FULL PAPER *Parasitology*

Effect of $CD4^+CD25^+$ T Cell-Depletion on Acute Lethal Infection of Mice with *Trypanosoma congolense*

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ABSTRACT. Despite the immense socio-economic repercussions of African trypanosomosis (AT), there is currently no effective control measure against the disease. Characterization of mechanisms governing resistance and/or susceptibility to AT could suggest interventions that might lead to more effective disease control. The present study was designed in an attempt to address the possible role of $CD4^+CD25^+$ T cells during an acute lethal infection of mice with *Trypanosoma congolense*, the causative agent of AT in domestic animals, through selective depletion using anti-CD25 monoclonal antibody. Accordingly, $CD4^+CD25^+$ T-cell-depletion resulted in a significant reduction or delay in parasitemia, pathology, and mortality, as compared to controls. The apparent resistance in $CD4^+CD25^+$-T-cell-depleted mice correlated with a profound suppression of Th2 cytokines *in vitro* and *in vivo*, culminating in a net Th1 cytokine environment. Cumulatively, these findings suggest that $CD4^+CD25^+$ T-cell- depletion improves the trypanotolerance of highly susceptible BALB/c mice acutely infected with the lethal *T. congolense*.

KEY WORDS: $CD4^+CD25^+$ T cell, depletion, Th1/Th2 cytokines, trypanosomosis, trypanotolerance.

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African trypanosomosis (AT), caused by various trypanosome species, is a tsetse-transmitted hemoprotozoan disease of sub-Saharan Africa with serious socio-economic repercussions [33]. In cattle, AT, mainly caused by *Trypanosoma congolense*, is characterized by emaciation, anemia and ultimately death [33]. Efforts to control AT have been hampered mainly by the trypanosomes' ability to undergo antigenic variation, resulting in failure to design an effective vaccine [22]. Furthermore, infection with African trypanosomes is associated with inhibition of lymphocyte proliferative response to antigens and mitogens, and this immunosuppression is thought to increase host susceptibility to secondary infection [5, 19, 28]. Consequently, characterization of mechanisms governing resistance and/or susceptibility to AT could suggest interventions that might lead to effective disease control.

Various animal models have provided conflicting evidence regarding the immunologic factors that influence the magnitude of resistance to AT [9, 18, 31, 33]. However, the overall host response to AT requires the contribution of variant-surface-glycoprotein-specific B and T cells [24, 27] as well as the macrophage/monocyte phagocyte system [6, 12]. In highly susceptible mice, $CD4^+$ T cells have been shown to mediate disease progression through excessive interferon gamma (IFN-γ) production [29]. In sharp contrast, resistance seems to be dependent on IFN-γ and interleukin (IL)-2 producing $CD4^+$ T cells in trypanotolerant mouse strains [27]. A subpopulation of $CD4^+$ T cells constitutively express CD25, the α-chain of IL-2 receptor [35]. In addition, conventional $CD4^+$ T cells (and minor populations of other lymphocytes and monocytes) also express CD25 following activation [23, 35]. Such $CD4^+CD25^+$ T cells have been linked with disease progression during tuberculosis [23], malaria [10], HIV AIDS [1], onchocerciasis [26] and leishmaniasis [3]. Furthermore, Zelenay *et al.* [35] reported that the newly activated $CD4^+CD25^+$ T cell subpopulation mostly contains pathological cells during experimental autoimmune encephalomyelitis. The possible role of $CD4^+CD25^+$ T cells during AT has not yet been elucidated. Therefore, the present study was designed in an attempt to address the role of $CD4^+CD25^+$ T cells during acute lethal *T. congolense* infection in mice, through selective depletion by intraperitoneal (i.p.) administration with anti-CD25 monoclonal antibody (mAb) [16]. We report that $CD4^+CD25^+$-T-cell-depletion results in a significant reduction in parasitemia, pathology, and mortality in infected mice. The possible role of $CD4^+CD25^+$ T cells during acute AT is discussed.

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MATERIALS AND METHODS

Animals and parasites: Eight-week-old female BALB/c mice (Nihon CLEA Inc., Tokyo, Japan) were inoculated i.p. with the lethal IL1180 strain of *T. congolense*. The lethal mutant strain, originally obtained from International Livestock Research Institute (Nairobi, Kenya) and kindly pro-

vided by Dr. Yoshisada Yabu (Dept. of Molecular Parasitology, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan), was generated by serial passage of the original low virulent IL1180 strain in mice over a prolonged period of time and maintained in our institute. All animal experiments were conducted in accordance with the Standards relating to the Care and Management of Experimental Animals of Obihiro University of Agriculture and Veterinary Medicine (Hokkaido, Japan).

Experimental design: A rat anti-mouse CD25 mAb (7D4, anti-CD25 mAb) [16] was used to deplete the mouse $CD4^+CD25^+$ T cells *in vivo* as previously described [10]. For each experiment, 10 BALB/c mice were divided into groups A and B (n=5). Group A mice were each treated i.p. with 1 mg of anti-CD25 mAb on days (D) -3, -1, and +3 post-infection (pi), while control group B mice received the same volume (500 μl) of PBS. On D0 pi, each mouse in both groups was inoculated i.p. with 2,000 *T. congolense* parasites. Parasitemia, weight gain, PCV, and survival rate were monitored during the course of infection. In parallel, on D7 pi, immune parameters were determined in splenocytes (SPC) or plasma from 5 group A or group B mice and compared with same parameters assessed in 5 group C (untreated and uninfected controls) mice. Results are representative of at least three similar independent experiments performed.

Flow cytometry analysis: In order to determine the level of $CD4^+CD25^+$ T cells or to confirm their depletion, SPC, prepared as previously described [4], from *T. congolense*-infected mice or mice treated with anti-CD25 mAb or PBS for 1 day, respectively, were double-stained with fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD25 (PC61, eBioscience, San Diego, CA, U.S.A.) and phycoerythrin (PE)-conjugated rat anti-mouse CD4 (GK1.5, BD Science, San Diego, CA, U.S.A.) mAbs at 4°C for 30 min. Flow cytometry and sorting were performed on a BD FACSCalibur using CellQuest software (BD Biosciense, Mountain View, CA, U.S.A.).

Preparation of soluble parasite antigens: At peak parasitemia, whole blood was collected from *T. congolense*-infected mice by cardiac puncture and parasites purified from the blood using DE52 anion-exchange column chromatography (Whatman, Brantford, UK) as previously described [13]. The purified parasites were washed three times with PBS and disrupted by 4-time repeated freeze-thawing and subsequent sonication. After centrifugation (10,000 × g, for 30 min at 4°C), the supernatant obtained was used as soluble total trypanosome lysates (TTL) in cell cultures and ELISA. The single lot preparation of TTL was used throughout the study.

Quantification of cytokine mRNA: Total RNA was extracted from TRIZOL (Invitrogen, Carlsbad, CA)-homogenized SPC from groups A (SPC-7D4-I), B (SPC-PBS-I), or C (SPC-N) mice according to the manufacturer's protocol and kept at –80°C until use. The specific primer pairs for mouse IFN-γ, tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS), IL-4, IL-10, IL-12p40, TGF-β1, and β-actin (R&D Systems, Abingdon, UK) were used to analyze respective mRNA expression by RT-PCR. RT-PCR was performed using a one-step RT-PCR kit (Takara Biomedicals, Shiga, Japan) according to the manufacturer's instructions, employing a 35-cycle program of denaturation at 94°C for 45 sec; annealing at 55°C for 45 sec; extension at 72°C for 45 sec. PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide and visualized by UV light.

Splenocyte cultures for determination of soluble cytokine production and lymphocyte proliferation: On D7 pi, 2×10^6 SPC-7D4-I, SPC-PBS-I, or SPC-N were cultured with or without 30 μg/m*l* TTL in 1 m*l* of RPMI 1640 medium (Sigma, St. Louis, MO, U.S.A.) supplemented with 10% fetal bovine serum and penicillin-streptomycin (100 U-100 μg/m*l*) in 24-well plates. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2, and culture supernatants were collected after 24–96 hr and used for determination of soluble cytokines as described below.

The lymphocyte proliferative response of 2.5 μg/m*l* concanavalin A (ConA)-stimulated or unstimulated SPC-7D4-I, SPC-PBS-I, or SPC-N was quantified using the Calbiochem bromodeoxyuridine Cell Proliferation Assay Kit (Calbiochem® Inc., La Jolla, CA, U.S.A.). Briefly, cells were incubated with or without ConA for 24 hr, then labeled with bromodeoxyuridine for 18 hr and assayed according to the manufacturer's protocol.

Quantification of soluble cytokines: Cytokines (IFN-γ, IL-4, and IL-10) were quantified in the culture supernatants or plasma by cytokine specific ELISA kits (Endogen, Rockford, IL, U.S.A.), following the manufacturer's protocols.

Quantification of antibody isotypes: ELISA plates were coated with 30 μg/m*l* TTL overnight at 4°C, washed with 0.05% Tween 20 in PBS, and then blocked with a blocking buffer (3% skim milk in PBS) at 37°C for 1 hr. Following 1 hr incubation of plates at 37°C with plasma samples diluted 1/25 in blocking buffer and subsequent washing, plates were incubated with horseradish-peroxidase-conjugated goat anti-mouse IgG1 or IgG2a (Bethyl Laboratories Inc. Montgomery, TX, U.S.A.) at 37°C for 1 hr. After washing, the assay was developed with 2,2,'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (Sigma).

Statistical analyses: Statistical analyses were assessed by a log-rank test for curve comparison using a PRISM computer program (GraphPad) to validate the data. *P*-values of <0.05 were considered statistically significant. Survival rates of infected mice were plotted according to the Kaplan-Meier method and compared statistically between groups A and B by a log-rank test (Mantel-Cox).

RESULTS

Depletion of $CD4^+CD25^+$ T cells reduces parasitemia development and increases survival rate of T. congolense-infected mice: In an initial experiment, we determined whether *T. congolense* infection had any effect on $CD4^+CD25^+$ T cells in the spleen compartment. By means

of flow cytometry, we noted that the absolute number of $CD4^+CD25^+$ T cells increased from $9.0 \pm 0.1 \times 10^5$ in SPC-N to $18.0 \pm 0.1 \times 10^5$ in SPC-I, indicating a significant increase ($p<0.01$) following infection.

We next depleted $CD4^+CD25^+$ T cells in mice using anti-CD25 mAb. In agreement with previous reports [10], $CD4^+CD25^+$ T cells were effectively depleted in SPC one day following the treatment (Fig. 1).

To investigate the possible role of $CD4^+CD25^+$ T cells in acute AT, we monitored the course of *T. congolense* infection in anti-CD25 mAb treated mice (group A). As compared to the highly susceptible control group B mice, depletion of $CD4^+CD25^+$ T cells resulted in lower development of parasitemia (Fig. 2A) and moderate but significant improvement in survival rate ($p<0.001$, Fig. 2B) in acutely infected mice. Furthermore, whereas control mice exhibited severe clinical manifestation of the disease by D5 pi, including huddling together with partially closed eyes, reduced movements, reduced food and water intake, and pilo-erection, only mild clinical signs were observed up to D8 pi in $CD4^+CD25^+$-T-cell-depleted mice (data not shown). However, after D9 pi, $CD4^+CD25^+$-T-cell-depleted mice also succumbed and later died in a similar pattern of control mice.

$CD4^+CD25^+$-T-cell-depletion correlates with reduction of trypanosome-induced pathology: We monitored the development of pathology in $CD4^+CD25^+$-T-cell-depleted mice during *T. congolense* infection. Whereas control mice exhibited progressive weight loss from D2 pi, $CD4^+CD25^+$-T-cell-depleted mice were gaining weight until D8 pi (Fig. 3A). Moreover, the development of anemia was mainly observed after D8 pi, with PCV values being significantly lower in control compared to $CD4^+CD25^+$-T-cell-depleted mice ($p<0.05$ and $P<0.001$ on D8 and D9 pi, respectively, Fig. 3B). Furthermore, splenomegaly was reduced in $CD4^+CD25^+$-T-cell-depleted mice compared to infected controls (Fig. 3C). However, both infected control ($p<0.05$) and $CD4^+CD25^+$-T-cell-depleted ($p<0.01$) mice developed significant splenomegaly by D7 pi as compared to uninfected controls. It is noteworthy that $CD4^+CD25^+$-T-cell-depleted mice also developed severe pathology prior to death.

We also determined the suppression level of ConA responses of SPC from $CD4^+CD25^+$-T-cell-depleted mice on D7 pi. As shown in Fig. 3D, compared to SPC-N, ConA-induced lymphocyte proliferation in both SPC-7D4-I and SPC-PBS-I was suppressed. However, the extent of T cell-suppression in SPC-PBS-I was significantly greater ($p<0.05$) as compared to that in SPC-7D4-I.

$CD4^+CD25^+$-T-cell-depletion correlates with suppression of Th2 cytokine levels in vitro and in vivo: To gain insights into the influence of $CD4^+CD25^+$ T-cell-depletion on the Th1/Th2 dichotomy during AT, we initially analyzed the alterations in cytokine mRNA expression in SPC collected on D7 pi. Figure 4A shows that, with the exception of TGF-β1 which was constitutively expressed, there were hardly any detectable cytokine transcripts in SPC-N. On the other hand, compared to those of SPC-PBS-I, SPC-7D4-I appeared to exhibit higher expression of IFN-γ, TNF-α, and iNOS mRNA levels. Interestingly, SPC-7D4-I had no detectable IL-10 transcripts, while their IL-4 and TGF-β1 mRNA levels tended to be lower compared to those of SPC-PBS-I (Fig. 4A).

We next examined the TTL-specific cytokine production (IFN-γ, IL-4 and IL-10) in culture supernatants of SPC from mice on D7 pi. SPC-N did not produce any detectable

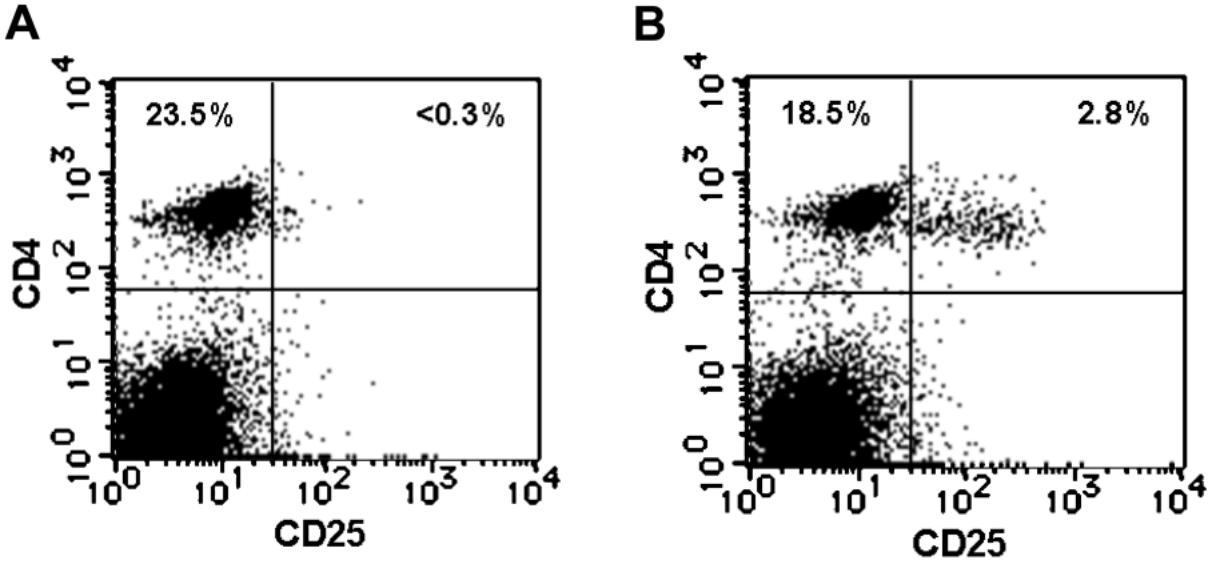

Fig. 1. Effect of anti-CD25 mAb treatment on $CD4^+CD25^+$ T cell population. BALB/c mice were treated i.p. with 1 mg of anti-CD25 mAb (group A) and depletion of $CD4^+CD25^+$ T cells was confirmed by splenocyte flow cytometry analysis one day after treatment (A). Splenocytes were double stained with PE-labeled anti-CD4 mAb (GK1.5) and FITC-labeled anti-CD25 mAb (PC61). Splenocytes from PBS-treated mice (group B) were used as controls (B).

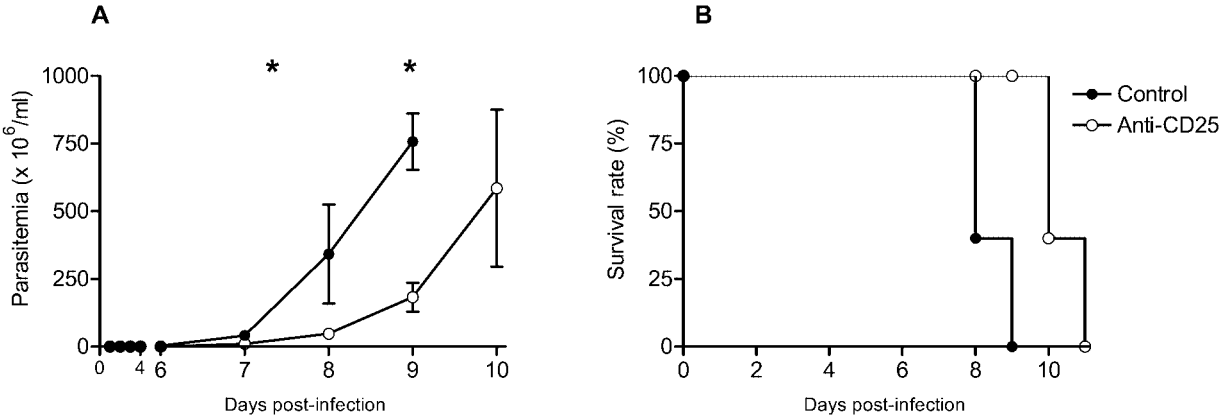

Fig. 2. $CD4^+CD25^+$ T-cell depletion reduces parasitemia development and delays mortality of *T. congolense*-infected mice. Mice in groups A and B were pre-treated i.p. with anti-CD25 mAb and PBS, respectively, on D-3, D-1, and D+3 pi with *T. congolense*. Parasitemia development (A) and survival rate (B) were monitored. Statistical analyses were assessed using a PRISM computer program. *P*-values of <0.05 were considered statistically significant. Data (mean ± SE; n=5) are representative of 4 independent experiments. Asterisks indicate statistically significant difference between groups A and B.

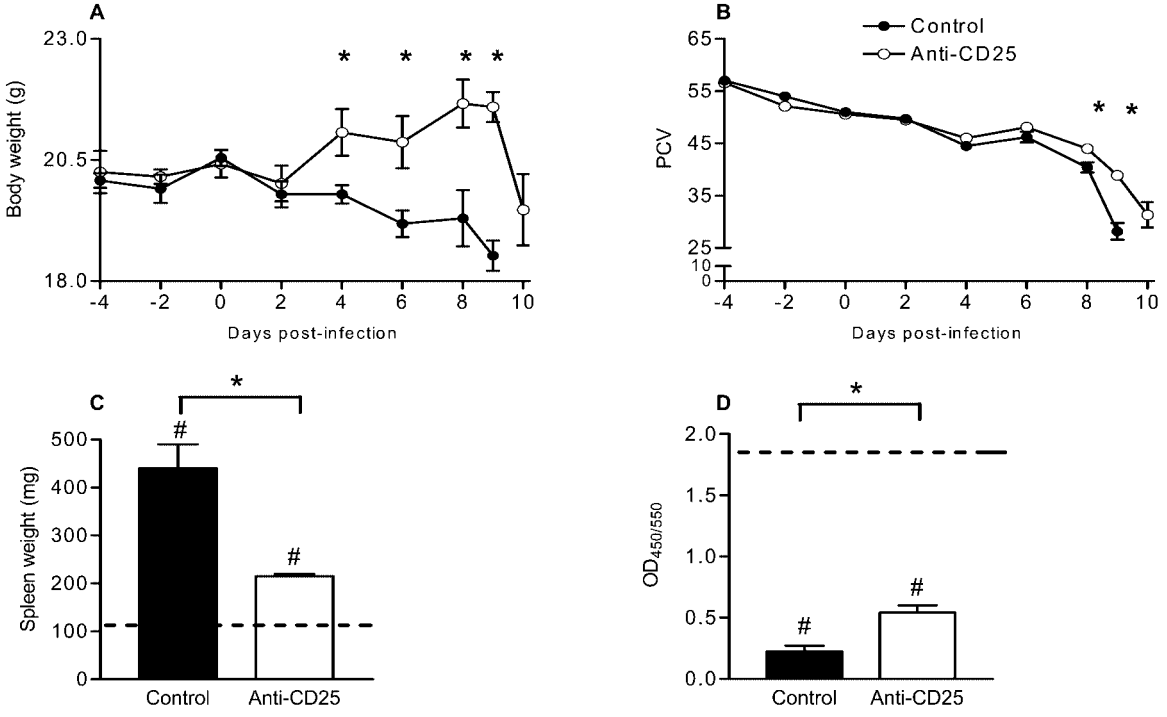

Fig. 3. $CD4^+CD25^+$ T-cell-depletion correlates with reduction of trypanosome-induced pathology. Development of emaciation (A) and anemia (B) were monitored in groups A and B mice at various times following infection with *T. congolense*. In addition, the degrees of splenomegaly (C) and parasite-induced immunosuppression of the splenocytes (D) from groups A and B mice were determined on D7 pi. Statistical analyses were assessed using a PRISM computer program. *P*-values of <0.05 were considered statistically significant. Data (mean ± SE; n=5) are representative of 3 independent experiments. Dashed horizontal lines represent the average values of spleen weights (C) and lymphocyte proliferation in splenocytes (D) from group C (untreated and uninfected) mice. Harsh (#) indicates statistically significant difference as compared to group C; Asterisks indicate statistically significant difference between groups A and B.

cytokines examined (dashed line in Fig. 4B and 4C). However, compared to SPC-PBS-I, IFN-γ levels were significantly augmented in SPC-7D4-I culture supernatants ($p<0.05$, Fig. 4B). In sharp contrast, TTL-activated SPC-PBS-I had significantly higher amounts of both IL-4 ($p<0.01$, Fig. 4C) and IL-10 (data not shown) compared to those of SPC-7D4-I. As illustrated in Fig. 4D, a net Th1-skewed cytokine response, characterized by higher IFN-γ/

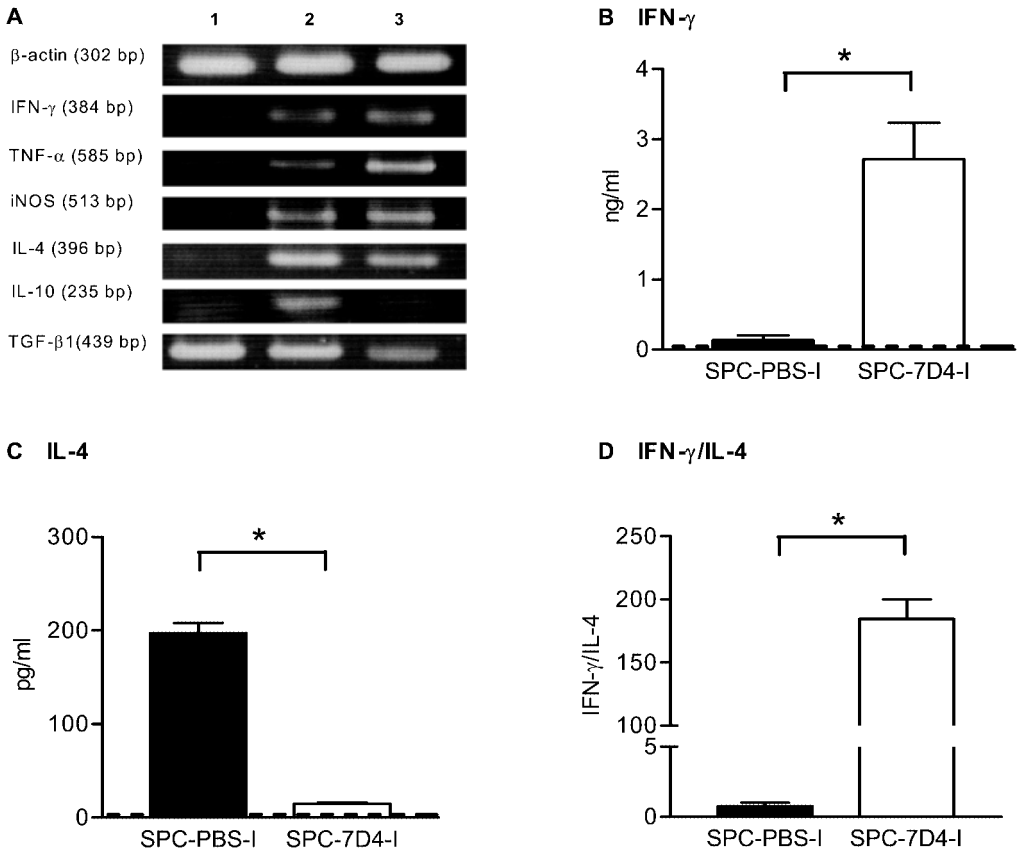

Fig. 4. CD4+CD25+ T-cell-depletion correlates with suppression of Th2 cytokine expression and production in the spleen compartment of *T. congolense*-infected mice. (A) On D7 pi, mRNA expression levels of *β*-actin, IFN-γ, TNF-α, iNOS, IL-4, IL-10, and TGF-*β*1 were determined in SPC-N (Lane 1), SPC-PBS-I (Lane 2), or SPC-7D4-I (Lane 3) by RT-PCR. The product size of each gene is shown in parenthesis. Levels (mean + SE; n=5) of IFN-γ (B) and IL-4 (C) were quantified in culture supernatants of splenocytes from groups A or B and IFN-γ/IL-4 (D) ratios calculated. Statistical analyses were assessed using a PRISM computer program. *P*-values of <0.05 were considered statistically significant. Dashed horizontal lines represent average cytokine levels in the culture supernatants of splenocytes from group C mice. Asterisks indicate statistically significant difference between groups A and B.

IL-4 ratio ($p<0.01$), was observed in SPC-7D4-I cultures, whereas SPC-PBS-I cultures exhibited a Th2 cytokine inclined response (lower IFN-γ/IL-4 ratio).

Cytokine levels in the plasma of mice were also determined on D7 pi. There were hardly any detectable cytokine levels in plasma of uninfected mice (dashed line in Fig. 5A-C). However, although both infected mouse groups had comparable amounts of plasma IFN-γ, infected CD4+CD25+-T-cell-depleted mice exhibited lower levels of IL-4 and IL-10 (Fig. 5A-C). Thus, as observed in the spleen, infected CD4+CD25+-T-cell-depleted mice exhibited a net Th1-inclined cytokine response (Fig. 5D).

To examine a possible link between TTL-specific antibody responses and the apparent skewed Th1 cytokine environment in CD4+CD25+-T-cell-depleted mice, we measured the TTL-specific IgG1 (Th2 indicator) and IgG2a (Th1 indicator) antibody titers in plasma on D7 pi. CD4+CD25+-T-cell-depleted mice exhibited a relatively stronger anti-TTL IgG2a and a weaker IgG1 antibody response compared to those of control mice (Fig. 6A-B). In agreement with Fig. 5D, CD4+CD25+-T-cell-depleted mice exhibited a Th1-inclined response as evidenced by higher IgG2a/IgG1 ratios ($p<0.05$) compared to controls (Fig. 6C).

DISCUSSION

Data from the present study indicate that infection with *T. congolense* increases the frequencies of CD4+CD25+ T cells. In an attempt to define the possible role of CD4+CD25+ T cells during murine AT, we selectively depleted these cells with anti-CD25 mAb prior to challenge with a lethal mutant strain of *T. congolense* IL1180. We observed that CD4+CD25+ T-cell-depletion enhanced trypanotolerance in infected mice as evidenced by significant reduction in parasitemia, pathology, as well as a moderate but significant increase in survival rate. In agreement with

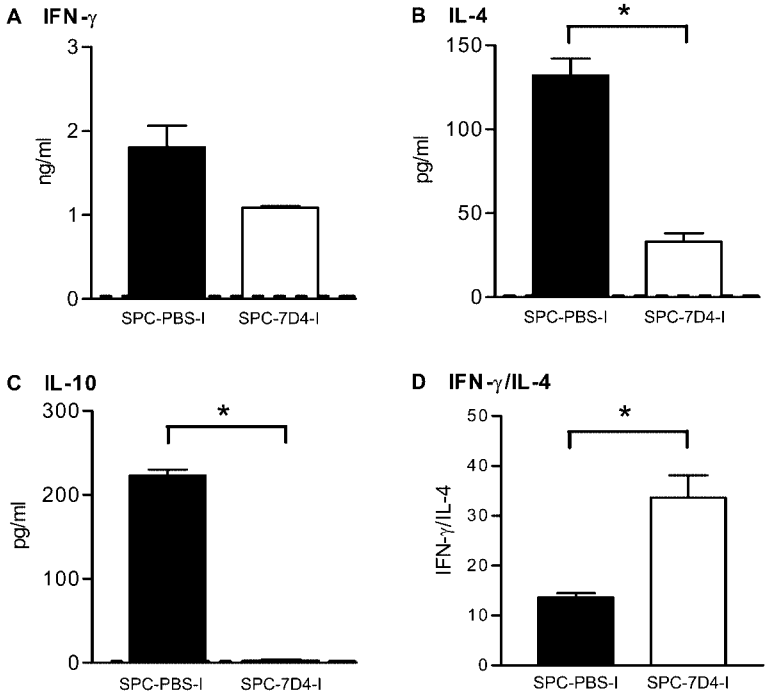

Fig. 5. $CD4^{+}CD25^{+}$ T-cell-depletion correlates with a skewed Th1 response in plasma from *T. congolense*-infected mice. On D7 pi, levels (mean + SE; n=5) of IFN-γ (A), IL-4 (B), and IL-10 (C) in the plasma of mice from groups A and B were quantified and IFN-γ/IL-4 (D) ratios calculated. Statistical analyses were assessed using a PRISM computer program. *P*-values of <0.05 were considered statistically significant. Dashed horizontal lines represent average cytokine levels in plasma from group C mice. Asterisks indicate statistically significant difference between groups A and B.

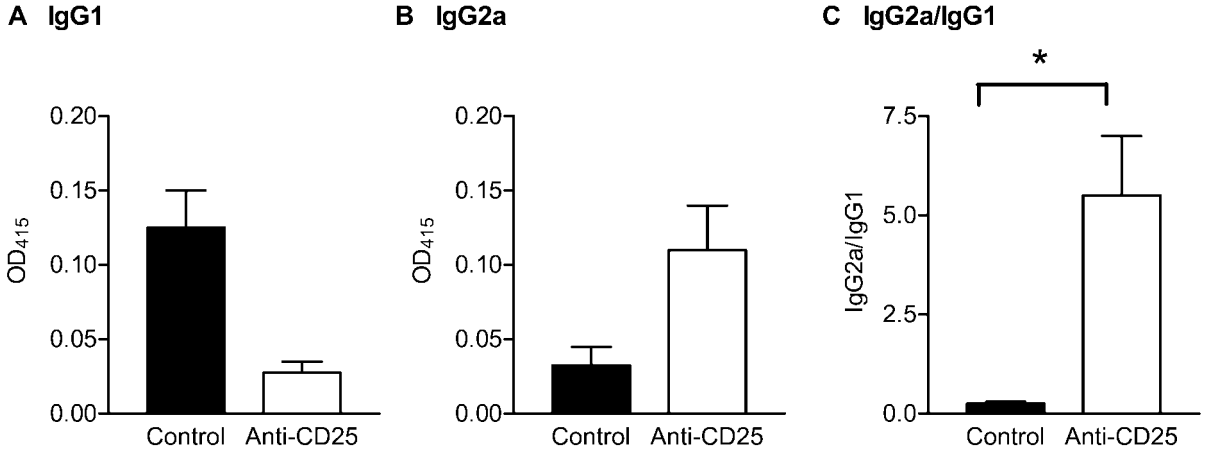

Fig. 6. CD4+CD25+ T-cell-depletion correlates with suppression of parasite-antigen-specific IgG1 and up-regulation of IgG2a titers in *T. congolense*-infected mice. Titers (mean + SE; n=5) of TTL-specific IgG1 (A) and IgG2a (B) antibodies were determined using plasma collected from groups A and B mice on D7 pi and IgG2a/IgG1 ratios (C) calculated. Statistical analyses were assessed using a PRISM computer program. *P*-values of <0.05 were considered statistically significant. Asterisks indicate statistically significant difference between groups A and B.

Tabel *et al.* [32], control BALB/c mice exhibited increased susceptibility to *T. congolense* infection as evidenced by earlier and more severe morbidity. In contrast, $CD4^+CD25^+$-T-cell-depleted mice remained clinically well until after one week pi, at which time the control mice had started dying. Furthermore, $CD4^+CD25^+$-T-cell-depleted mice exhibited a steady weight gain, whilst control mice were gradually becoming emaciated.

To gain insights into mechanisms underlying the apparent enhanced trypanotolerance in $CD4^+CD25^+$-T-cell-depleted mice, we analyzed the modulation of various immune responses during the course of infection. In agreement with Oldenhove *et al.* [21], we demonstrated a profound suppression of Th2 cytokines that culminated into a net Th1 cytokine environment during the period when $CD4^+CD25^+$-T-cell-depleted mice exhibited resistance to the lethal infection. Indeed, Th1 cytokines are reportedly protective against AT through their contribution to the control of the initial and most aggressive parasitemic waves [9, 17, 19, 27]. The enhanced proinflammatory response (increased expression of IFN-γ, TNF-α, iNOS and secretion of IFN-γ) following anti-CD25 mAb treatment appears to be beneficial in our model and could be at the basis of the observed increased trypanotolerance. It seems that this treatment regime preferentially induces trypanosome-antigen-specific Th1 cells to secrete increased IFN-γ levels which in turn classically activate macrophages (Mø) [7, 17] as evidenced by up-regulation of both TNF-α and iNOS mRNA levels in splenocytes from treated mice. In agreement with previous reports, such classically-activated Mø could contribute to parasite elimination through enhanced phagocytic and microbicidal activities [18, 30, 31] and also through enhanced expression and secretion of trypanotoxic TNF-α and NO molecules during the initial stage of the infection [7, 15, 34]. Cumulatively, these findings suggest that during *T. congolense* infection, the beneficial anti-trypanosome Th1 responses are generally suppressed, at least in part, by cells within the $CD4^+CD25^+$ T cell subpopulation in mice as is the case in tuberculosis and onchocerciasis [23, 26]. However, the role of $CD4^+CD25^+$ T-cells in various parasitic infections seems to vary according to parasite species and even strains [10, 14]. Although we cannot exclude the possibility that our results are only applicable to immune responses against the particular parasite strain used in this study, our current hypothesis is that the resultant Th1 cytokine milieu following $CD4^+CD25^+$ T cell-depletion is protective.

$CD4^+CD25^+$ T cells posses both effector and regulatory functions [23, 35]. The latter subpopulation occurs naturally in mice and man and accounts for 5–10% of circulating $CD4^+$ T cells in healthy subjects [23]. Such regulatory T cells (TR) suppress the activation and proliferation of effector $CD4^+$ and $CD8^+$ T cells [2, 25]. In the present study, depletion of $CD4^+CD25^+$ T cells enhanced trypanosome-antigen-specific IFN-γ production and inhibited IL-10 and IL-4 secretion. This indicates that the increased frequencies of $CD4^+CD25^+$ T cells during AT are not simply a reflection of excessive immune activation, but that a subset of this population may have immunoregulatory properties.

Interestingly, Guillams *et al.* [8] recently demonstrated that T_R contribute to trypanotolerance in *T. congolense*-infected C57Bl/6 mice. Considering the possibility of T_R being involved in our model, the discrepancy in their and our findings could be based on the differences in the mouse strains used in the two studies. In the relatively resistant C57Bl/6 model, exhibiting a type-I cytokine environment during early-stage *T. congolense* infection, T_R play a beneficial role of suppressing excessive and sustained proinflammatory responses and hence control immunopathology [8, 20]. In contrast, in an extremely *T. congolense* susceptible BALB/c model which secretes high levels of mixed type-I/type-II cytokine [20, 32], a pro-inflammatory response is desirable for parasite control during early stage of infection. In this model, T_R could be undesirable as they contribute towards production of suppressive type-II cytokines such as IL-4. That could explain the skewed proinflammatory response which in turn contributed to enhanced trypanotolerance following depletion of T_R in BALB/c mice. However, it is noteworthy that T_R are not exclusively contained within the CD25-expressing subset but rather in the Foxp3-expressing subset, which may be $CD25^-$ [35].

Conversely, this study identified $CD4^+CD25^+$ T cells to contribute to pathogenesis of AT, possibly through their expression and secretion of elevated levels of suppressive cytokines (IL-10, TGF-β, and IL-4). It is conceivable to speculate that trypanosomes may deliberately activate $CD4^+CD25^+$ T cells to induce a favorable immune response for their survival. For instance, IL-10 is documented to have a profound suppressive effect on both the production and functional activity of IFN-γ [11]. Thus, a simultaneous increase in both IL-10 and IFN-γ levels renders the latter functionally ineffective. Interestingly, a sudden increase in circulating Th2 cytokines was observed in $CD4^+CD25^+$-T-cell-depleted mice at the time they succumbed to the lethal *T. congolense* infection (data not shown).

It is noteworthy that $CD4^+CD25^+$ T-cell-depletion in this study did not result in sustained protection in acutely *T. congolense*-infected mice. According to Zelenay *et al.* [35], this may not be surprising as $CD4^+CD25^+$ cell pool progressively recover normal frequency in less than a month after depletion. On the other hand, that could also suggest that it may truly be T_R responsible for the effect, but since the method used in this study could not depleted all the T_R cells, but only $CD4^+CD25^+$ cells [35], the protective effect could not be maximized or sustained. Considering the severity and acute nature of this strain of *T. congolense* infection in BALB/c mice, $CD4^+CD25^+$ T cells could play a significant role in the pathogenesis of AT. This study suggests that additional immunoregulatory events may also be critical. This notion is further supported by our observation that, although significantly less depressed than in control mice, mitogen-induced lymphocyte proliferation remained largely suppressed in the $CD4^+CD25^+$-T-cell-depleted mice. Indeed, although our data suggest that $CD4^+CD25^+$ T cells

may be a source of suppressive Th2 cytokines, it is well established that other cell types, including Mø, $CD4^+$ and $CD8^+$ T cells, may also secrete suppressive cytokines [27, 31].

In conclusion, we demonstrated that depletion of $CD4^+CD25^+$ T cells can evoke a temporary but significant anti-trypanosome protection in infected mice. Accordingly, $CD4^+CD25^+$ T-cell-depletion leads to a biased Th1 cytokine phenotype that may be responsible for the significant reduction of *T. congolense* growth, reduced pathology, and a significant delayed mortality. Conversely, our data suggest that during acute *T. congolense* infection, activation of $CD4^+CD25^+$ T cells contributes to trypanosusceptibility. Further studies should be directed at characterizing more extensively the phenotype of $CD4^+CD25^+$ T cells during the course of AT.

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