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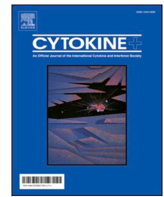

# Interleukin 17 producing T cell responses in human chronic trichinellosis-insight from a case study

Chiara Della Bella [a,b], Chiara Medici [d,1], Sofia D'Elios [e], Marisa Benagiano [b], Alessandra Ludovisi [c], Maria Angeles Gomez-Morales [c], Mario M. D'Elios [a,b], Fabrizio Bruschi [d,*]

[a] *Department of Molecular and Developmental Medicine, University of Siena, Italy*
[b] *Department of Experimental and Clinical Medicine, University of Firenze, Italy*
[c] *Department of Infectious Diseases, Istituto Superiore di Sanità, European Union Reference Laboratory for Parasites, Rome, Italy*
[d] *Department of Translational Research, N.T.M.S., Università di Pisa, Pisa, Italy*
[e] *Department of Clinical and Experimental Medicine, University of Pisa, Italy*

A R T I C L E I N F O

*Keywords:*
*Trichinella pseudospiralis*
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A B S T R A C T

*Introduction:* We studied the cellular immune response in a patient infected since 10 months (along with other 51 people) during a trichinellosis outbreak caused by *Trichinella* spp.
*Methods:* A 46 years old female resulted serologically positive for trichinellosis. We isolated peripheral blood mononuclear cells (PBMCs) and incubated them with excretory/secretory antigens (ESA) of *Trichinella spiralis* (T1) or *Trichinella pseudospiralis* (T4) to produce antigen specific T cell lines and clones, analysed for the phenotype (T helper or cytotoxic cells), for their T4 or T1 antigens specificity and for their cytokine profile (IFNγ, IL-17A, IL-4) by flow cytometry, thymidine incorporation assay and ELISpot.
*Results:* The test performed using ESA from T1 or T4 has identified the species responsible for infection as *T. pseudospiralis* since the proliferative responses (evaluated by CFSE, Carboxyfluorescein succinimidyl ester, FACS analysis) was higher for T4 (72,8%) than T1 (23.6 %) antigen. The cell lines produced significant levels of IFNγ, IL-4 and IL-17A after stimulation. From the T cell line obtained in response to T1 ESA, as regards CD4 + cells, 12 % Th2, 22.8 % Th1, 6.6 % Th17, 6 % Th0, 2.2 % Th1/Th17 and 0.7 % Th2/Th17, were obtained. From the T1-specific TCL we generated 15 clones. From the TCL specific for T4 ESA, as regards CD4+, 15.2 % Th2, 27.1 % Th1, 3 % Th17, 10.3 %Th0, 1.9 % Th1/Th17 and 1 % Th2/ Th17 were obtained. From such TCL 4 clones were isolated, 1Th2, 1 Th1, 1 Th17, 1 Th1/Th17 and no Th0 nor Th2/Th17.
*Conclusions:* By cellular immunology techniques the species responsible of the infection resulted *T. pseudospiralis*, confirming the results previously obtained by serology. For the first time it was revealed in a human chronic infection the presence of Th17 cells.

## 1. Introduction

Human trichinellosis is caused by the consumption of raw or undercooked meat containing living larvae of the parasitic nematodes *Trichinella* spp. After ingestion of infected meat, muscle larvae develop to the adult stage in a row of columnar epithelial cells in the small intestine. Once mature, adult worms mate and the females begin to shed, the newborn larvae (NBL) which migrate across the lamina propria of the villus into the lymph and bloodstream. However, only the NBL that reach skeletal muscles develop to the infective stage. The genus *Trichinella* is a relatively large complex of ten distinct species and three genotypes, with a geographic distribution worldwide. Two morphologically distinguishable (i.e. nonencapsulated as *Trichinella pseudospiralis* and encapsulated as *Trichinella spiralis*) phenotypes of *Trichinella* are known, according to the appearance of first-larvae (L1) within muscle cells of infected hosts. The extensive genetic variability within the *Trichinella* complex is of major biological significance and interest, and can reflect among others divergent host–parasite relationships [1].

Diagnosis of human trichinellosis is based on serological tests such as ELISA and Western blot (Wb) as well as on direct diagnosis which can be

* Corresponding author.
*E-mail address:* fabrizio.bruschi@unipi.it (F. Bruschi).
[1] Present address: Division of Clinical Pathology, ASL 5, La Spezia, Italy.

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carried out when patients are available to undergo muscle biopsy [2].

Despite the fact that patients rarely accept this procedure, the muscle biopsy have several advantages compared with serological tests, allowing confirmation of diagnosis and isolation and typing of the isolate when the infected meat responsible for the outbreak is not available. Molecular tools are able to genotype even one single larva [3].

The knowledge of the cellular immune response to this parasitic nematode derives mainly from studies carried out in experimental infection in rodents. In mice, T cell responses are readily generated in the mesenteric lymph nodes (MLN) during a primary infection producing Type 2 cytokines including IL-4 and IL-5, together with IL-9 [4]. However, *Trichinella* infection also generates a population of so-called natural Th1 cells in the thymus, which are expanded by the presence of IL-4, associated with expression of low affinity/low avidity T cell receptors and production of IFN-γ upon stimulation [5]. As regards humans, the cellular immune response to the parasite was evaluated in very few studies which highlight the increased CD8-T-cell expression and a type 2 cytokine pattern during the muscular phase of *Trichinella* infections [6]. A dominance of a peripheral Th2 phenotype in infected humans was also highlighted in data from a study by Della Bella et al. who generated *Trichinella*-specific T cell clones from peripheral mononuclear cells (PBMC) in individuals that had been infected since 3 months with *T. britovi* and presented elevated peripheral IgG1, IgE, and eosinophilia [7]. It is important to underline the capacity of *T. spiralis* ESA to induce an immunomodulatory network (which encompasses Th2- and Treg-type responses) that may have beneficial effect on the outcome of some disorders [8].

In the present study, we report the results obtained from the evaluation of the cellular immune response in one patient infected during an outbreak caused by *Trichinella pseudospiralis,* occurred in Genoa, Italy [9], through the induction of specific *T. pseudospiralis* and *T. spiralis* specific T cell clones.

## 2. Materials and methods

### 2.1. Case history

Two weeks after consumption of the infected meat) the patient suffered of abdominal discomfort and diarrhea followed then by neck and vertebral muscle myalgias with fever (around 38.7 °C).

After consultation of her physician, it was diagnosed a flu syndrome for which analgesic and antipyretics drugs were administered, with a light amelioration of the symptomatology which, however reappeared at the end of the treatment.

In the meanwhile some people who had participated at the same dinner were hospitalised and to carry out the epidemiological survey, our patient was contacted by the Service of Infectious diseases and invited to underlie some laboratory tests which revealed a leukocytosis (20,300/mmc.) with 49.6 % (10.080/mmc) of eosinophils, whereas CPK and LDH serum levels were normal, the serology for trichinellosis resulted positive, for all these reasons a treatment with albendazole (800 mg/die) and prednisone (25 mg./die) were administered for a period of 10 days. At the end of the treatment the patient was asymptomatic and eosinophils declined to 15 %. After 90 days post infection leucocytes were 13,800 (neutrophils 10,800, eosinophils 300/mmc.).

### 2.2. Chest Rx and echocardiogram resulted negative

After several months from infection the patient still suffered a general malaise and lack of appetite with a loss of 5 kg never recovered in comparison with the pre-infection period.

#### 2.2.1. Samples

Blood samples were collected ten months after infection (chronic phase of the infection) and used to obtain serum and PBMCs from the patient who agreed to participate to the study, involved in the Genoa Outbreak [9].

#### 2.2.2. Western blot

A serum sample from the donor was tested by Wb to confirm the presence of anti-*Trichinella* IgG using a high-sensitive revelation system based on chemiluminescence [10].

#### 2.2.3. Trichinella antigens

Excretory/secretory antigens (ESA) used in this study were from *T. pseudospiralis* (T4) and *T. spiralis* (T1) muscle larvae [2].

### 2.3. Characterization of fresh PBMCs phenotype and specificity for T4 or T1 ESAs

A whole blood sample (10 mL) from the *Trichinella*- infected donor was collected in lithium-heparin tubes. Heparinized blood was layered on to Ficoll-Hypaque density gradient (Lymphoprep, Alere Technologies, Oslo, Norway) and centrifuged at 2,200 rpm for 25 min in order to isolate PBMCs, which were then harvested, washed and counted. A part of the PBMCs was frozen in DMSO 10 % at −80 °C for future use.

$5 \times 10^5$ fresh PBMCs/well were seeded in a second plate after CFSE (Carboxyfluorescein succinimidyl ester) staining (CellTrace CFSE dye, Invitrogen, USA), following manufacturer's instructions. These cells were cultured in triplicate for 48 h in medium alone, medium with 10 μg/ml of T4 ESA, or 10 μg/ml of T1 ESA in RPMI 1640 (BioConcept AG, Allschwil, Swiss) complete medium (with L-glutamine 1 %, beta-mercaptoethanol 1 %, Na-pyruvate 1 %, non-essential aminoacids 1 %, penicillin 50,000U, and streptomycin 50 mg) supplemented with 5 % human serum (Sigma Aldrich, St. Louis, Missouri, US)

After 48 h of T1 or T4 ESA incubation of CFSE-labelled fresh PBMCs, a FACS analysis was performed. T cell phenotype was characterized by human anti-CD3 Pacific blue (BioLegend, 300431, intended use Flow Cytometry (FC), dilution 1:100), anti-CD4 PE (BioLegend, 344602, intended use FC, dilution 1:100) and anti-CD8 APC (BioLegend, 301014, intended use FC, dilution 1:100) labelling by FACS analysis (BD FACS CantoII and the FACSDiva software; Becton Dickinson, Franklin Lakes, NJ, USA). In addition, the CFSE-labelled fresh PBMCs response to ESAs was tested by CFSE proliferation assay [11]. 5,000 events were acquired and the CFSE signal was evaluated on the gated T $CD4^+$ and T $CD8^+$ subpopulations. A positive proliferative response was named when the % of events was found over the basal proliferation threshold.

Three matched healthy individuals were also tested for study control.

### 2.4. Generation of Trichinella pseudospiralis and T. spiralis-specific T cell lines

In order to generate T4 and T1 ESA specific T cell lines (TCL), $1.5 \times 10^6$ fresh PBMCs/ml/well were seeded in a 24 multiwell plate and cultured for 5 days with 10 μg/ml of T4 or T1 ESA in RPMI 1640 complete medium supplemented with 5 % human serum. On day six and thereafter at 3-days interval, cell lines were supplemented with human recombinant IL-2 (20 UI/ml) (PeproTech, London, UK).

### 2.5. Proliferative response to T4 or T1 ESA by Trichinella-induced cell lines

On day 12, T cell blasts obtained from the T1-induced and T4-induced lines were tested for their response to T1 or T4 ESA respectively, by CFSE proliferation test. In detail, $2 \times 10^5$ T cell blasts of each *Trichinella*-induced cell line, were labelled with CFSE and incubated at 37 °C under 5 % $CO_2$ with complete medium or with T1 or T4 ESA (10 μg/ml). After 5 days of culture, cells were stained with anti-CD3 Pacific blue, anti-CD4 PE and anti-CD8 APC and tested for their proliferation to the T4 and T1 ESAs following the CFSE signal by flow cytometry. 5,000 events were acquired and the CFSE signal was evaluated on the gated T $CD4^+$ and T $CD8^+$ subpopulations. A positive response is defined as the

% of events over the basal proliferation threshold.

### 2.6. Determination of the phenotype and the cytokine profile of the *T. pseudospiralis* and *T. spiralis* specific T cell lines

$10^6$ T cell blasts of T1-induced and T4-induced lines were incubated at 37 °C, 5 % $CO_2$ in humidified atmosphere for 5–6 h with complete medium and the Golgi block Brefeldin A or stimulated with Leukocyte Activation Cocktail with BD GolgiPlug (BD Biosciences, Franklin Lakes, New Jersey, US), containing PMA (Phorbol 12-Myristate 13-Acetate) mixed with Ionomycin and Brefeldin A [12]. Cells were then washed and labelled for external markers with anti-human CD4 PerCP (BioLegend, 980820, intended use FC, dilution 1:100) or anti-human CD8 PerCP (BioLegend, 980916, intended use FC, dilution 1:100) fluorochrome-conjugated antibodies, according to manufacturer's specifications (BioLegend, San Diego, California, US). Fixation/Permeabilization solution (BD Biosciences, Franklin Lakes, New Jersey, US) was then applied on all the samples to allow the following intracellular staining of anti-human IFN-γ FITC (BioLegend, 317306, intended use FC, dilution 1:100), anti-human IL-4 PE (BioLegend, 344602, intended use FC, dilution 1:100) and anti-human IL-17A Pacific blue (BioLegend, 512312, intended use FC, dilution 1:100) fluorochrome-conjugated antibodies. Labelled T cells were finally washed and flow cytometric analysis was carried out on BD FACS Canto II using the FACSDiva software (Becton Dickinson, Franklin Lakes, NJ, USA).

### 2.7. *T. pseudospiralis* and *T. spiralis*-specific T cell clones

T cell clones were generated from T4-induced and T1-induced lines by high-efficiency cloning procedure [13]. In particular, after serial dilutions, 0.3 cell/well were stochastically seeded in four 96 round bottom micro-well plates, together with $10^5$ irradiated PBMCs as feeder, phytohaemagglutinin (PHA) as non-specific T cell receptor mitogen (Gibco, Thermo Fisher Scientific,Waltham, Massachusetts, US) (0.5 % v/v) in complete medium with human recombinant IL-2 (50 U/mL). After two weeks of medium refresh, T cell clones were chosen by optical microscopy and screened for their helper or cytotoxic function by CD4 or CD8 fluorochrome-conjugated monoclonal antibodies labelling for FACS analysis (eBioscience, San Diego, CA, US).

The obtained T cell clones were further tested for their responsiveness to *Trichinella* antigens by measuring their [$^3$H]-thymidine (Perkin Elmer, Waltham, MA, US) uptake after 60 h stimulation with complete medium, or with T1 or T4 antigen (1 μg/mL) in the presence of irradiated autologous mononuclear cells as APCs [14]. A mitogenic index (MI), calculated as the ratio between mean values of counts per minute (CPM) obtained in stimulated cultures and those obtained in the presence of complete medium, a value greater than 3 was considered a positive result. All experiments were performed in triplicate.

### 2.8. Assessment of the cytokine profile of *T. pseudospiralis* and *T. spiralis*-specific T cell clones by ELISpot

To estimate the cytokine production of the obtained *Trichinella*-specific T cell clones upon antigen stimulation, highly sensitive cytokine enzyme-linked immunosorbent spot (ELISpot) assays were applied (Mabtech, Stockholm, Sweden) [15]. $10^5$ T cell blasts of each clone (duplicate samples) were co-cultured in 0.2 ml of medium with 1.5 × $10^5$ irradiated autologous APCs in the absence or presence of *T. pseudospiralis* or *T. spiralis* (10 μg/ml) or the positive control for cytokine production included in each kit. Cell were incubated at 37 °C, 5 % $CO_2$ in humidified atmosphere for 24–48 h in microplates coated with anti-IFN-γ or anti-IL-4 or anti-IL-17 antibodies. At the end of the incubation time spots were revealed following manufacturer's specifications and the number of spot forming cells (SFC) was counted using the AID automated ELISpot reader by the AID ELISpot Software Version 3.2.3. Results were expressed as number of SFC per million cells.

The functional classification of the TCD4$^+$ clones obtained was carried out as follows: T helper 1 (Th1) clones were those producing IFN-γ, but not IL-4, nor IL-17. T helper 0 (Th0) clones were those producing both IFN-γ and IL-4. T helper 2 (Th2) clones were those producing IL-4, but not IFN-γ nor IL-17. T helper 17 (Th17) clones were those producing IL-17, but not IFN-γ, nor IL-4. Th1/Th17 were clones with a mixed production of IFN-γ and IL-17 but not IL-4, similarly Th2/Th17 clones were lymphocytes producing both IL-4 and IL-17 but not IFN-γ. Evenly, for TCD8$^+$ cytotoxic lymphocytes the classification was analogous.

### 2.9. Statistical analysis

Descriptive statistics (mean ± SD, percentage frequency) was applied to summarize data set. IBM®SPSS Statistic (version 28.0) was used to analyze the statistical data.

## 3. Results

### 3.1. Serological reactivity to Trichinella antigens

Fig. 1 shows the pattern of reactivity of the serum sample from the patient with crude *T. spiralis* antigens. This pattern is similar to that observed in serum samples from individuals with confirmed trichinellosis caused by *T. pseudospiralis* and clearly differs from the pattern of reactivity observed for control serum samples from individuals with confirmed trichinellosis caused by *T.spiralis*.

### 3.2. Characterization of the *T. spiralis* and *T. pseudospiralis*-specific T cells in fresh PBMCs

Fresh lymphocytes were stimulated with T1 and T4 antigens and then characterized by FACS analysis. Within the T cell population of the infected patient, CD3$^+$ CD4$^+$ CD8$^-$ were 62.5 % and CD3$^+$ CD4$^-$ CD8$^+$ were 33.9 %.

The proliferation test with CFSE was performed on fresh T cells in order to evaluate *Trichinella* antigenic specificity. In the CD4$^+$ gate of fresh PBMC sample we found 1.5 % of T1-specific T cells and 4.8 % of T4-specific T cell. Whereas in the CD8$^+$ gate of the same fresh PBMCs sample the T1-specific T cells were 0.3 % and the T4-specific T cell were 0.9 %.

With regard to the three study healthy control subjects, their fresh PBMCs FACS characterization showed a mean of 59.4 ± 3.6 % of CD3$^+$ CD4$^+$ CD8$^-$ and 28.2 ± 2.5 % of CD3$^+$ CD4$^-$ CD8$^+$.

Both CD4 and CD8 fresh cells from all the three healthy controls showed no proliferation in response to T4 nor T1 ESA.

### 3.3. *T. pseudospiralis* and *T. spiralis* specific T cell lines

Two TCL were raised with T1 or T4 *Trichinella* antigen, alternatively. The TCL specifically induced by T4 *T. pseudospiralis* ESA consisted of 72.8 ± 5.4 % of CD3$^+$CD4$^+$ and 18.5 ± 2.1 % of CD3$^+$CD8$^+$ T cells; whereas, that specifically induced by T1 *T. spiralis* ESA consisted of 23.6 ± 1.9 % of CD3$^+$CD4$^+$ and 7.4 ± 1.8 % of CD4$^+$CD8$^+$T cells (Table 1). Fig. 2 and Table 2 show results related to cytokine production by T1- and T4-specific TCL.

### 3.4. Characterization of the *T. spiralis* and *T. pseudospiralis*-specific T cell clones

Twenty-eight CD4$^+$ and no CD8$^+$ T cell clones were obtained from the T1-specific TCL, antigen specificity assessed for T1 ESA evidenced a MI in the range of 6.8–17.5 for 15 out of the 28 T cell clones. From the T4-specific TCL, nine CD4$^+$ and 2 CD8$^+$ T cell clones were obtained, from which 4/9 (44 %) were specific for T4 ESA, with MI from 7.3 to 15.1, whereas no CD8$^+$ clone showed specific proliferation to this antigenic preparation. The cytokine profiles of each specific clone,

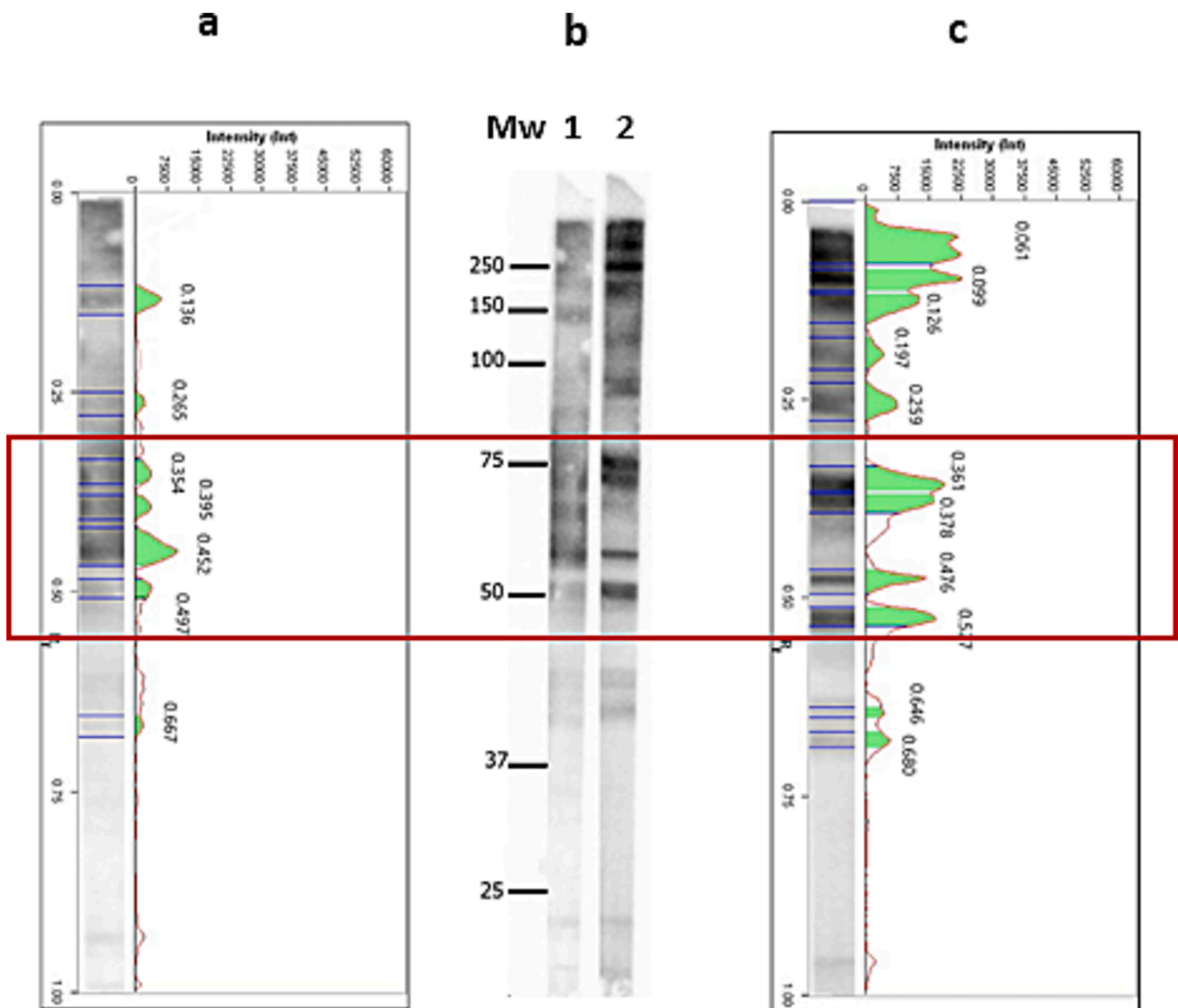


**Fig. 1.** Western blot patterns, signal intensities and relative migrations values of *Trichinella spiralis* antigens recognized by human anti-*Trichinella*-specific IgG. Panel a: *T. spiralis* excretory/secretory antigens recognized by a serum sample (reference serum) from an individual infected with *T. pseudospiralis*; panel c: *T. spiralis* crude worm extract (CWE) recognized by a serum sample (reference serum) from an individual infected with *T. pseudospiralis*; panel b, proteins from *T. spiralis* ESA (lane 1) and from *T. spiralis* CWE (lane 2) recognized by specific IgG present in the serum sample from the *T. pseudospiralis* infected donor. Lane Mw: molecular weights in kDa. Red box refers to the highest differences detected on the western blot patterns.

**Table 1**
CFSE proliferation test. Flow cytometric analysis of T1-induced and T4-induced T cell lines in order to assess their *Trichinella* antigenic specificity. 5,000 events were acquired and the CFSE signal was evaluated on the gated T CD4$^+$ (T helper) and T CD8$^+$ (T cytotoxic) populations.

| T cell lines | CD3$^+$CD4$^+$ proliferating cell (%) | CD3$^+$CD8$^+$ proliferating cell (%) |
|---|---|---|
| T1 *T. spiralis* – induced cell line | 23.6 ± 1.9 | 7.4 ± 1.8 |
| T4 *T. pseudospiralis* – induced cell line | 72.8 ± 5.4 | 18.5 ± 2.1 |

evaluated by ELISpot assay for IFN-γ, IL-4 and IL-17, are detailed in Table 3.

## 4. Discussion

Parasites have developed a variable array of mechanisms to evade or modulate the host's immune response thus establishing infection [16,17]. Generally, helminth infections are frequently long lasting and can inhabit immunocompetent as well as immunocompromised hosts. Therefore, it could be deduced that these parasites might have developed the production of modulatory molecules modifying host responses and consequently promoting their own survival [18].

A predominant T helper 2 (Th2) response throughout parasitic infections has been broadly reported, even though the exact mechanism initiating this response has not been fully clarified [19]. This ability of parasites to skew immune responses towards Th2 responses and chronic infection may be as beneficial to the hosts as well as to the parasites.

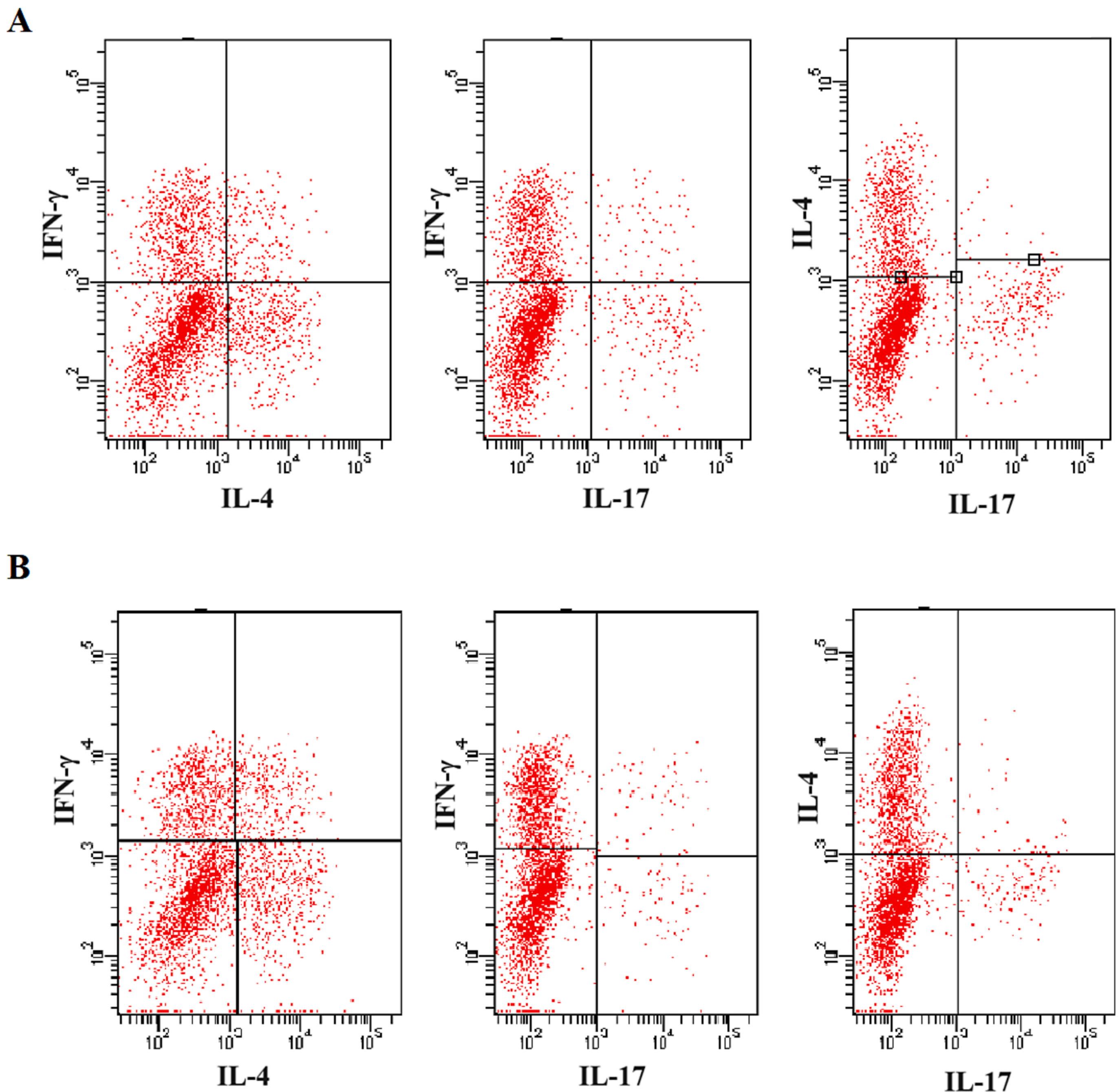


**Fig. 2.** Flow cytometric analysis of cytokine production by T1-specific and T4-specific T cell lines. Intra-cytoplasmatic staining for IFN-γ, IL-4 and IL-17 detection was performed. 5,000 events were acquired and cytokine related signal was plotted in the $CD4^+$ gate of T1-specific T cell line (A) and T4-specific T cell line (B). Data are not shown for $CD8^+$ analysis.

Prior immunological studies have demonstrated that Th2 – mediated allergies could be alleviated by parasites that induce Th1 cell responses. However, recently, it has been postulated that Th2-cell responses induced by helminths might be accompanied by inhibition of Th2-mediated inflammatory disorders through mechanisms including other non-Th1 cell subsets e.g. regulatory T cells [20]. This Type 2 immune response consist of activation of CD4+ Th2 cells, employment of eosinophils, basophils and mast cells, in addition to the Th2 cytokine profile, all of which aid in preventing excessive inflammatory reactions [21,22]. Moreover, the interaction between host and helminth-secreted products promotes the activation of dendritic cells (Dcs) which in turn induce the differentiation of naïve T-helper cells into Th2, activating regulatory T cells, B reg and alternatively activated macrophages [23].

*T. spiralis* and its secretory products can suppress inflammatory responses and induce Th2-type immune responses, as determined by the elevated Th2-associated cytokine levels observed in infected animals [24,25]. The Th1 phenotype immune response is induced during the intestinal stage and predominantly induces a Th2-type immune response during the muscle phase. The role of the Th1-type immune response contributes to parasites elimination. Th2-type immune responses may help alleviating tissue damage and strengthen tissue repair [26]. The muscle phase of the infection is further characterized by the existence of Treg cells. Treg-type immune responses play a key role in the anti-inflammatory effects of helminth infection [27,28]. IL-17 is a key cytokine for host protection against mucosal infections [29] as well as a crucial cytokine either in multiple autoimmune [30] or inflammatory

**Table 2**
Data summary of flow cytometric analysis for the cytokine production of T1-specific and T4-specific T cell lines. Percentage of T helper (CD4$^{+}$) and T cytotoxic (CD8$^{+}$) lymphocytes specific for *Trichinella* antigens in each cell line obtained. Five thousand events were acquired and IFN-γ, IL-4 and IL-17 production were estimated in the CD4$^{+}$ or CD8$^{+}$ gate of each cell line.

| T4 *Trichinella pseudospiralis*-specific TCL | | | | | | |
|---|---|---|---|---|---|---|
| | Th0 | Th2 | Th1 | Th17 | Th1/Th17 | Th2/Th17 |
| CD4$^{+}$ (82.8 %) | 10.3 % | 15.2 % | 27.1 % | 3.0 % | 1.9 % | 1.0 % |
| | **Tc0** | **Tc2** | **Tc1** | **Tc17** | **Tc1/Tc17** | **Tc2/Tc17** |
| CD8$^{+}$ (6.8 %) | 3.3 % | 0.0 % | 29.8 % | 0.3 % | 0.3 % | 0.3 % |

| **T1 *Trichinella spiralis*-specific TCL** | | | | | | |
|---|---|---|---|---|---|---|
| | **Th0** | **Th2** | **Th1** | **Th17** | **Th1/Th17** | **Th2/Th17** |
| CD4$^{+}$ (80.0 %) | 6.0 % | 12.0 % | 22.8 % | 6.6 % | 2.2 % | 0.7 % |
| | **Tc0** | **Tc2** | **Tc1** | **Tc17** | **Tc1/Tc17** | **Tc2/Tc17** |
| CD8$^{+}$ (8.2 %) | 11.7 % | 22.2 % | 8.0 % | 2.6 % | 1.1 % | 1.1 % |

**Table 3**
Cytokine profile of T4-specific and T1-specific CD4+ T cell clones. Each obtained *Trichinella*-specific T cell clone was evaluated for its phenotype by flow cytometry using external staining with anti-CD4 fluorochrome-conjugated antibodies. IFN-γ, IL-4 and IL-17 production were determined by ELISpot assay following stimulation with T4 or T1 antigen, respectively.

| T4 *Trichinella pseudospiralis*-specific T cell clones | | | | | | |
|---|---|---|---|---|---|---|
| | Th0 | Th2 | Th1 | Th17 | Th1/Th17 | Th2/Th17 |
| **CD4$^{+}$** | 0/4 | 1/4 | 1/4 | 1/4 | 1/4 | 0/4 |

| **T1 *Trichinella spiralis*-specific T cell clones** | | | | | | |
|---|---|---|---|---|---|---|
| | **Th0** | **Th2** | **Th1** | **Th17** | **Th1/Th17** | **Th2/Th17** |
| **CD4$^{+}$** | 1/15<br>6.7 % | 5/15<br>33.3 % | 7/15<br>46.7 % | 2/15<br>13.3 % | 0/15<br>0.0 % | 0/15<br>0.0 % |

diseases [31].

The IL-17 family is constituted by several moleculs (from IL-17A to IL-17F) which, by binding to specific receptors (IL-17RA to IL-17RE), express their biological functions. The most studied IL-17 family member is IL-17A and along with IL-17F, by binding to two receptors (IL-17RA or IL-17RC) promotes its biological activities [32].

This family is mainly involved in the host defence mechanisms against various infectious agents (bacteria, fungi and helminths) by stimulating the production of cytokines and chemokines, recruiting inflammatory cells such as neutrophils, inducing anti-microbial proteins and modifying T-helper cell differentiation [32].

IL-17 as well as IL-23 (which is involved in the activation of Th17 cells) resulted increased in the intestine of *T. spiralis* experimentally infected animals. It has been also shown that *in vitro* IL-17 can induce a smooth muscle hyper-contractility [33].

Furthermore, increased TGF-β production and higher numbers of Th17 cells and IL-17 levels are associated with worm expulsion and hypercontractility of the small intestinal muscle [34]. T regulatory cells and IL-17 play also a role in regulating weight loss due to intestinal infection [34].

In a recent study, the immunomodulatory effect of a 53 kDa protein secreted by *T. pseudospiralis* (Tpp53) were investigated on a Th17-related disease model using an Imiquimod (IMQ)-induced psoriasis model. As a result, it was found that the administration of recombinant Tpp53 (rTpp53) on the skin could ameliorate IMQ-induced psoriasis, as revealed by the improvement of pathological lesions of psoriasis (epidermis hyperplasia and parakeratosis, acanthosis, epidermal extension, and inflammatory infiltration), and the inhibited expression of IL-23/IL-17 axis related cytokines and chemokines in psoriasis skins. [35].

Little is known about the modulation of the Th17 response by *Trichinella*, and few *Trichinella*-derived molecules have been identified to be responsible for immunomodulation. IL-17 is a critical cytokine in the pathogenesis of psoriasis. In skin psoriasis, Th17-cell-produced IL-17A and IL-17F mainly act on keratinocytes to induce the production of various inflammatory mediators and facilitate the abnormal proliferation of keratinocytes DCs also produce the cytokines IL-6, IL-1β and TNF-α to further enhance Th17-cell differentiation from CD4+ cells, which produce high levels of IL-17A, IL-17F, IL-22 and TNF-α. The result of this study provide new insights into parasitic immunomodulation through the Th17 signalling pathway [35].

Current knowledge indicates that, after a transitory initial Th1 response, mice immune system raises and maintains a Th2 polarization lasting through time [4]. The lack of information about the characterization of the T cells involved in immune response to human trichinellosis has encouraged our studies.

Following our results which, demonstrated in humans a mixed Th1/Th2 response of T cell clones specific for *Trichinella britovi* in five infected patients, among which none resulted capable to produce IL17 [7].

The present study aimed to confirm a mixed Th1/Th2 response in human trichinellosis and to further investigate the possible presence of IL-17 producing cells.

More recently, it was observed that both CXCL10 and CCL2 which can be considered markers of Th1 and Th2 polarization, respectively, are increased in the sera of trichinellosis patients, confirming the mixed reaction [36].

The present study, characterizing the immune reponse in chronic phase of human trichinellosis, revealed IL-17 production from *T. spiralis* and *T. pseudospiralis* specific T cell lines and T cell clones obtained from the peripheral blood sample of the patient. The limit of our study is represented by the fact that it was not possible to study other patients, however the isolation of Th17 cell clones show the possibility of occurrence of such response.

In the 2017 study, no IL-17 was produced by *T. britovi*-specific T cell clones and the lack of IL-17 production was related to the time of blood collection probably because no IL-17 production can be detected in early phases of infection. The present investigation focused on T1-specific and T4-specific T cell clones, assessed by the ELISpot highly sensitive methodology to detect antigen-specific responses, revealed the production of IL-17.

Our results showed the appearance of multifunctional CD4+ and CD8+ cells expressing two cytokines (Th1/Th17 and Th2/Th17, Tc1/Tc 17 and Tc2/Tc17 respectively) in *T. pseudospiralis* and *T. spiralis* specific T cell lines. These cells capable of simultaneously producing more than one cytokine have been described and associated with a protective immune response in the context of infectious diseases [37].

For the first time in human trichinellosis immune response depiction, a IL-17 production was detectable in T limphocytes isolated from peripheral blood of an infected patient.

CFSE test to T1 and T4 antigens detected a significantly higher proliferation to *T. pseudospiralis* compared to *T. spiralis*. Considering a sequence homology between the two species, we can assert that the patient infecting parasite was *T. pseudospiralis*.

Our result is in agreement with those obtained by Gomez-Morales et al., [8] which showed serologically a typical *T. pseudospiralis* pattern of antigens recognised by sera of the patients involved in the Genoa outbreak.

The analysis of cellular immune response versus specific antigens can be considered a valid alternative to obtain species identification to muscle biopsy, a sort of *liquid biopsy*, a procedure well known in clinical oncology [38,39].

Thus, ELI Spot and FACS analysis could be considered as novel tool for trichinellosis screening to support diagnosis.

The fact that in chronic infection it is possible to isolate T cell clones producing IL-17 might help in understanding the mechanisms underlying autoimmune reactions which can be observed in human trichinellosis [40,41]. In this latter study a progressive increase of anti-GSTO1-1 antibodies was detectable from 4 weeks to 16 weeks after the infection. It is now well clear that IL17 has a relevant role in the determination of autoimmune responses [30,42].

Now the search of the molecules present in E/S of *Trichinella* muscle larvae, capable to induce Th17 production is the next step of research.

## Ethics statement

The present work did not involve experiments on humans or animals. The privacy of the patient was protected by ensuring anonymity and confidentiality in both data management and reporting. No identifying information is contained in the manuscript. The subject participating to the study gave informed consent. The study was conducted along to the guidelines of Helsinki Declaration.

## CRediT authorship contribution statement

**Chiara Della Bella:** Methodology, Investigation. **Chiara Medici:** Methodology, Investigation. **Sofia D'Elios:** Writing – review & editing. **Marisa Benagiano:** Methodology. **Alessandra Ludovisi:** Methodology. **Maria Angeles Gomez Morales:** Writing – review & editing, Writing – original draft, Supervision. **Mario M. D'Elios:** Writing – review & editing, Writing – original draft, Conceptualization. **Fabrizio Bruschi:** Writing – review & editing, Writing – original draft, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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