Nonencapsulated *Trichinella pseudospiralis* Infection Impairs Follicular Helper T Cell Differentiation with Subclass-Selective Decreases in Antibody Responses

Kazunobu Asano,^a Zhiliang Wu,^a Piyarat Srinontong,^a Takahide Ikeda,^{a,b} Isao Nagano,^a Hirokuyi Morita,^b Yoichi Maekawa^{a,c}

Department of Parasitology and Infectious Diseases, Gifu University Graduate School of Medicine, Gifu, Japan^a; Department of General Internal Medicine, Gifu University Graduate School of Medicine, Gifu, Japan^b; Domain of Integrated Life Systems, Gifu Center for Highly Advanced Integration of Nanosciences and Life Sciences (G-CHAIN), Gifu University, Gifu, Japan^c

Infectious microorganisms often modify host immunity to escape from immune elimination. *Trichinella* is a unique nematode of the helminth family, whose members parasitize the muscle cells inside the host without robust eliminative reactions. There are several species of *Trichinella*; some develop in muscle cells that become encapsulated (e.g., *Trichinella spiralis*) and others in cells that do not encapsulate (e.g., *Trichinella pseudospiralis*). It has already been established that *Trichinella* infection affects host immune responses in several experimental immune diseases in animal models; however, most of those studies were done using *T. spiralis* infection. As host immune responses to *T. spiralis* and *T. pseudospiralis* infections have been reported to be different, it is necessary to clarify how *T. pseudospiralis* infection influences the host immune responses. In this study, we investigated the influence on host humoral immunity in *T. pseudospiralis*-infected mice. We demonstrated that *T. pseudospiralis* infection decreased antigen-specific IgG2a and IgG2b antibody (Ab) production in mice immunized with a model antigen. This selective decrease in gamma interferon (IFN- γ)-dependent Ab production was not due to a decrease in IFN- γ production, and we instead found impaired follicular helper T (T_{fh}) cell differentiation. The affinity maturation of antigen-specific Ab tended to be delayed but was not significant in *T. pseudospiralis*-infected mice. We also observed that CD11b⁺ spleen cells in *T. pseudospiralis*-infected mice expressed CD206 and PD-L2, the phenotype of which was M2 macrophages with weak production of interleukin-6 (IL-6), possibly resulting in impaired T_{fh} differentiation. Taken together, our results indicate that nonencapsulated *Trichinella* infection induces selective dampening in humoral immunity with the suppression of T_{fh} differentiation.

Trichinella species are parasitic nematodes for many mammalian hosts, including humans (1). After the ingestion of muscle tissues contaminated with the muscle larval form of *Trichinella*, the larvae develop into adult worms by molting in the small intestine of the host. Female adult worms invade the intestinal tissue to release newborn larvae. Newborn larvae systemically migrate in the host body to striated muscle cells, resulting in survival for many years (2). *Trichinella* are thought to establish long-term parasitism by escaping elimination through alterations in host immune responses (3, 4). There are several species of *Trichinella*; some develop in muscle cells that become encapsulated (e.g., *Trichinella spiralis* and *T. britovi*) and others in cells that do not encapsulate (e.g., *Trichinella pseudospiralis*). Although both types of *Trichinella* survive in the host muscle tissues for long periods of time, the strategies for their survival may be different. Indeed, the infected host mounts an immune response to the infection with both types of *Trichinella*, but each immune response is different, possibly due to the different forms of immunomodulation shown by each *Trichinella* type (3–5). For example, stronger inflammatory responses are evoked in *T. spiralis* infection than in *T. pseudospiralis* infection, while nonencapsulated *T. pseudospiralis* infection induces more interleukin-10 (IL-10) production than *T. spiralis* infection (4, 5).

Several studies support the idea of *Trichinella* infection affecting the host immune responses to immunized antigens (Ag) or self-antigens in immunological disorder models. Saunders et al. reported that *Trichinella* ameliorated type-1 diabetes in an animal model (6). Zhao et al. demonstrated the modulation of inflammatory bowel disease in a mouse model following *Trichinella* infec-

tion (7). Moreover, Aranzamendi et al. reported that *Trichinella* infection protected the host against allergic airway inflammation (8). It is obvious that *Trichinella* infection affects the host immune responses, but most studies regarding immunomodulatory actions by *Trichinella* were done using *T. spiralis*, and studies of immunomodulation by *T. pseudospiralis* are available in limited numbers only (9). As the immunomodulatory activity of *T. pseudospiralis* may be different from that of *T. spiralis* as mentioned above, it is necessary to make clear the mechanisms underlying the immunomodulation by *T. pseudospiralis*. In particular, the influence of *T. pseudospiralis* infection on humoral immunity is unknown, even though there are several reports regarding *T. spiralis* infection modifying Ag-specific antibody (Ab) production (8, 10–12).

Humoral immunity is controlled by effector CD4⁺ T cells. Upon Ag stimulation, CD4⁺ T cells differentiate into one of several different effector cells such as Th1 and Th2. In addition to Th1

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Address correspondence to Yoichi Maekawa, ymaekawa@gifu-u.ac.jp.

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and Th2, follicular helper T (Tfh) cells are known to preferentially control humoral immunity phenotypes, including germinal center formation, class switch recombination, and the affinity maturation of immunoglobulin (13). Tfh cells secrete IL-4 and IL-21 to control their functions. IL-6 signaling, IL-21 signaling, and inducible costimulator (ICOS) signaling are critical mediators for Tfh cell differentiation. Several studies have indicated that *Trichinella* infection affects Th1 as well as Th2 responses (14, 15), whereas the influence of trichinellosis on Tfh responses has not yet been assessed.

In the present study, we demonstrated alterations in Tfh cell differentiation in *T. pseudospiralis*-infected mice by evaluating the change in antibody production. Tfh cell differentiation was impaired with the induction of M2 macrophages by nonencapsulated *Trichinella* infection in mice.

MATERIALS AND METHODS

Mice. Female BALB/c and C57BL/6 mice (6 to 8 weeks old) were purchased from Japan SLC (Hamamatsu, Japan), and ovalbumin (OVA)-specific I-A^b-restricted T cell receptor (TCR), OT-II transgenic mice (C57BL/6 background, Thy1.1[−] Thy1.2⁺) were from Taconic Associates LLC (Hudson, NY). Thy1.1 mice on the C57BL/6 background were provided by Y. Itoh (Shiga Medical University). We used BALB/c mice as the host animal for most experiments. In the OT-II T-cell transfer experiment and Ab affinity maturation experiment, we used the C57BL/6 mouse strain. All mice were maintained at the Animal Resources Center of Gifu University Graduate School of Medicine under pathogen-free conditions in individual ventilated cages and were fed sterile food and water. All animal care and experimental procedures were approved by the Committee for Animal Research and Welfare of Gifu University.

Infection with muscle larvae. BALB/c as well as C57BL/6 mice were used to harvest muscle larvae. We used BALB/c-derived parasites for infection of the BALB/c strain, while C57BL/6-derived parasites were used for infection of the C57BL/6 strain. The mature muscle larvae were recovered from mice orally infected with *T. pseudospiralis* (ISS13) at 48 days or later after infection performed by the conventional pepsin digestion method (16). The concentration of larvae in the solution was assessed. Mice were orally infected with 200 *T. pseudospiralis* larvae using a feeding needle.

Immunization. Mice were intraperitoneally immunized with 1 µg ovalbumin (OVA; Sigma-Aldrich, St. Louis, MO) absorbed in 200 µl aluminum hydroxide (alum) (InVivoGen, San Diego, CA). A second immunization was performed in the same manner as the primary immunization 10 days later. In the NP-KLH immunization, 10 µg of NP₁₄-KLH (Biosearch Technologies Inc., Novato, CA) was absorbed in alum and inoculated into mice intraperitoneally. In some experiments, naive OT-II TCR T cells (Thy1.1[−] Thy1.2⁺) were purified from spleen cells of OT-II TCR Tg mice using a naive CD4⁺ T cell purification kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and 1 × 10⁶ purified OT-II T cells were adaptively transferred into Thy1.1 mice previously infected with *T. pseudospiralis* 10 days before.

Enzyme-linked immunosorbent assay (ELISA). In order to measure the amounts of antigen-specific antibodies in serum, 96-well microtiter plates were coated with OVA in coating buffer. After blocking was performed with blocking buffer (phosphate-buffered saline [PBS]–1% bovine serum albumin [BSA]), serially diluted sera were applied to the plate. Two hours after incubation at room temperature, the plate was washed and biotinylated anti-mouse IgG1, IgG2a, and IgE and horseradish peroxidase (HRP)-conjugated anti-mouse IgG2b were added to the plate. All Abs detecting immunoglobulin were purchased from Invitrogen (Carlsbad, CA). Incubation with biotinylated Abs was followed by incubation with HRP-conjugated streptavidin (Invitrogen). After several washes of the plate, the remaining HRP was detected by adding TMB (3,3',5,5'-tetramethylbenzidine) (Sigma-Aldrich) as a substrate. After the reaction

had been terminated by addition of 2 M sulfuric acid, optimal density (OD) was measured with a Multiskan JX plate reader (LabSystems, Helsinki, Finland) set to 450 nm. In order to detect cytokines in the supernatant, all antibodies used were purchased from BioLegend (San Diego, CA). Ninety-six-well microtiter plates were coated with purified anti-mouse gamma interferon (IFN-γ) (XMG1.2) or IL-4 (11B11). After blocking, culture supernatants were subjected to several dilutions and incubated at room temperature for 2 h. Plates were then washed, and biotinylated Abs detecting IFN-γ (R4-6A2) or IL-4 (BVD6-24G2) were added. The detecting Abs were visualized by incubation with HRP-conjugated streptavidin and TMB. The detection of NP-specific IgG1 was performed as described previously (17). Briefly, sera were collected 20 and 30 days after the NP₁₄-KLH immunization. NP₄-BSA (high affinity) or NP₂₆-BSA (total) (Biosearch Technologies) was coated on 96-well enzyme immunoassay (EIA)/radioimmunoassay (RIA) plates (Corning Inc., NY) with 50 mM carbonate/bicarbonate buffer, followed by blocking with 0.1% BSA–phosphate-buffered saline with Tween 20 (PBS-T). After blocking was performed, serially diluted serum samples were applied. The remaining Abs to NP were measured by the use of biotinylated anti-mouse IgG1 followed by HRP-conjugated streptavidin.

RNA isolation and quantitative real-time PCR. Total RNA for the quantitative measurement of gene expression was isolated from whole spleen cells with TRIzol reagent (TaKaRa Bio Inc., Shiga, Japan) according to the manufacturer's instructions. Reverse transcription (RT) was performed using Prime reverse transcriptase (TaKaRa) according to the manufacturer's instructions. Quantitative real-time RT-PCR was performed with a Thermal Cycler Dice Real Time system (TaKaRa). Primers were designed based on the published sequences in GenBank, including the IL-6 gene (*IL6*) (NM_031168.2 [TTCCATCCAGTTGCCTTCTTG/CATTTCCACGATTTCCCAGAG]) and the GAPDH gene (*Gapdh*) (glyceraldehyde-3-phosphate dehydrogenase) gene (NM_001289726.1 [GGCATTGTGGAAGGGCTCAT/GACACATTGGGGGTAGGAACAC]). Real-time RT-PCR was run using a SYBR Premix Ex Taq kit (TaKaRa). Specific external controls were constructed for target genes. A 10-fold serial dilution (10¹ to 10⁷ copies/2 µl) of each standard DNA was used to generate standard curves for each gene. Expression levels are represented as the copy number of the target gene/10⁶ copies of the housekeeping gene (*Gapdh*).

Flow cytometry. All antibodies for cell surface staining were used in a 1:200 dilution. Fluorochrome-conjugated monoclonal antibodies specific to mouse Thy1.1 (OX-7), Thy1.2 (30-H12), PD-1 (29F.1A12), CXCR5 (L138D7), CD4 (GK1.5), CD11b (M1/70), CD206 (C068C2), and PD-L2 (TY25) were purchased from BioLegend. All samples were resuspended in PBS-based staining buffer containing 2% fetal calf serum (FCS) and 0.01% NaN₃ (Sigma-Aldrich), preincubated at 4°C for 15 min with 2.4G2 supernatants, and then washed and stained with specific monoclonal Abs (MAbs) at 4°C for 20 min. Data were collected on a FACSCanto II instrument (BD Biosciences, Franklin Lakes, NJ) and analyzed using FlowJo (Tree Star, OR) software.

Statistical analysis. In all experiments, the significance of differences between groups was calculated using the Student *t* test with Excel software for unpaired data. Differences were considered significant when the *P* value was <0.05. No test of normality or power analysis was conducted, and no animals were excluded from the experiments.

RESULTS

***T. pseudospiralis* infection affected antigen-specific antibody responses.** Although it has already been established that *Trichinella* infection in experimental animal models affects several immune responses, including type 1 diabetes, inflammatory bowel disease, and asthma, most of those studies were performed by using *T. spiralis*. The changes induced in the host immune responses, including humoral immune responses induced by *T. pseudospiralis* infection, are currently unclear. In order to address this issue, we immunized *T. pseudospiralis*-infected mice with a model antigen and

FIG 1 *T. pseudospiralis* infection decreased OVA-specific IgG2a and IgG2b production. *T. pseudospiralis*-infected (Tp+OVA, $n = 5$) or uninfected (OVA, $n = 5$) BALB/c mice were intraperitoneally immunized twice with ovalbumin (OVA), 10 and 20 days after being infected. Ten days (d) after the second immunization, OVA-specific IgG1 (A), IgE (B), IgG2a (C), and IgG2b (D) levels were measured by ELISA. UI, unimmunized naive mice group. The data are representative of results of at least 3 independent experiments. *, $P < 0.05$.

examined its influence on antigen-specific antibody production. BALB/c mice were orally infected with 200 muscle larvae of *T. pseudospiralis*. Ten days after infection, infected as well as uninfected mice were intraperitoneally immunized with OVA absorbed in alum. A second immunization was given 10 days later. Ten days after the second immunization, mice were sacrificed and serum samples were collected in order to assess OVA-specific antibody responses with ELISA (Fig. 1). No significant changes were observed in OVA-specific IgG1 or IgE levels resulting from *T. pseudospiralis* infection (Fig. 1A and B). On the other hand, we found that levels of OVA-specific IgG2a and IgG2b were significantly decreased in *T. pseudospiralis*-infected mice (Fig. 1C and D). These results suggest that *T. pseudospiralis* infection does not suppress whole-antibody responses.

We then investigated whether *T. pseudospiralis* infection modulates the differentiation of effector CD4⁺ T cells. Upon antigen stimulation, CD4⁺ T cells differentiate into functional subtypes such as Th1 and Th2 cells. Th1 and Th2 cells secrete cytokines, including gamma interferon (IFN- γ) and interleukin-4 (IL-4), respectively. These secreted cytokines regulate the class switch recombination of immunoglobulins. Class switching to IgG2a and IgG2b is controlled by IFN- γ , whereas IL-4 produced by Th2 cells induces IgG1 and IgE class switching. In order to examine whether antigen-specific cytokine production was affected by *T. pseudospiralis* infection, we isolated spleen cells from OVA-immunized control and *T. pseudospiralis*-infected BALB/c mice and stimulated them with OVA *in vitro*. Three days after the stimulation, we measured levels of IFN- γ as well as IL-4 in the culture supernatants (Fig. 2). IFN- γ and IL-4 were produced in both groups but not at significantly different amounts. Taken together, these results indicate that the decrease in levels of OVA-specific IgG2a and

FIG 2 Levels of production of OVA-specific IFN- γ and IL-4 from spleen cells from *T. pseudospiralis*-infected and control OVA-immunized mice were similar. *T. pseudospiralis*-infected or uninfected BALB/c mice ($n = 5$ each) were intraperitoneally immunized twice with ovalbumin (OVA) 10 and 20 days after being infected. Ten days after the second immunization, spleen cells were stimulated with OVA at 100 μ g/ml for 3 days. After the culture experiment, levels of IFN- γ (A) and IL-4 (B) in culture supernatants were measured with ELISA. Open columns, naive mice; filled columns, OVA-immunized mice; hatched columns, *T. pseudospiralis*-infected OVA-immunized mice. The data are representative of results of at least 3 independent experiments.

IgG2b observed in *T. pseudospiralis*-infected mice did not appear to be due to a decrease in the production of IFN- γ by Th1 cells.

***T. pseudospiralis* infection suppressed the differentiation of CD4⁺ T cells to follicular helper T cells.** In order to elucidate the mechanism by which *T. pseudospiralis* infection decreases antigen-specific IgG2a and IgG2b production, we focused on follicular helper T (Tfh) cells, a subset of CD4 helper T cells. Tfh cells are known to regulate antigen-specific humoral immunity. Ten days after *T. pseudospiralis* infection, Thy1.1⁺ Thy1.2⁻ C57BL/6 mice were adaptively transferred with purified naive OVA-specific TCR transgenic OT-II T cells (Thy1.1⁻ Thy1.2⁺) and immunized intraperitoneally with OVA/alum (Fig. 3A). Ten days after the immunization, mice were sacrificed and spleen cells were analyzed for the differentiation of transferred OT-II cells to Tfh by flow cytometry (Fig. 3B and C). We identified Tfh cells as cells expressing PD-1 and the chemokine receptor CXCR5 in the CD4⁺ Thy1.1⁻ Thy1.2⁺ population (OT-II population). We found that approximately 5% of OT-II cells were of the Tfh phenotype in control mice, whereas few Tfh cells were detected in *T. pseudospiralis*-

FIG 3 Impaired differentiation of follicular helper T cells in *T. pseudospiralis*-infected mice. One million purified naive OT-II TCR T cells (Thy1.1⁺ Thy1.2⁺) were adaptively transferred into control uninfected C57BL/6 (Thy1.1⁺ Thy1.2⁺) (n=3) mice or C57BL/6 (Thy1.1⁺ Thy1.2⁺) mice that had been infected with *T. pseudospiralis* 10 days before (*n* = 3). Simultaneously with OT-II T cell transfer, mice were intraperitoneally immunized with OVA as described for Fig. 1. (A) Purity data. (B) Ten days after the immunization, mice were sacrificed and spleen cells were analyzed for the differentiation of transferred OT-II Tfh cells by flow cytometry for expression of PD-1 and CXCR5 in the CD4⁺ Thy1.1⁺ Thy1.2⁺ population. (C) Frequencies of Tfh cells in OT-II cells. Open columns, uninfected; filled columns, *T. pseudospiralis*-infected. *, *P* < 0.05. (D) *T. pseudospiralis*-infected or uninfected C57BL/6 mice (*n* = 5 each) were immunized intraperitoneally with NP₄-KLH absorbed in alum. At 20 days and 30 days after the immunization, NP-specific IgG1 levels in serum were measured using ELISA against NP₄ (high affinity) as well as NP₂₆ (total). The affinity maturation of NP-specific IgG1 is shown as NP₄/NP₂₆ ratios. The higher value represents the maturation of Ab affinity. Open column, uninfected mice; filled column, *T. pseudospiralis*-infected mice. The data are representative of results of at least 3 independent experiments.

ralis-infected mice. We then examined the affinity maturation of antigen-specific immunoglobulin because Tfh cells control affinity maturation in the germinal center. Ten days after the *T. pseudospiralis* infection, C57BL/6 mice were immunized intraperitoneally with NP₁₄-KLH-alum. We then evaluated the affinity maturation of NP-specific IgG1 antibodies by measuring the ratio of high-affinity IgG1 (against NP₄) to total IgG1 (against NP₂₆) with ELISA 20 and 30 days after the immunization (Fig. 3D). The ratio of NP₄/NP₂₆ tended to be lower (but not to a statistically significant degree) in *T. pseudospiralis*-infected mice than in control mice 20 days after the immunization, whereas the ratios were not significantly different between the two groups 30 days after the immunization.

Macrophages in *T. pseudospiralis*-infected mice were skewed to the M2 type with insufficient IL-6 to induce Tfh differentiation. We then examined the mechanism underlying the impaired differentiation of Tfh cells in *T. pseudospiralis*-infected mice. As IL-6 and IL-21 are crucial cytokines in Tfh differentiation, we examined IL-6 expression levels in spleen cells in *T. pseudospiralis*-infected BALB/c mice (Fig. 4B). Expression of IL-6 in spleen cells was increased by the immunization with OVA, whereas *T. pseudospiralis* infection suppressed these immuniza-

tion-induced increases. IL-6 is strongly produced by activated macrophages; therefore, we examined the activation status of macrophages in *T. pseudospiralis*-infected BALB/c mice by analyzing the expression of CD206 and PD-L2 with a flow cytometer (Fig. 4B). It has already been established that there are two types of activated macrophages, M1 and M2 macrophages. Upon exposure to lipopolysaccharide (LPS) and IFN- γ , monocytes differentiate into M1 macrophages that produce IL-6 and TNF- α and are involved in inflammatory responses. On the other hand, M2 macrophages induced by IL-4 produce small amounts of IL-6. M2 macrophages are characterized by stronger expression of CD206 and PD-L2 than M1 macrophages. CD11b⁺ cells in the spleen exhibited stronger expression of CD206 and PD-L2 than thioglycolate-induced M1 macrophages, indicating that *T. pseudospiralis* infection induced monocytes to differentiate into M2 macrophages without the production of IL-6.

DISCUSSION

In the present study, we demonstrated that Tfh differentiation was impaired in *T. pseudospiralis*-infected mice and is accompanied by decreased production of antigen-specific IgG2a and G2b. As IL-6 is a critical cytokine for the differentiation of Tfh, the weak pro-

FIG 4 *T. pseudospiralis* infection induced M2-like macrophage activation with weak IL-6 production. (A) Ten days after the immunization of *T. pseudospiralis*-infected BALB/c mice ($n = 3$), IL-6 expression in spleen cells was measured by quantitative RT-PCR. *, $P < 0.05$. (B) The expression of CD206 and PD-L2 in CD11b⁺ cells in the spleen was tested by flow cytometry. Thioglycolate-induced peritoneal (*ip*) CD11b⁺ cells were used as M1 macrophages. The data are representative of results of at least 3 independent experiments.

duction of IL-6 in spleen cells may not be sufficient to complete Tfh differentiation in *T. pseudospiralis*-infected mice. IL-6 is known as a proinflammatory cytokine that is produced by macrophages, in particular, M1 macrophages. Our results showed that CD11b⁺ cells in the spleens of *T. pseudospiralis*-infected mice preferentially expressed CD206 and PD-L2 in contrast to thioglycolate-induced M1 macrophages, indicating that macrophage activation was skewed to M2 in *T. pseudospiralis*-infected mice. Therefore, we speculate that *T. pseudospiralis* infection selectively reduced levels of antigen-specific IgG2a and IgG2b without a reciprocal increase in IgG1 or IgE levels by modulating Tfh differentiation through M2 macrophage induction.

Helminth infection, including trichinellosis, modifies host immunity in several manners such as Th2 deviation, regulatory T cell activation, and regulatory B cell induction (11, 18, 19). Helminth infection generally induces strong Th2 responses and polyclonal IgE production. However, in the present study, we did not observe a strong Th2 response to immunized antigens in *T. pseudospiralis*-infected mice. Tfh differentiation was instead affected by the *T. pseudospiralis* infection. As Tfh differentiation is strongly dependent on IL-6, decreases in IL-6 production in the spleen as a priming site for CD4⁺ T cells is a reasonable outcome for impaired Tfh differentiation in *T. pseudospiralis*-infected mice. The mechanism by which the production of IgG2a and IgG2b was selectively decreased in *T. pseudospiralis*-infected mice has not yet been elucidated. In the *T. spiralis*-infected model, Boitelle et al. reported, similarly to our findings, that immunized Ag-specific IgG2a levels were markedly decreased but that the decrease of IgG1 levels was moderate compared with the results seen with control uninfected mice. They also showed that decreases in levels of IFN- γ and increases in levels of IL-5 and IL-13 from immunized Ag-stimulated peripheral lymph node cells were seen in *T. spiralis*-infected mice, and this cytokine profile was different from our findings. This

difference in levels of cytokine production may be dependent on the properties of the parasites used and/or the timing of immunization after infection. We immunized infected mice 10 days after infection, and they immunized infected mice only 1 day after infection. Otherwise, impaired Tfh differentiation may be unique to *T. pseudospiralis* infection and may not occur in *T. spiralis* infection. Class switching to IgG2a and IgG2b is dependent on the presence of IFN- γ , which is known as a Th1 cytokine. However, IFN- γ is secreted not only from Th1 cells but also from Tfh cells (20, 21). In the class switch recombination of immunoglobulin, T cells producing cytokines involved in these processes must be close to B cells in germinal centers (GCs). As Tfh cells are the only effector CD4⁺ T cells that are capable of migrating into GCs, the generation of IFN- γ -secreting Tfh cells may have been selectively impaired in *T. pseudospiralis*-infected mice, resulting in a decrease in IFN- γ -dependent Ab production. IFN- γ production in the Ag-stimulated spleen cells of *T. pseudospiralis*-infected mice was similar to that of wild-type (WT) mice; therefore, most IFN- γ -secreting cells may be Th1 but not IFN- γ -secreting Tfh. We need to investigate these possibilities in more detail in future studies.

Similarly to helper T cells, macrophages also differentiate into at least two distinct effectors, including classically (M1) and alternatively (M2) activated macrophages (22, 23). Several studies have indicated that excretory-secretory (ES) products, particularly the 53-kDa protein TsP53 from *T. spiralis*, attenuate experimental colitis and the LPS-induced damage from endotoxemia in mice and also induce macrophage activation to M2 (24–26). TsP53-induced M2 macrophage activation is reported to be STAT6 dependent but not IL-4R α independent. *T. pseudospiralis* also produces a counterpart of TsP53 in *T. spiralis* (27), and this counterpart protein may play a similar role in modulating macrophage activation into M2, resulting in the weak production of IL-6, as shown in this study. We are now investigating this issue by

using a recombinant 53-kDa ES protein from *T. pseudospiralis*. Nevertheless, ES products and a particular component of ES products are reported to be involved in the modification of innate immune responses as well as of Th1/Th2 paradigm-related adoptive immune responses (28).

Do Tfh cell-related immune responses affect the parasitism of *Trichinella* in the host? Are Tfh cells harmful to *Trichinella*? A previous study reported a role for Tfh cells in a *Schistosoma japonicum* infection model (29). That study found that when ICOS-deficient mice with impaired Tfh differentiation were infected with *S. japonicum*, amelioration of liver pathology was observed, and also that the adoptive transfer of Tfh cells into ICOS-deficient mice reestablished the liver pathology with enhanced cell accumulation in granulomas, indicating that Tfh cells are involved in the pathology of helminth infection. As a robust pathological reaction may not be induced even when *Trichinella* parasites reside in host tissues, *Trichinella* infection may suppress Tfh responses to the parasite itself. Further investigations are needed in order to answer these questions.

Trichinella infection is still an active form of infection, particularly in eastern European and Russian areas. Therefore, elucidating the precise mechanisms underlying the pathogenesis and immunomodulation of trichinellosis is important for controlling the infection. On the other hand, modulatory effects by *Trichinella* on host immunity have the potential to be applied to immune-related diseases such as autoimmunity or allergies. Our results provide an insight into the treatment of inflammatory disorders. Intensive studies that clarify the underlying mechanisms are needed for the development of therapies.

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K.A. and Y.M. designed all experiments, and K.A. performed all experiments. Z.W. assisted with all experiments, and P.S. performed cell-transfer experiments. T.I. and H.M. assisted with the flow cytometry experiments, and I.N. prepared parasites. K.A. and Y.M. wrote the manuscript.

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