Parasitol Res (2013) 112:3041–3051
DOI 10.1007/s00436-013-3488-y

ORIGINAL PAPER

Preventive effect of pidotimod on reactivated toxoplasmosis in mice

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Received: 20 April 2013 / Accepted: 22 May 2013 / Published online: 18 June 2013

Abstract As one of food-borne parasitic diseases, toxoplasmosis entails the risk of developing reactivation in immunocompromised patients. The synthetic dipeptide pidotimod is a potent immunostimulating agent that improves the immunodefenses in immunodepression. To investigate the efficacy of pidotimod as a preventive treatment, we used a murine model of reactivated toxoplasmosis with cyclophosphamide (CY)-induced immunosuppression. Pidotimod administration significantly restored the body weight and spleen organ index, increased survival time (from 70 to 90 %), and decreased the parasitemia (from 80 to 35 %) of CY-induced mice with reactivated toxoplasmosis. Cytokine profiles and $CD4^+$ T cells subpopulation analyses by Cytometric Bead Array and flow cytometry demonstrated that pidotimod treatment resulted in a significant upregulation of pro-inflammatory cytokines (IFN-γ, TNF-α, and IL-2) and Th1 cells (from 3.73±0.39 to 5.88±0.46 %) after CY induction in infected mice. Additionally, histological findings and parasite DNA quantification revealed that mice administered with pidotimod had a remarkable reduction of parasite burden (two-log) and amelioration of histopathology in the brains. The in vitro studies showed that pidotimod significantly restored concanavalin A-induced splenocyte proliferation and pro-inflammatory cytokines in the supernatants of splenocyte culture. It could be concluded that the administration of pidotimod in immunocompromised mice significantly increases the Th1-biased immune response, prolongs survival time, and ameliorates the load of parasites in the blood. This is the first report of the preventive effect of pidotimod on reactivated toxoplasmosis.

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Introduction

Toxoplasma gondii is an obligatory intracellular parasite with global distribution. Human beings can be infected with *T. gondii* by ingestion or handling of food or water that is contaminated with oocysts shed by cats or by eating undercooked or raw meat containing tissue cysts. Primary infection is usually subclinical, but in some patients, recrudescence of tissue cysts can be present, such as cancer chemotherapy, HIV/AIDS, or organ transplant

recipients (Gharavi et al. 2011; Israelski and Remington 1993; Xavier et al. 2013). In these processes, toxoplasmosis almost always happens as a result of the reactivation of latent infection.

Currently used therapy for acute toxoplasmosis in humans includes sulfa–pyri combination; however, these drugs can have serious side effects (Katlama et al. 1996; Meneceur et al. 2008). The aqueous extracts of *Astragalus membranaceus* and *Scutellaria baicalensis* Georgi proved to be the effective adjuvant herbal agents in treating acute toxoplasmosis (Yang et al. 2012). So far, no approach for reactivated toxoplasmosis of latent infection in both animals and humans has been reported. The synthetic dipeptide pidotimod (3-L-pyroglutamyl-L-thiaziolidine-4-carboxylic acid) is a potent immunostimulating agent both in vitro and in vivo (Chiarenza et al. 1994). In particular, the administration of pidotimod results in a significant augmentation of immune response and counteraction to glucocorticoid-induced thymocyte apoptosis (Giagulli et al. 2009; Migliorati et al. 1992). However, little is known about the general mechanism of action of this synthetic compound in reactivated toxoplasmosis. Herewith, we used a murine model of reactivated toxoplasmosis induced by cyclophosphamide (CY) and investigated the efficacy of pidotimod as an oral preventive agent.

Materials and methods

Experimental animals

A total of 350 female, 8- to 12-week-old specific pathogen-free BALB/c mice were purchased from the animal facilities of Vital River, Beijing, China. They were acclimatized for at least 7 days before the experiments and all appeared normal and healthy on examination. The protocol was approved by the Institutional Review Board of the Institute of Biomedicine at Anhui Medical University (Permit Number AMU26-100311).

Parasites and infections

BALB/c mice were orally infected with 200 μl of brain homogenate containing 20 parasite cysts (PRU strain, genotype II) to set up latent infection. Loss of body weight was monitored and expressed as the percentage of baseline starting weight. Mice were monitored daily for survival.

Study design

Seven experimental groups (50 mice in each) were investigated. Among them, 20 mice in each group were set aside for survival monitoring. CY (Endoxan, Baxter Oncology GmbH, Halle, Germany) was given intraperitoneally every second day to induce immunosuppression starting at day 0 (Fig. 1). The experimental groups were as follows: control group; group A: noninfected mice with normal saline (NS, 0.9 % saline solution) only; group B: noninfected mice with CY (100 mg/kg body weight) and NS; group C: noninfected mice with pidotimod (Wanshining, Shandong, China) at a dose of 400 mg/kg body weight and injected intraperitoneally with CY as previously described (Du et al. 2008; Frawley et al. 2011); group D: infected mice with NS; group E: infected mice with CY and NS; and group F: infected mice with pidotimod and CY (Table 1). All mice were given amoxicillin and clavulanic acid (Youlinjia, Hubei, China) at 1.0 g/L drinking water throughout the experiment in order to prevent unwanted bacterial infection (Sumyuen et al. 1996). Animals were sacrificed under anesthesia after various interval days of CY administration. Body weight loss and the relative organ index of the spleen (the ratio of organ weight to body weight, in milligrams per gram) were calculated for each animal.

Parasitemia and parasite burden

For parasitemia determination, 200 μl of blood was collected following strict decontamination procedures. DNA templates were extracted with a DNA extraction kit (QIAGEN GmbH, Hilden, Germany) following the instructions of the manufacturer. To measure the amount of parasite burden in the brain, 50 mg of tissue was digested and DNA was extracted and determined with UV spectrophotometry based on the OD ratio at 260 and 280 nm (the absorbance ratio was 1.8–2.0). An ABI prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) was used for 40 cycles of polymerase chain reaction (PCR). The cycle threshold values (Ct) indicative of the quantity of the target gene were determined. In this assay, an increase of fluorescent signal above a preset threshold within 38 PCR cycles was considered positive (i.e., $Ct<38$). The thermal cycling conditions were programmed according to the manufacturer's instructions.

Analyses of $CD4^+$ T cell subpopulations by flow cytometry

Fresh single-cell suspensions of leukocytes from the spleen were prepared. Intracellular cytokine staining cells were stimulated for 5 h with 40 ng/ml PMA (Sigma-Aldrich, St. Louis, MO, USA) and 1 mg/ml ionomycin (Sigma-Aldrich) in the presence of brefeldin A (Sigma-Aldrich) at a final concentration of 3 μg/ml. Cells were harvested, extracellularly stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD4, fixed and permeabilized with IntraPrep™ Permeabilization Reagent (Beckman Coulter, Marseille, France), and then intracellularly stained with APC-conjugated anti-IFN-γ, PE-conjugated anti-IL-4, and PerCP-Cy5.5-conjugated anti-IL-17A (eBioscience, San Diego, CA, USA) according to the manufacturer's recommendations. For Treg cell analysis, cells in a single

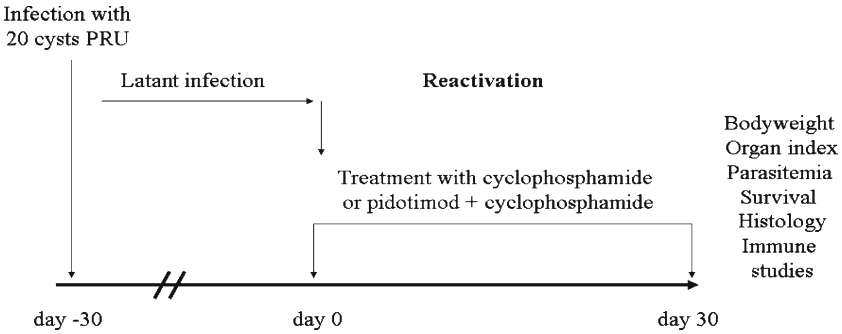

Fig. 1 Murine model of reactivated toxoplasmosis in CY-induced immunocompromised mice

suspension were stained with FITC-conjugated anti-CD4 and PE-conjugated anti-CD25, followed by intracellular staining with PE-Cy5-conjugated anti-foxhead box protein 3 (Foxp3) following the manufacturer's protocol. Stained cells were analyzed by FACS Canto II (BD Biosciences, San Jose, CA, USA). The data were analyzed using the CellQuest Pro software (BD Biosciences).

Quantitation of cytokine production in sera

The concentration of IL-2, IL-4, IL-10, IL-17, IFN-γ, and TNF-α cytokines was measured in serum samples from each animal using the Cytometric Bead Array (CBA) Mouse Cytokines Kit (BD Biosciences) following the manufacturer's instructions. The standard curve ranged from 1 to 5,000 pg/ml, and values over 1.0 pg/ml were regarded as positive.

Splenocyte proliferation assay and cytokine analysis

For in vitro splenocyte proliferation studies, all mice were sacrificed under anesthesia and the spleens were removed aseptically. Splenocytes were washed with PBS and cultured in RPMI-1640 medium containing 10 % fetal calf serum (FCS; HyClone, Logan, UT, USA) at a density of 1×10^6 cells/ml with concanavalin A (Con A; Sigma-Aldrich) (5 μg/ml) in a 96-well plate. Cell proliferation was measured by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) assay after 72 h culture. Supernatants were collected and analyzed for cytokine production (IL-2, IFN-γ, and TNF-α) by CBA as described previously.

Table 1 Experimental design of the study

Groups[a]	*T. gondii* infected	Treatment
Control	No	None
A	No	NS
B	No	CY+NS
C	No	PT+CY
D	Yes	NS
E	Yes	CY+NS
F	Yes	PT+CY

NS normal saline (0.9 % saline solution, orally), *CY* cyclophosphamide (100 mg/kg body weight, intraperitoneally), *PT* pidotimod (400 mg/kg body weight, orally)

[a] Seven experimental groups of 50 mice each were investigated. Additionally, 20 mice in each group were set for survival time monitoring

Quantification of transcription factors mRNA by quantitative real-time PCR

Total RNA from the splenocytes was isolated with TRIzol (Invitrogen, Carlsbad, CA, USA). RNA was converted to cDNA using the RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's protocol. Primers and TaqMan probes were synthesized using an ABI synthesizer and gene expression assay probes at ShineGene (Shanghai, China). The primers/probe assay IDs are AA14903 (T-bet), AA14906 (GATA binding protein 3 [GATA3]), AA14912 (Foxp3), and AA14918 (β-actin) (Song et al. 2011). An ABI Prism 7500 sequence detection PCR system was used to perform real-time PCR with cDNA and Premix Ex TaqTM (Takara, Dalian, China) in order to detect the mRNA expression of mouse transcription factors. The thermal cycling conditions were programmed following the manufacturer's instructions. The factors mRNA expression levels at each time point were normalized against β-actin (endogenous control). A fivefold dilution series of pooled cDNA from each sample was used to construct a relative standard curve against which β-actin and mRNA expression were normalized.

Histology and immunohistochemistry

Brains were excised from each mouse, fixed in a solution containing 4 % buffered formaldehyde, and embedded in paraffin. Sagittal sections and cross-sections (4 μm) were stained with hematoxylin and eosin (H&E) or by an immunoperoxidase method with rabbit anti-*T. gondii* antibody (Abcam, Cambridge, UK). Sections stained with H&E were observed for histological changes.

Statistical analysis

The statistical difference between the changes in the experimental groups was determined by the means and standard deviations using Student's *t* test and between three groups by the one-way analysis of variance. Intergroup survivals were compared by log-rank test of Kaplan–Meier survival curves. All statistical analyses were performed using GraphPad Prism and SPSS. $P<0.05$ was considered to be statistically significant.

Results

Effect of preventive treatment with pidotimod on body weight and spleen organ index

Figure 2a shows that mice of the CY treatment groups (B, C, E, and F) had a notable decrease in body weight compared to the control. Body weight was partially restored ($P<0.05$) when mice were treated with pidotimod. The spleen organ indices were significantly and synchronously reduced by CY administration. The organ indices of the spleen were significantly restored with pidotimod treatment ($P<0.01$) (Fig. 2b).

Effect of preventive treatment with pidotimod on parasitemia and mice survival

Parasitemia was measured in all animals, three mice for each time, starting on the first day after *T. gondii* infection. As shown in Fig. 3a, parasitemia appeared only between days 5 and 12 postinfection during the acute phase of infection. The differences in parasitemia at day 7 after CY injection were shown to be statistically significant between pidotimod-treated and pidotimod-untreated infected mice. Since day 17 after CY injection, parasitemia has become detectable in pidotimod-treated mice, and then the parasite burden in the blood increased gradually in both pidotimod-treated and pidotimod-untreated infected mice ($P<0.01$). The percentage of parasitemia increased up to 80 % of pidotimod-untreated mice and to 35 % of those treated with pidotimod within 30 days (Fig. 3b). Thus, pidotimod treatment significantly decreased the parasite load in the blood.

The survival of animals that had been injected with CY followed by pidotimod treatment is presented in Fig. 3c. Pidotimod treatment increased the survival of CY-induced immunocompromised mice ($P<0.001$). Thirty percent of the animals without pidotimod treatment died within 30 days after CY injection, whereas 90 % of the pidotimod-treated mice survived the injection for more than 30 days.

Effect of preventive treatment with pidotimod on histological changes

Figure 4a shows that the histological findings in brains from days 1 to 28 after CY injection paralleled with the results of parasitemia and time of survival as described previously. The brain lesions were characterized by inflammatory cell infiltration, glial nodules, vascular cuffing with lymphocytes, and focal mononucleated cell infiltration in the meninges. Diffuse infiltration of mononucleated cells was also notable. Quantification of parasite DNA at day 7 after CY injection revealed a two-log increase in parasite burden in the brain of mice without pidotimod treatment compared to those with pidotimod treatment (Fig. 4b).

Effect of preventive treatment with pidotimod on cytokine production

To determine the influence of pidotimod treatment on cytokine production in CY-induced immunosuppressed mice, the cytokine profiles in sera were analyzed using CBA assays. As shown in Fig. 5a, the data revealed that IL-2, IL-4, IL-10, IL-17, IFN-γ, and TNF-α were decreased in CY-induced immunosuppressed mice compared to the control group. Comparatively, IFN-γ production was notably restored with pidotimod treatment, whereas no significant differences of IL-4 and IL-10 expression were observed between CY-induced mice treated with or

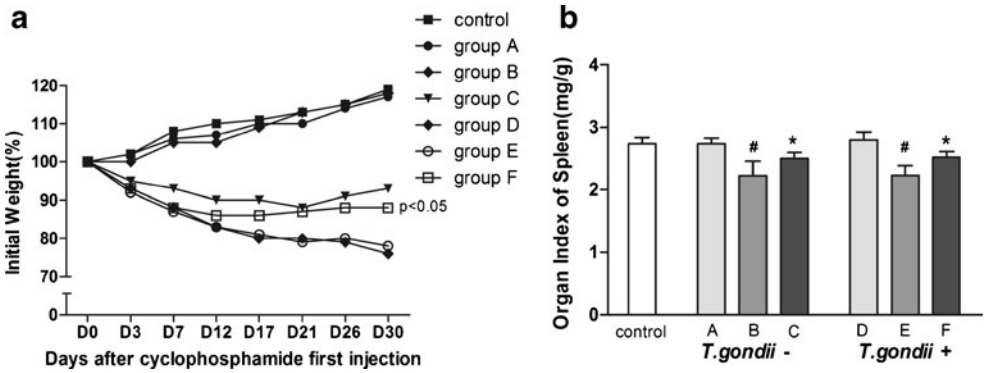

Fig. 2 Effects of pidotimod on body weight (**a**) and spleen organ index (ratio of spleen weight to body weight) (**b**) on immunocompromised mice induced by CY. The results are presented as the mean± standard error of the mean (SEM) of 20 animals per group. #$P<0.01$ versus the control; *$P<0.01$ versus CY-induced mice treated without pidotimod

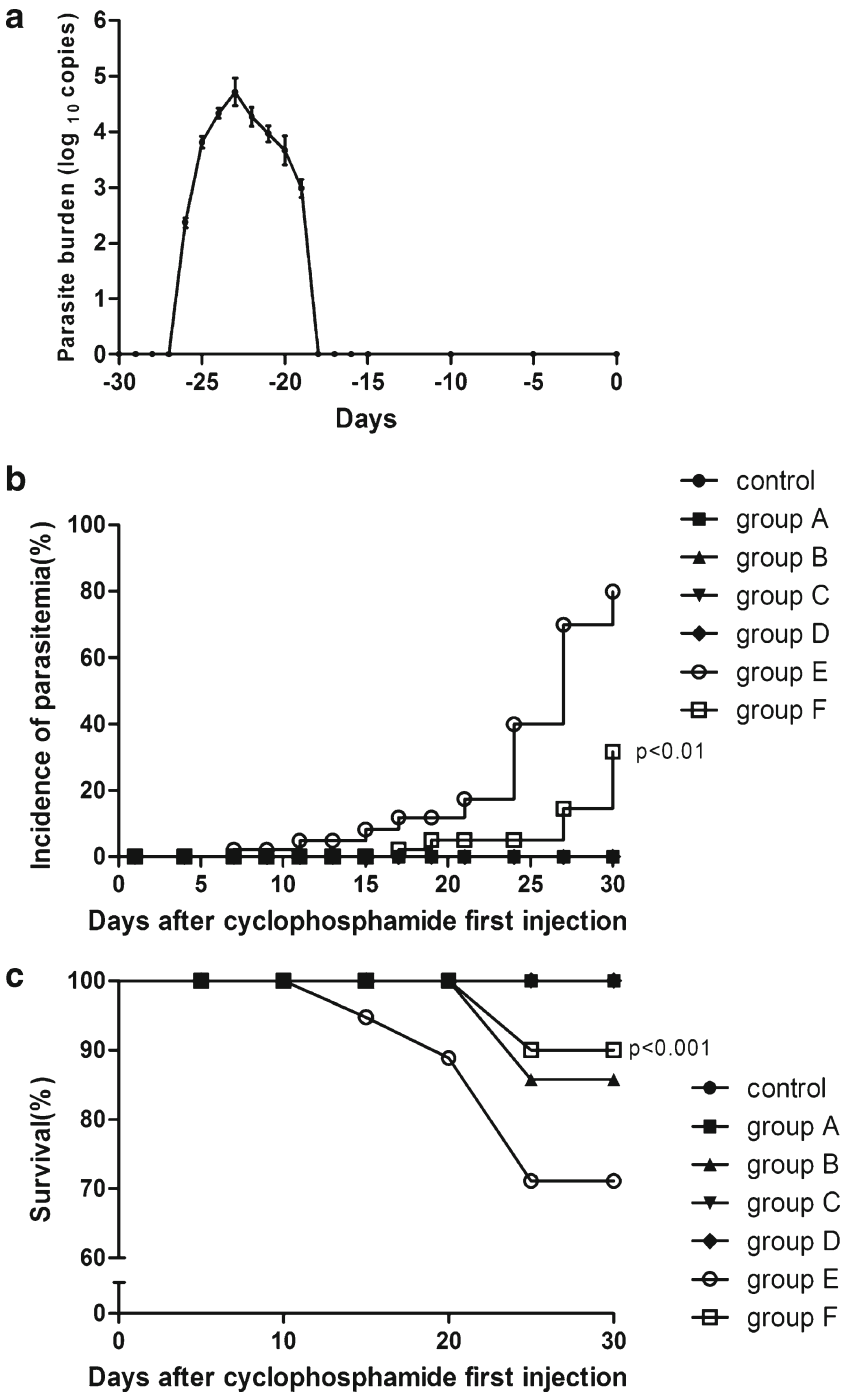

Fig. 3 Parasitemia and survival of CY-induced immunocompromised mice. **a** Arithmetic means±SEM (n=3) of parasite burden of BALB/c mice infected with 20 *T. gondii* cysts during the acute phase of the infection. **b** Parasitemia in mice treated with or without pidotimod after CY injection. **c** Survival of mice treated with or without pidotimod after CY injection. The parasitemia and the survival curve only differ significantly in *T. gondii*-infected groups of mice

without pidotimod, suggesting a Th1 dominant response. Moreover, we also observed restoration of TNF-α and IL-2 in pidotimod-treated mice. No significant difference was noted in the production of IL-17A between pidotimod-treated and pidotimod-untreated groups in CY-induced immunosuppressed mice. Additionally, analyses of pro-inflammatory cytokines (IFN-γ, IL-2, and TNF-α) in splenocyte culture supernatants revealed a remarkable recovery of cytokine production with pidotimod treatment in CY-induced immunosuppressed mice (Fig. 5b).

Effect of pidotimod on splenocyte proliferation

The effects of pidotimod on Con A-stimulated mouse spleen lymphocyte proliferation were assessed by MTT. The results indicated that pidotimod greatly restored the proliferative response which had been diminished by

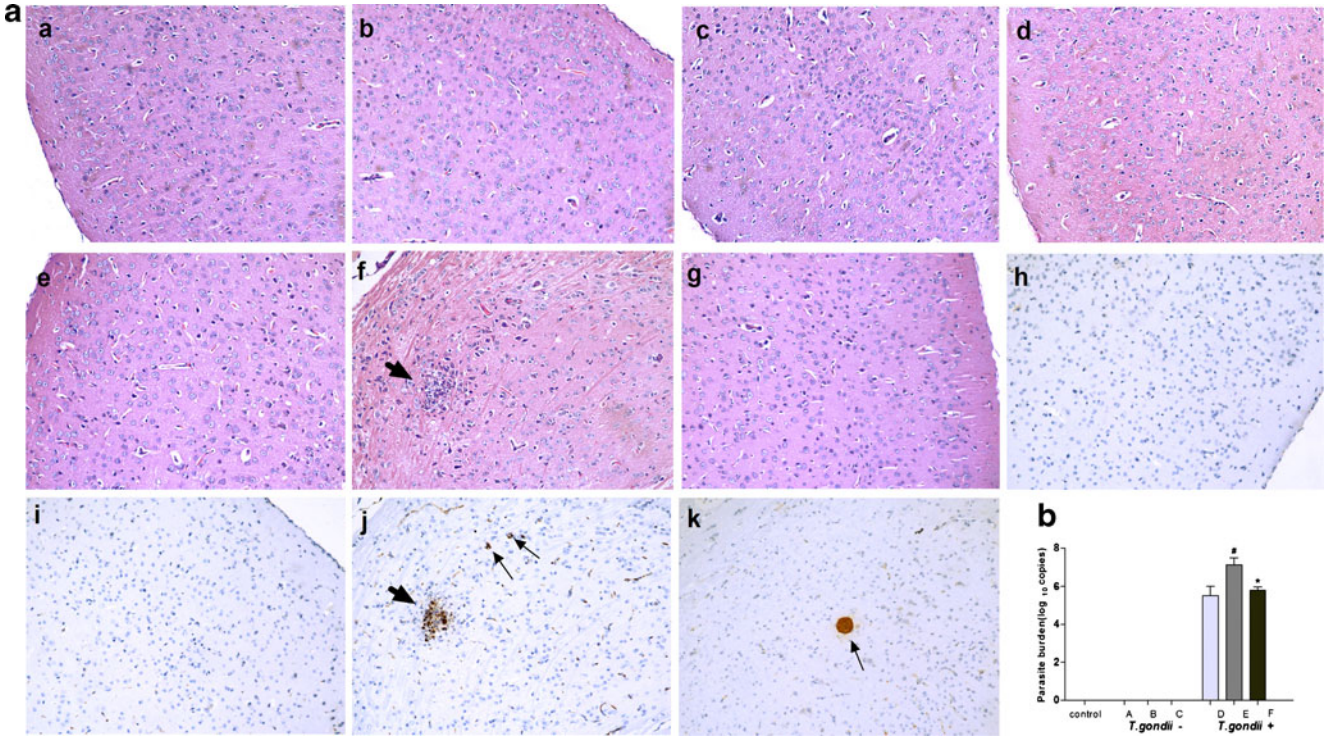

Fig. 4 Histological changes in the brains of mice injected with CY. **a** Histopathology of brains in mice with reactivated toxoplasmic encephalitis at day 7 after the initiation of preventive treatment. Magnification, ×200. Presence of mononucleated inflammatory cell infiltration in the inflammatory foci (*large arrows*); cyst and liberated tachyzoites (*small arrows*) in the brains. *a*, *h* Control mice; *b* noninfected mice with NS; *c* noninfected mice with CY and NS; *d* noninfected mice with pidotimod and CY; *e*, *i* infected mice with NS; *f*, *j* infected mice with CY and NS; *g*, *k* infected mice with pidotimod and CY. The sections shown are representative for at least three mice per group. **b** CY-induced immunosuppressed mice had an approximately two-log increase in parasite burden in the brain in comparison to pidotimod-treated mice at day 7 after CY injection. Data are presented as the mean±SEM. $^{\#}P<0.01$ versus *T. gondii*-infected mice with NS only; $^{*}P<0.01$ versus CY-induced mice treated without pidotimod

CY-induced immunosuppression in Con A-stimulated mice ($P<0.01$) (Fig. 5c).

Effect of preventive treatment with pidotimod on $CD4^+$ T cell subpopulation and inflammatory mediators

As shown in Fig. 6a–c, the mice of CY treatment groups had a significant decrease in $CD4^+$ T cells compared to the control. The percentage of Th1 was remarkably recovered in pidotimod-treated mice with CY-induced immunosuppression. Interestingly, an increase of Treg cells was also observed simultaneously in this group. No significant difference in the mean frequencies of Th2 and Th17 cells was found with relation to pidotimod treatment. The total number of splenocytes was also increased significantly in pidotimod-treated CY-induced mice ($P<0.01$) (data not shown). The absolute number of each Th subset was increased correspondingly.

The mRNA expression of specific transcription factors such as T-bet, GATA3, and Foxp3 of Th1, Th2, and Treg cells in the spleens of mice were examined using quantitative real-time PCR. As shown in Fig. 6d, those transcription factors were decreased in CY-induced immunocompromised mice compared to the control. Pidotimod treatment significantly recovered the production of transcription factor T-bet. It was also found that the level of Foxp3, but not GATA3, was upregulated with pidotimod treatment in CY-induced immunosuppressed mice.

Discussion

T. gondii primary infection is usually asymptomatic in most immunocompetent individuals and usually progresses uneventfully to latent infection in a matter of months. In immunocompromised hosts, however, toxoplasmosis almost always constitutes a severe and even fatal consequence due to the reactivation of latent infection (Montoya and Liesenfeld 2004; Scholer et al. 2001). Depletion of immune cells in the setting of chronic infection rapidly leads to the reactivation of infection and death (Gazzinelli et al. 1992). CY is a DNA alkylating agent frequently used as an anticancer chemotherapy. It has been known that CY can also kill normal cells, including immune cells (Giampietri et al. 1978; Mazur et al. 2002). As a synthetic substance with

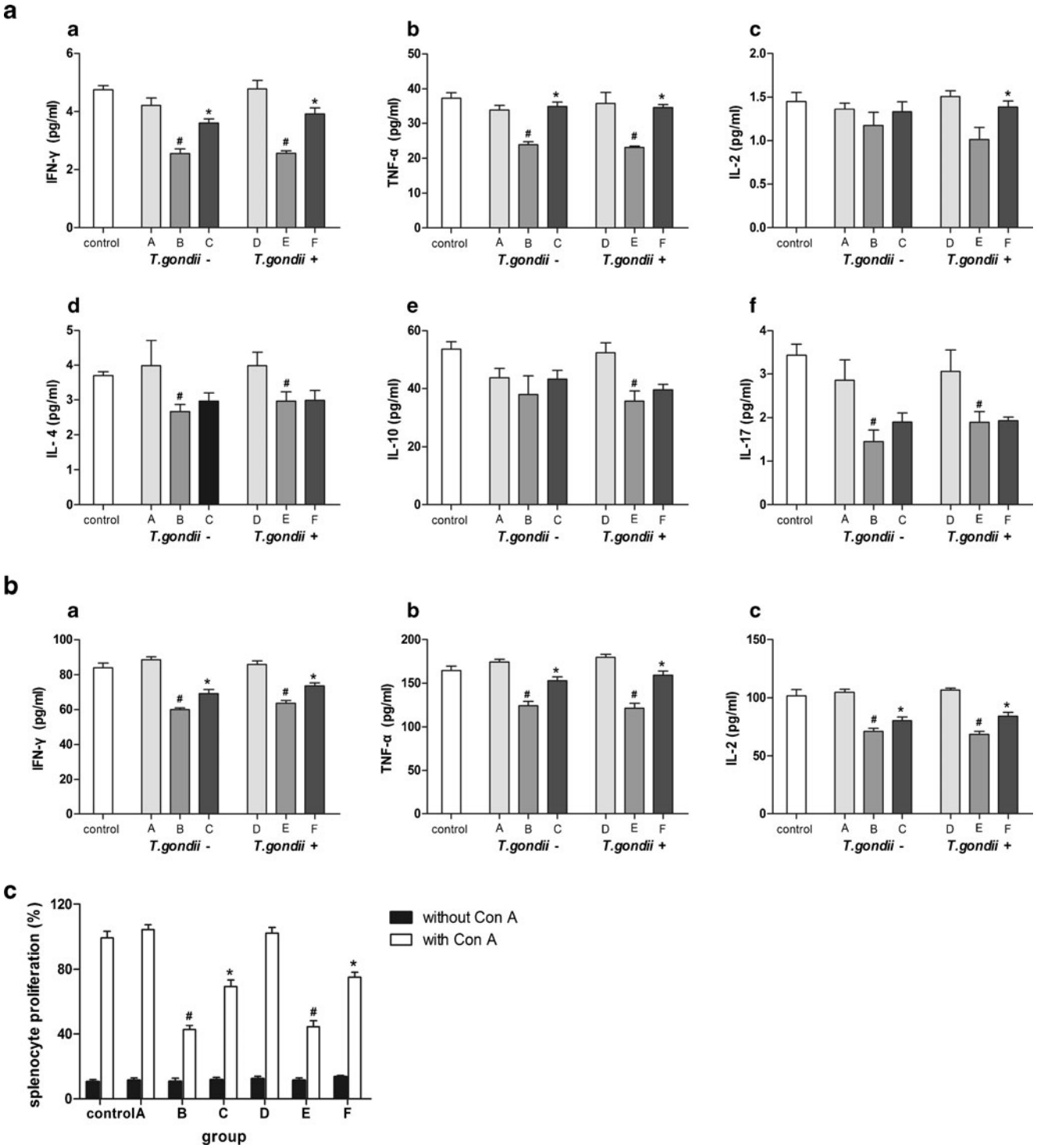

Fig. 5 Effects of pidotimod on cytokine production and splenocytes proliferation. **a**, **b** Sera and splenocyte culture supernatants were analyzed for cytokines by the CBA. Note that the levels of the Th1 signature cytokine IFN-γ and the pro-inflammatory cytokines TNF-α and IL-2 were strongly recovered, while the sera levels of Th2 signature cytokines IL-4 and IL-10 had no significant change in mice treated with pidotimod. **c** In vitro splenocytes proliferation was analyzed by MTT and expressed as percentage values, taking the control group with Con A as 100 %. Results show the mean cytokine concentration±SD. Three mice were analyzed per group. $^{\#}P<0.01$ versus the control; $^{*}P<0.01$ versus CY-induced mice treated without pidotimod. All data are representative of three repeated experiments with three mice

important immunomodulatory properties, pidotimod is able to induce a protective effect in immunosuppressed hosts (Migliorati et al. 1992, 1994). It has been reported that the combination of pidotimod and RGAP acts synergistically in

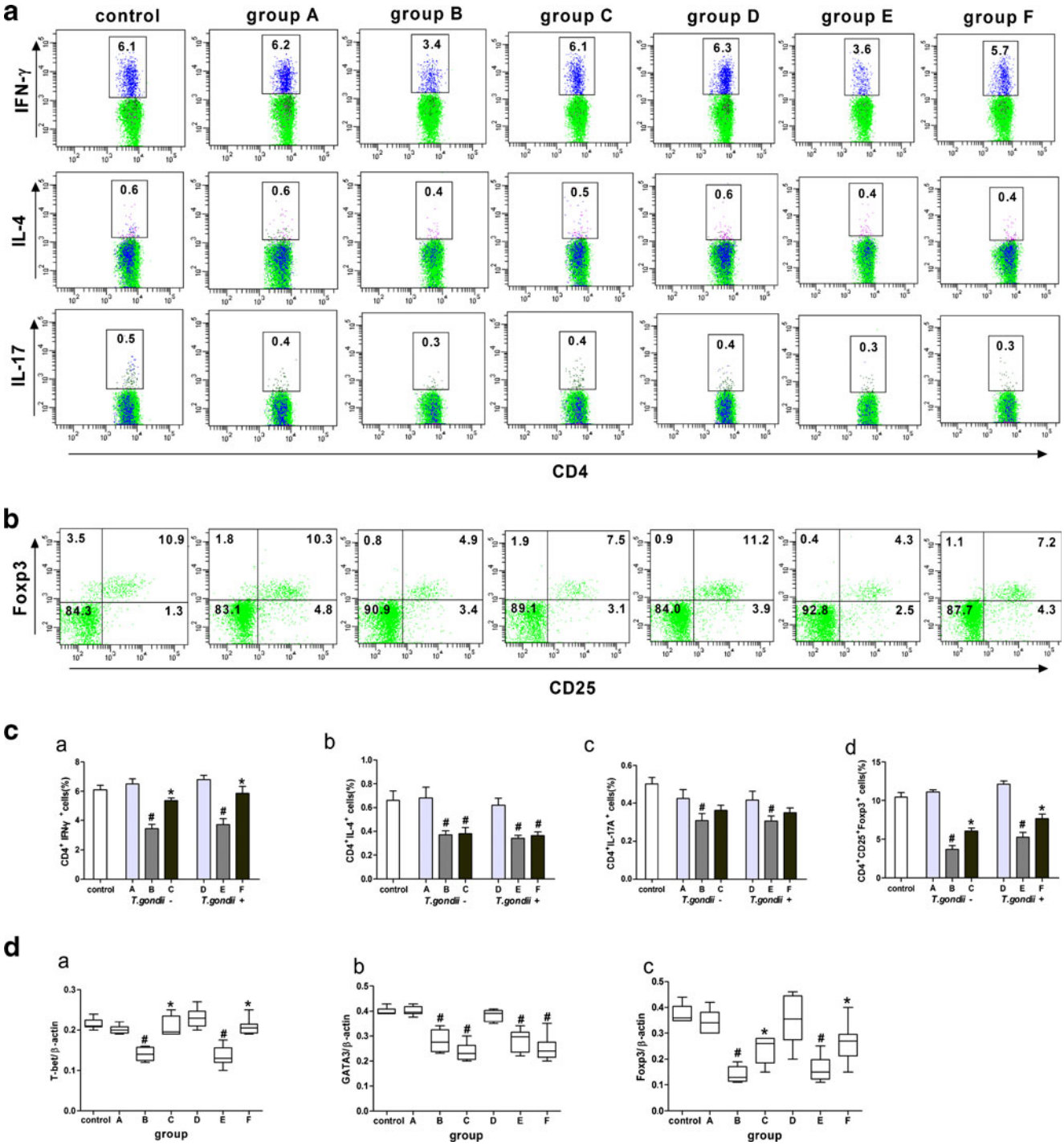

Fig. 6 Pidotimod-treated mice show recoveries of Th1 and Treg cells. **a**–**c** Analysis of $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}4^+$, $CD4^+IL\text{-}17A^+$, and $CD4^+CD25^+Foxp3^+$ T cells in splenocytes of mice. The percentage of $CD4^+IFN\text{-}\gamma^+$ T cells and $CD4^+CD25^+Foxp3^+$ T cells was recovered in pidotimod-treated CY-induced mice, while the percentage of $CD4^+IL\text{-}4^+$ and $CD4^+IL\text{-}17A^+$ T cells remained unchanged. **d** The mRNA expression of transcription factors was quantified by real-time PCR. Values along the vertical axis represent relative expression levels normalized to β-actin. Three mice were analyzed per group. $^{\#}P<0.01$ versus the control; $^{*}P<0.01$ versus CY-induced mice treated without pidotimod. Data (in **a**) are representative of three experiments

enhancing the immunity in CY-induced immunosuppressed mice (Du et al. 2008). Di Renzo et al. (1997) observed in vitro that peripheral blood mononuclear cell proliferation and IL-2 production had been notably restored when treated with pidotimod in immunocompromised patients. The present investigation demonstrated a clear prophylactic effect of

pidotimod on reactivated toxoplasmosis in immunosuppressed mice. The results were obtained in a murine model of reactivated toxoplasmosis, imitating the course of disease in immunocompromised patients, including the presence of parasitemia, pathological lesions in the brain, survival time, and immune status of hosts.

Transmission of extracellular and intracellular parasites in the blood following oral ingestion, leading to dissemination within the host, is a prerequisite for the establishment of *T. gondii* infections. Thus, parasitemia plays an important role in pathogenesis during acute toxoplasmosis and in the reactivation of latent infections (Barragan and Sibley 2002; Lachenmaier et al. 2011). Our experiment disclosed a preventive effect of pidotimod on the course of reactivation. Pidotimod treatment significantly alleviated the parasitemia induced by the immunosuppressive agent, with these findings reaching statistical significance in experimental mice on day 17 after CY injection. Thus, the beneficial efficacy of pidotimod administration may preclude the latency from reactivation following the establishment of chronic toxoplasmic infection in the host.

Latent *T. gondii* infection is usually benign in immunocompetent subjects with a Th1-mediated/classically activated macrophage immune response, which confers a lifelong protection, regardless of whether the host presents resistant or susceptible MHC haplotypes (Cortez et al. 2009; Jensen et al. 2011; Suzuki et al. 1994). Both in vivo and in vitro studies indicate that IFN-γ and TNF-α are crucial cytokines that mediate the resistance of the host against *T. gondii* infection. Neutralization of endogenous IFN-γ during latent infection may induce reactivated toxoplasmosis and even death due to toxoplasmic encephalitis (Dellacasa-Lindberg et al. 2007; Silva et al. 2009). Similarly, neutralization of TNF-α leads to the reactivation of chronic toxoplasmosis and a lethal exacerbation of the disease (Gazzinelli et al. 1993; Silva et al. 2009). In the present work, we demonstrated that pidotimod is able to strongly reverse the decrease of IFN-γ and TNF-α production induced by CY. Thus, pidotimod treatment significantly inhibits the reactivation of chronic toxoplasmosis and enhances the survival of animals with CY injection. It was observed that Th1 rather than Th2 cell subpopulation showed a significant recovery in its signature cytokine IFN-γ. We also assessed the mRNA expression profiling of T-bet, Foxp3, and GATA3 genes to identify the efficacy of pidotimod treatment on reactivated toxoplasmosis in immunosuppressed mice. Consistent with the flow cytometry observation of T cell subpopulations in the spleen, T-bet and Foxp3 expressions were markedly elevated with pidotimod treatment in CY-induced immunosuppressed mice. No significant recovery of GATA-3, however, was seen as a master Th2 transcription factor (Ho et al. 1991). IL-17 has been implicated in the pathology associated with various forms of infection-induced inflammation (Kelly et al. 2005; Yoshida et al. 2007). It has been shown to be one of the major cytokines involved in the development and recruitment of neutrophils. The initial innate immune response led by neutrophils has been reported to be critical for the successful resolution of the acute *Toxoplasma* infection. In contrary to a recent report that IL-17A contributed to the pathology of acute *T. gondii* infection (Guiton et al. 2010), no significant changes were noted in the Th17 signature cytokine IL-17 in the present study. The time point of IL-17 detection during latent *T. gondii* infection rather than acute *T. gondii* infection may account for the discrepancy of the IL-17 levels. So far, little has been known regarding the dynamics of IL-17 expression during the chronic phase of *T. gondii* infection.

Although current antibiotic therapy, including sulfadiazine, pyrimethamine, ciprofloxacin, and epiroprim (Jost et al. 2007; Mehlhorn et al. 1995; Meneceur et al. 2008; Zhao et al. 2010), is available for toxoplasmosis, it suffers from problems caused by induction of bone marrow suppression, allergic reactions, and other adverse drug reactions (Katlama et al. 1996); more effective treatments with fewer side effects would be beneficial. Pidotimod has been shown to be safe in humans and has little genotoxicity (Adams et al. 1994), neurotoxicity, and other toxic effects in all animal species tested (Coppi et al. 1994). Furthermore, it has neither antimicrobial activity nor interaction with some of the most common therapeutics (antibiotics, antihypertensives, anticarcinogens, and nonsteroidal anti-inflammatory agents) (Manzardo et al. 1994). Our experiments also showed that pidotimod had no visible parasiticidal activity in survival and duplication of tachyzoites in vitro (data not shown). The present approach suggested that pidotimod has potentiality in the prophylactic strategy for reactivated toxoplasmosis in individuals with accompanying immunosuppression.

Conclusions

The murine model of reactivated toxoplasmosis described here proved valuable for studying the efficacy of immunoregulatory drugs for preventive effect on reactivated toxoplasmosis. The present work showed that pidotimod administration has a significant benefit for the alleviation of parasitemia and prolonging of survival time in mice with reactivated toxoplasmosis through enhancing Th1-mediated cellular immunity. Mice treated with pidotimod had a remarkable reduction of parasite burden and amelioration of histopathology in the brains. Furthermore, the in vitro studies showed that pidotimod significantly restored Con A-induced splenocyte proliferation and pro-inflammatory cytokines in the supernatants of splenocyte culture.

Acknowledgments This work was funded by the National Basic Research Program of China (973 Program, Grant No. 2010CB530001) and Natural Science Foundation of China (Grant Nos. 81171605 and 81271864). Our thanks to Prof. Geoff Hide at University of Salford, Salford, UK for his kind language polishing and comments.

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