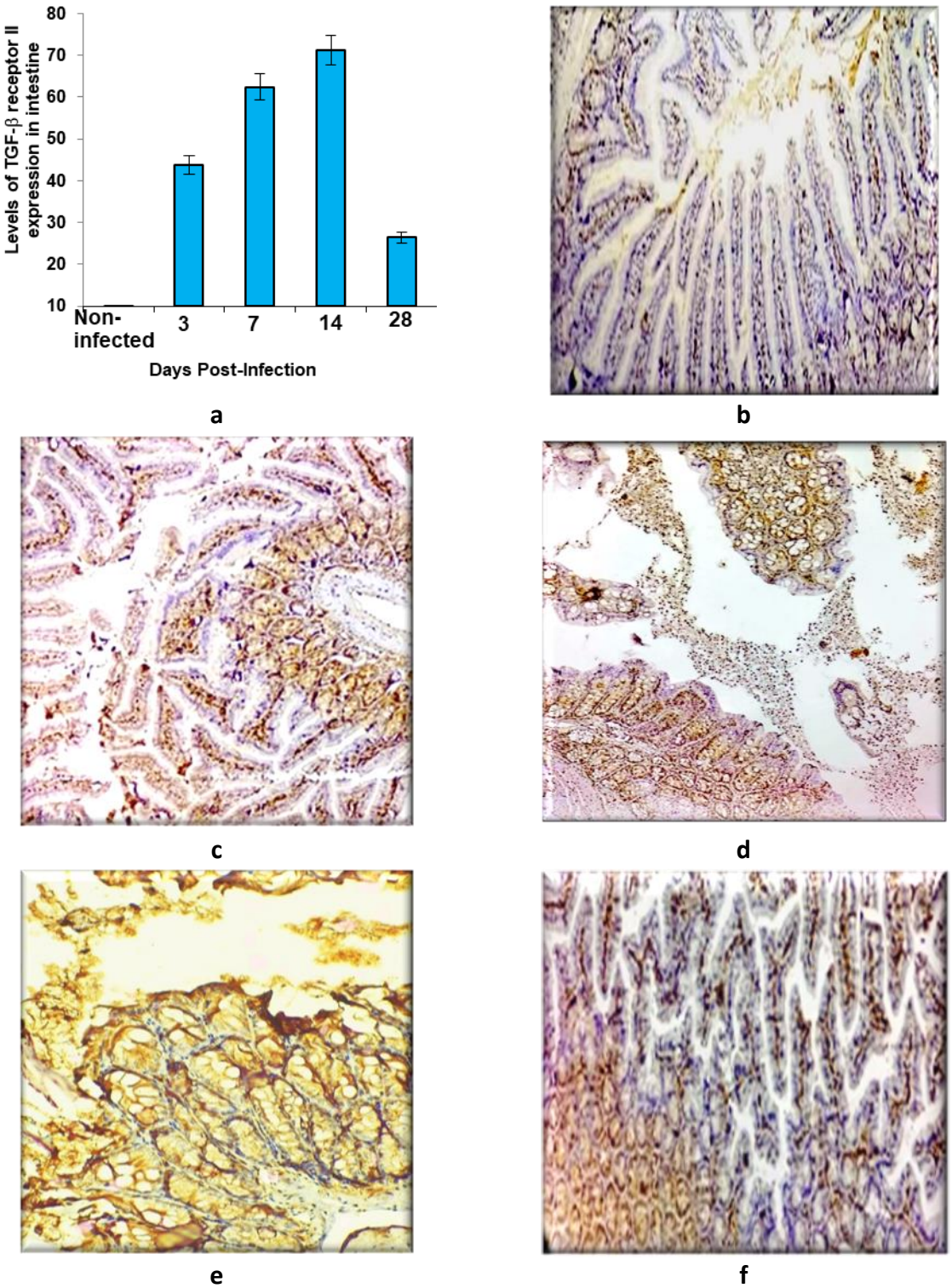

Figure 4. Immunohistochemical analysis of TGF-β receptor II in the small intestine at various time points post-infection with *T. spiralis.* (a) A histogram demonstrated that *T. spiralis* infection enhanced transforming growth factor-β (TGF-β) receptor II expression peaking around 7-14 dpi (r = 0.998, p<0.001). (b) Intestinal sections from the normal control group demonstrated weak TGF-β receptor expression in the intestinal epithelial cells and villous core inflammatory cells. (c) At 3 dpi, mild TGF-β receptor expression was observed in both the epithelial cells and villous core inflammatory cells. (d) By 7 dpi, moderate expression of the TGF-β receptor was evident in these cell populations. (e) At 14 dpi, mice exhibited strong TGF-β receptor expression in the epithelial cells and villous core inflammatory cells. (f) By 28 dpi, TGF-β receptor expression was significantly reduced in both epithelial cells and villous core inflammatory cells compared with the earlier infected groups.

Histopathological Examinations

Histological analysis of small intestine and skeletal muscle sections collected at different time points post-infection (PI) was conducted using hematoxylin and eosin (H&E) staining to characterize the tissue alterations associated with *T. spiralis* infection (Figure 5 a–h). At 3 dpi, intestinal sections demonstrated notable pathological changes, including marked inflammatory cell infiltration within the lamina propria, early villous shortening, and focal epithelial desquamation, indicating the onset of acute mucosal injury. By 7–14 dpi, these alterations became more pronounced, with extensive villous blunting, crypt hyperplasia, and dense subepithelial inflammatory infiltrates, reflecting sustained immune activation during the intestinal phase of infection. By 28 dpi, the intestinal mucosa exhibited near-complete restoration of villous architecture, with only minimal residual inflammatory infiltration.

In contrast, skeletal muscle sections harvested at 28 dpi exhibited typical features of the muscle phase, characterized by well-developed nurse cell–larva complexes. These complexes were accompanied by mild to moderate perilarval inflammatory infiltrates composed predominantly of mononuclear cells, along with early fibrosis surrounding the encapsulated larvae. Notably, the degree of inflammatory damage appeared attenuated in the muscle tissues relative to the intestinal lesions observed during earlier stages. This reduction in tissue injury corresponded temporally with the up-regulation of TGF-β expression, suggesting a role for TGF-β in dampening excessive inflammation and promoting tissue remodeling during the chronic phase of infection.

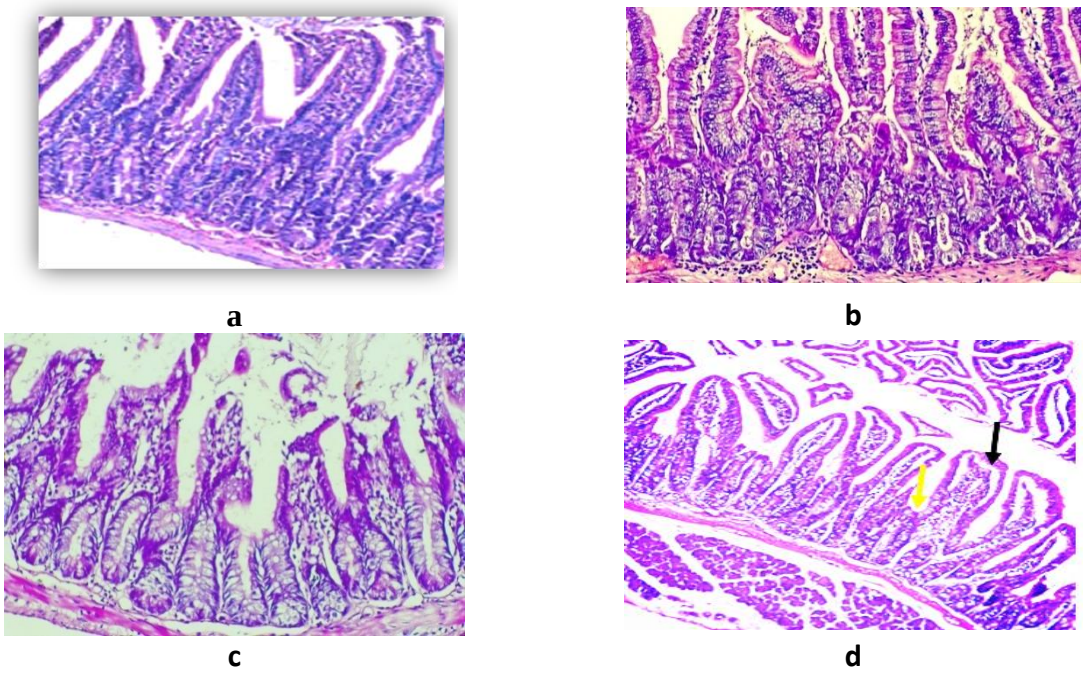

Figure 5. Histopathological changes in the small intestine and skeletal muscle during *T. spiralis* infection. (a) Intestinal section from the normal control group showing intact villous architecture composed of tall columnar epithelial cells with numerous goblet cells. Crypts were regularly arranged, and the villus-to-crypt ratio was preserved, with minimal collagen fibers in the mucosa and sub mucosa (hematoxylin and eosin, H&E, ×200). (b) At 3 dpi, intestinal sections displayed early inflammation with villous distortion, villous atrophy, ulceration of villous tips, inflammatory cell infiltration (often mixed, including eosinophils), and numerous *T. spiralis* larvae/adult worms embedded in or adhering to epithelial cells (H&E, ×200). (c) At 7 dpi, sections showed severe ulceration, blunting and shortening of villi, pronounced villous atrophy, and intense lymphocytic (and eosinophilic) infiltration in the lamina propria, with numerous adherent/intraepithelial *T. spiralis* stages (H&E, ×100). (d) At 14 dpi, focal mild shortening and broadening of villi were observed, with residual mild ulceration and mild inflammatory infiltrates, consistent with the beginning of recovery as adult worm expulsion occurs (H&E, ×200).

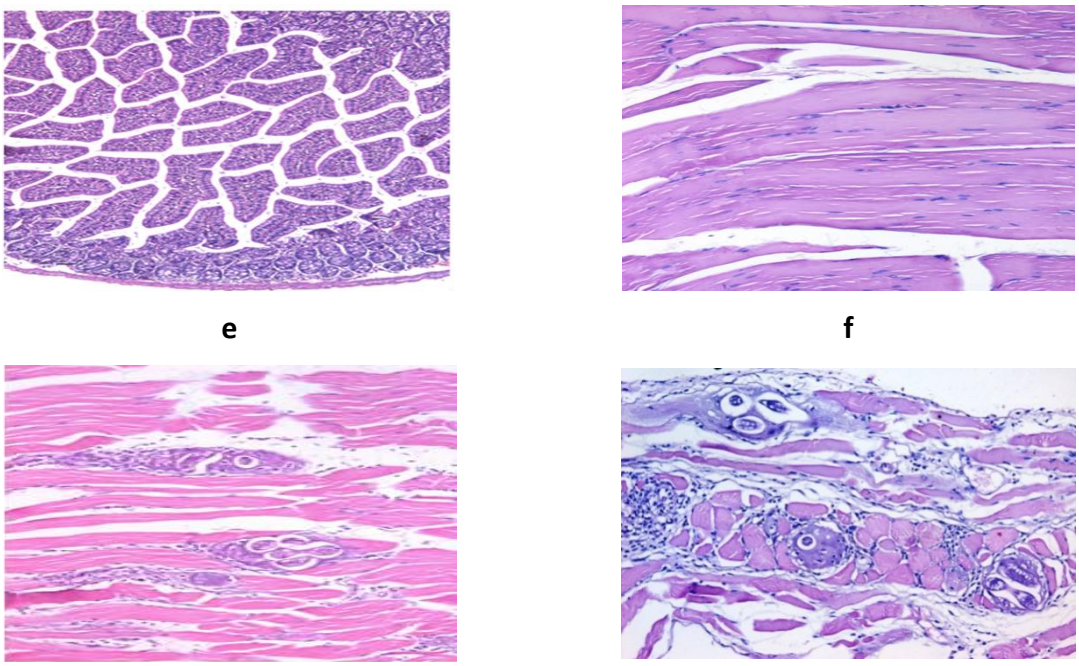

Figure 5. Continued. Histopathological changes in the small intestine and skeletal muscle during *T. spiralis* infection. (e) At 28 dpi, the intestinal mucosa demonstrated nearly restored villous architecture with only minimal inflammatory infiltration (H&E, ×100). (f) Skeletal muscle from the normal control group exhibited normal architecture, with elongated acidophilic muscle fibers, peripheral basophilic nuclei, and clear transverse striations (H&E, ×200). (g, h) Skeletal muscle of infected mice at 28 dpi showed multiple encysted *T. spiralis* larvae, viable and degenerating, surrounded by marked lymphocytic infiltration (H&E, ×100 and ×200).

Discussion

T. spiralis is a major foodborne helminth capable of establishing chronic infection through modulation of host immune responses. IFN-γ and TGF-β represent two opposing immunological axes that influence early inflammatory control and later regulatory adaptation. This study aimed to elucidate the temporal dynamics of IFN-γ, TGF-β, and intestinal TGF-β receptor II expression during *T. spiralis* infection and to correlate these changes with parasite burden and tissue pathology.

The temporal distribution of adult worms and muscle larvae observed in this study aligns with the classical biphasic developmental pattern of *T. spiralis*. The significant increase in adult worm numbers between 3 and 7 dpi reflects the successful establishment and rapid maturation of the parasite within the intestinal mucosa. The subsequent sharp decline in adult worm burden by 14 dpi, followed by complete clearance by 28 dpi, is consistent with the host's effective intestinal expulsion mechanisms. These findings paralleled earlier studies documenting worm burden peak around 7dpi.[19,20]

The early detection of larvae at 7 and 14 dpi corresponds to the migratory phase, during which newborn larvae disseminate hematogenously and initiate encystment in skeletal muscle. By 28 dpi, the high larval burden (approximately 1×10^4 larvae per mouse) confirms efficient larval production and successful establishment of the chronic muscle phase. The magnitude of larval recovery at this time point is consistent with previous experimental infections and reflects the substantial reproductive output of adult females during their peak at 7 dpi.[21-23]

Collectively, these findings demonstrated the synchronized progression of *T. spiralis* from intestinal maturation to systemic larval dissemination and muscle encystment. The distinct kinetics of adult worm decline and larval accumulation highlighted the coordinated host–parasite interactions governing the transition

between the intestinal and muscular phases of infection.

The temporal cytokine patterns observed in this study provided important insights into the immunological strategies engaged during *T. spiralis* infection. The pronounced but short-lived elevation of IFN-γ, peaking at 7 dpi, corresponds to the early intestinal phase when larval penetration and epithelial disruption elicit strong pro-inflammatory and Th1-driven responses. This early activation is likely crucial for initiating host defense mechanisms aimed at limiting parasite establishment and is consistent with previous reports describing transient Th 1 up-regulation during the early stage of infection.[13,24,25,26]

However, the subsequent decline in IFN-γ from 14 dpi onward suggested a tightly regulated attenuation of Th1 activity as the infection progresses. This down-modulation coincides with the onset of the muscle phase, during which excessive inflammatory responses would risk exacerbating tissue damage.

In contrast, the sustained increase in TGF-β from 3 dpi through 28 dpi underscores its central role in shaping a regulatory and tissue-protective immune environment. The continuous elevation of TGF-β suggests that *T. spiralis* actively promotes regulatory pathways to counterbalance early inflammation, thereby facilitating tissue repair, reducing immunopathology, and ensuring its own persistence. This pattern is consistent with earlier studies the well-documented capacity of *T. spiralis* to induce an immunoregulatory milieu, enabling long-term host–parasite coexistence.[8,27,28]

Collectively, these data highlighted a finely orchestrated transition from an early pro-inflammatory phase to a dominant regulatory phase during *T. spiralis* infection. Such coordinated modulation of host immunity likely represents a key mechanism underlying the parasite's ability to establish chronic infection while minimizing tissue injury.

Our findings indicated that TGF-β receptor II expression in the intestine is dynamically regulated during *T. spiralis* infection and closely reflects the immunological demands of each developmental stage of the parasite. During the early intestinal phase (7–14 dpi), we observed a marked up-regulation of TGF-β receptor II. This increase is consistent with the heightened need for TGF-β–mediated signaling during initial host–parasite interactions, where modulation of epithelial and immune cell responses supports both tissue repair and the coordinated immune mechanisms involved in adult worm expulsion. Notably, this period coincides with the timeframe in which adult worms are typically cleared from the murine intestine, suggesting that enhanced TGF-β pathway activity may contribute to the regulation of local immunity and maintenance of mucosal homeostasis.

By 28 dpi, when the infection has shifted to the muscle phase and nurse cell formation is prominent, intestinal expression of TGF-β receptor II had substantially declined. This reduction likely reflects the diminished immunological activity within the intestine once adult worms were eliminated and the host's immune focus has transitioned toward the skeletal muscle. The down regulation at this stage is consistent with the cessation of intestinal pathology and the reduced requirement for TGF-β–driven regulatory signaling in the gut environment.

Collectively, these observations highlighted the stage-dependent nature of TGF-β receptor expression during *T. spiralis* infection. The pronounced early up-regulation followed by subsequent decline underscores the adaptability of host regulatory pathways and supports the concept that TGF-β signaling plays a pivotal role in shaping the intestinal immune response during the acute phase of infection. These results align with those of Chaimon et al., 2024, who reported that *T. spiralis* TGF-β homologs interact with host receptors, activate TGF-β/Smad signaling, and promote collagen capsule formation in muscles.[29] Collectively, the data underscore TGF-β as a central immunomodulatory mediator facilitating parasite persistence while limiting host tissue damage.[8,27,30] Understanding this regulatory axis may inform strategies to modulate immune responses in helminth infections or related inflammatory disorders.

Histological analysis in the present study revealed that *T. spiralis* infection induced

progressive, tissue-specific pathology. Intestinal lesions peaked between 7 and 14 dpi, characterized by villous shortening, blunting, ulceration, and inflammatory infiltration, with near-complete recovery by 28 dpi. In skeletal muscles, encysted larvae were observed at 28 dpi, surrounded by lymphocytic and mixed inflammatory infiltrates, reflecting the host immune response. Interestingly our results of serum cytokine analysis showed a significant negative correlation between IFN-γ and TGF-β ($r = -0.42$, $p = 0.012$), with IFN-γ peaking during the early intestinal phase (~7 dpi) and declined thereafter, while TGF-β progressively increased during the muscle phase (14–28 dpi), indicating a shift toward immune regulation and tissue repair. These findings highlighted the coordinated temporal relationship between parasite-induced tissue pathology and modulation of host immune responses. These findings are consistent with earlier studies, highlighted the dynamic intestinal and muscular pathology associated with trichinosis. [25,29,31,32.]

The present study provided compelling evidence that *T. spiralis* employs highly coordinated immunomodulatory strategies to regulate host cytokine responses, particularly TGF-β and IFN-γ, throughout the course of infection. Early induction of IFN-γ –mediated Th1 immunity contributed to initial parasite containment during the intestinal phase. In contrast the elevated TGF-β and its receptor expression highlighted a central immunomodulatory axis exploited by the parasite during chronic infection. This dynamic interplay between pro-inflammatory and regulatory cytokines reflected an evolutionarily optimized mechanism of immune modulation that enables *T. spiralis* to evade host defenses while limiting excessive tissue pathology. Overall, these findings emphasized the central roles of TGF-β and IFN-γ in shaping the immunological trajectory of trichinellosis. A deeper understanding of the molecular pathways governing this cytokine balance may advance the development of targeted immunotherapeutic strategies for parasitic infections and broader inflammatory disorders.

Author Contributions

AFAE, MME; Planned and participated in the study design. Performed the laboratory work. Made critical reviews and approved the final version. IRS, MSE; Planned and participated in the study design. Analyzed the data. Wrote and reviewed the manuscript. Made critical reviews and approved the final version. TKZ; The corresponding author. Planned and participated in the study design. Writing of the manuscript, data interpretation. Made critical reviews and approved the final version.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the Research Ethical Committee at the Faculty of Medicine, Al-Azhar University, Cairo, Egypt. (Approval date March 2024).

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