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Regulatory T Cells Prevent Control of Experimental African Trypanosomiasis

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African trypanosomes are single-cell, extracellular blood parasites causing profound immunosuppression. Susceptible BALB/c mice infected s.c. into a footpad with $10^4$ Trypanosoma congolense die with fulminating parasitemia within 10 days. We injected BALB/c mice 2 days before such an infection with different doses of a depleting mAb specific for CD25, a surface marker of regulatory T cells (Tregs). Pretreatment with a low, optimal dose of anti-CD25 resulted in a dramatic effect, in that the infected mice did not develop parasitemia, as well as eliminated all parasites and showed no signs of disease. Their spleens showed a 100% reduction of CD4+CD25$^{\text{high}}$ T cells and overall a 70% reduction of CD4+CD25$^{\text{low}}$Foxp3$^+$ T cells 7 days postinfection. The protective effect of treatment with an optimal dose of anti-CD25 could be reversed by administration of $l$-N6-(1-iminoethyl) lysine, a specific inhibitor of inducible NO synthase or administration of anti-CD8 Ab. Analysis of the cytokine patterns and cell surface marker in infected mice pretreated with anti-CD25 Abs pointed to a potential NKT cell response. We then conducted infections in C57BL/6 $^{\text{a}}$ mice that differ from Th1 cells in that they adhere to glass, plastic, and nylon wool (10, 14).

The subpopulation of pathogenic T cells producing most of the IFN-$\gamma$ in the T. congolense-infected BALB/c mice are MHC class-II-restricted CD4$^+$ T cells that differ from Th1 cells in that they adhere to glass, plastic, and nylon wool (10, 14).

We found that CD4$^+$ T cells mediate immunosuppression in BALB/c mice infected with T. congolense (10). CD4$^+$ CD25$^+$ regulatory T cells have been shown to prevent the development of autoimmune disease (15) as well as to modulate the immune responses to a number of infectious agents (16, 17). The following experiments were conducted to examine the potential role of natural T regulatory cells in modulating infections by T. congolense in susceptible BALB/c mice. We attempted to eliminate CD25$^+$ regulatory T cells through treating mice with mAb to CD25 (anti-CD25) before infection. Considerations led us to carefully examine the effects of administering different amounts of anti-CD25 mAb. A number of different T cell types, including activated T cells, express CD25 at different levels. Administering different amounts of depleting anti-CD25 Ab is therefore likely to affect different CD25$^+$ T cell types differently (18). We found that administration of an optimal (low) dose of anti-CD25 Abs prevented disease and death in highly susceptible BALB/c mice infected with T. congolense. The treatment led to complete elimination of the parasites.

Materials and Methods

Mice

Female, 8- to 10-wk-old BALB/c AnNCrlBR (BALB/c) mice and 5- to 6-wk-old female outbred Swiss white mice (CD1) were purchased from the Animal Resource Center of the University of Saskatchewan (Saskatoon, Canada). Female, 8- to 10-wk-old C57BL/6$^{\text{a}}$ BALB/c mice were purchased from The Jackson Laboratory. The mice were kept in polycarbonate cages on sawdust, and allowed free access to food and water throughout the experiments, according to the recommendations of the Canadian Council of Animal Care.

Parasite

T. congolense, Trans Mara strain, variant antigenic type TC13 was used in this study. The origin of this parasite strain has been described previously.

Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

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1 This work was funded by the Canadian Health Research Institute/Regional Partnership Program.

2 Address correspondence and reprint requests to Dr. Henry Tabel, Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B4, Canada. E-mail address: henry.tabel@usask.ca

3 Abbreviations used in this paper: VSG, variant surface glycoprotein; i.f.p., into a footpad; i.NIL, i.-N6-(1-iminoethyl) lysine; Treg, regulatory T cell; wt, wild type.

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Frozen stabilates of parasites were used for infecting CD1 mice immunosuppressed with cyclophosphamide, and passages were made i.p. every third day as described previously (19). The parasites purified from the blood of infected CD1 mice by DEAE-cellulose chromatography (20) were used for infecting BALB/c mice.

Infection, estimation of parasitemia, and survival time

For the experiments, mice were infected s.c. into a hind footpad (i.f.p.) with $10^4$ T. congolense variant antigenic type TC13. A drop of blood was taken from the tail vein of each infected mouse. The parasitemia was estimated by counting the number of parasites present in at least 10 fields at $\times 400$ magnification by phase-contrast microscopy. A count of 256 parasites per field is equivalent to $10^9$ parasites/ml (21). To test for infection of the blood of anti-CD25 Ab-treated mice, which were subsequently infected i.f.p. with T. congolense but did not develop detectable parasitemia, three blind passages were conducted. At day 7 postinfection, 0.5 ml of blood taken from the vena cava was injected i.p. into an immunosuppressed mouse. After 3 days, 0.5 ml of blood was taken from this mouse and injected into another immunosuppressed mouse. The procedure was repeated once more. Parasitemia in these mice was assessed as shown above. The survival time was defined as the number of days postinfection that the infected mice remained alive. Moribund mice were euthanized.

Hybridomas

The rat hybridoma PC61 (IgG), secreting an anti-CD25 mAb and rat hybridoma TIB210 (IgG), anti-mouse CD8, were purchased from the American Type Culture Collection (ATCC, Manassas, Virginia).

Abs and reagents

The following Abs were purchased from BD Pharmingen: purified anti-mouse CD16/CD32 (FcγRII Receptor, Clone: 2.4G2); FITC-conjugated rat anti-mouse CD4 (RM4-5); PE-conjugated rat anti-mouse CD25 (53-6.7); PE Cy5-conjugated rat anti-mouse Foxp3 (H57-597); PE-conjugated rat anti-mouse CD122 (TM-β1); and OptEIA sets of anti-mouse IFN-γ, IL-10, IL-6, TNF-α, and MCP-1. Monoclonal anti-murine IFN-γ Ab (XMG1.2), used for the in vivo treatment, was a gift of Dr. C. Havele, University of Saskatchewan (Saskatoon, Canada). Mouse Treg Staining Kit was purchased from eBioscience. Griess reagent system for detection of nitrite and control rat IgG was purchased from Sigma-Aldrich Canada. L-N6-(1-imminoethyl) lysine (L-NIL) was purchased from A.G. Scientific.

FIGURE 1. Treatment with an optimal dose (45 µg) of anti-CD25 mAb PC61 results in a complete absence of parasites in the blood of T. congolense-infected mice. Groups of 5 to 10 BALB/c mice were treated i.v. with control rat IgG (A) and varying doses of rat anti-mouse CD25 mAb PC61 (B–F) on day −2. They were then infected s.c. with $10^4$ TC13 in a hind footpad on day 0. Parasitemia was monitored after the infection. Note: 1/5 of the mice in the 85 µg (C), 4/10 in 15 µg (E), and 10/10 in 45 µg (D) mAb-treated groups did not show any detectable parasitemia throughout the experiment (termination on day 90).

FIGURE 2. Pretreatment of highly susceptible BALB/c mice with an optimal low dose of anti-CD25 mAb PC61 results in a complete protection against TC13 infection. Groups of 5 to 10 BALB/c mice were treated i.v. with either rat control IgG or varying doses of mAb PC61 on day −2. They were then infected s.c. with $10^4$ TC13 in a hind footpad on day 0. Survival of mice was monitored after infection.
Treatment of mice with anti-CD25 mAb PC61, anti-CD8 mAb TIB 210, anti-IFN-γ mAb XMG1.2, and L-NIL

Groups of BALB/c mice were injected i.v. with 300, 85, 45, 15, 3, or 1 μg of mAb PC61 or rat control IgG on day −2. Some groups of mice were injected i.v. with 150 μg of anti-CD8 on day −2. For in vivo anti-IFN-γ treatment, some groups of mice were injected i.p. with 200 μg of anti-IFN-γ/mouse/day on days 0, 2, and 4 postinfection. Some groups of mice were injected i.p. with L-NIL (10 mg/kg body weight) daily from day 0 to 5 postinfection.

Flow cytometry

Spleen cells were collected from *T. congolense*-infected BALB/c mice. The cells were incubated (15 min at 4°C) with purified anti-mouse CD16/CD32 (2.4G2) mAb to block nonspecific binding Ab to Fc receptors, washed with staining buffer, resuspended in staining buffer, and surface stained with the relevant appropriate Abs. Intracellular staining for Foxp3 was performed using the Treg staining kit (eBioscience) in accordance with the manufacturer’s recommendations. In brief, cells were treated with fixative/permeabilization buffer to fix and permeabilize the cells. Intracellular staining was then performed using PE-Cy5-conjugated anti-Foxp3 Ab and PE-conjugated rat IgG1 (isotype-matched control Ab). Samples were resuspended in FACS staining buffer and analyzed on a FACScan flow cytometer using CellQuest software (BD Biosciences).

Splenic cultures for measurement of cytokine synthesis

Spleen cell suspensions were prepared from *T. congolense*-infected BALB/c mice on varying days postinfection. Cells were cultured in 24-well tissue culture plates at an optimal cell density of 5 × 10^6 cells/ml (1 ml/well) in a humidified incubator containing 5% CO₂. The culture supernatant fluids were collected after 48 h and centrifuged at 1,500 g for 10 min. The supernatant fluids were stored at −35°C until used for cytokine assays.

Cytokine assays

Cytokines IFN-γ, IL-10, IL-6, TNF-α, and MCP-1 in culture supernatant fluids were determined by routine sandwich ELISA using OptEIA sets from BD Pharmingen and Immuno-4 plates (Dynax Technologies), according to the manufacturer’s protocols. Each sample was tested for each cytokine in triplicate.

Measurement of nitrite production

Nitrite concentrations in the culture fluids harvested after 24 h were determined by the “Griess reagent system” from Promega as described previously (22). In brief, 50 μl of culture supernatant fluids were incubated with an equal volume of sulfanilamide solution (1% sulfanilamide, Sigma-Aldrich in 5% phosphoric acid, Sigma-Aldrich) for 5–10 min at room temperature, protected from light. Then, 50 μl of the NED (Sigma-Aldrich) (0.1% N-1-naphthylethylenediamine dihydrochloride in water) was added.
and incubated for another 10 min at room temperature, protected from light. The absorbance was measured at 550 nm in a microELISA reader. Nitrite levels were determined by comparison with a sodium nitrite (Sigma-Aldrich) standard curve. The sensitivity of this assay was 2.5 μM.

Statistical analysis
Data are represented as means ± SE. Significance of differences was determined by ANOVA using StatView SE 1988 Software (Abacus Concepts) or a log-rank test for curve comparison using a GraphPad Prism computer program (BD Biosciences).

Results
Treatment with an optimal, low dose of a monoclonal anti-CD25 Ab before infection results in a complete protection against T. congolense infection and elimination of the parasites
BALB/c mice were given different amounts of monoclonal anti-CD25 Ab (mAb PC61) i.v. 2 days before s.c. infection i.f.p. with 10⁶ T. congolense, clone TC13. Mice pretreated with control Ab died between days 8 and 10 with fulminating parasitemia (>10⁹ parasites/ml blood) (Fig. 1A). Treatment of BALB/c mice with a single dose of 300 μg anti-CD25 Ab led to partial control of parasitemia (Fig. 1B) and enhanced survival time (Fig. 2). Pretreatment with a lower dose of anti-CD25 Ab (85 μg) led to a greater reduction of parasitemia (Fig. 1C) and an even longer survival time (Fig. 2). Treatment of BALB/c mice with a single dose of 45 μg anti-CD25 Ab, which was found to be the optimal dose, led to complete control of the infection without any detectable parasitemia (Fig. 1D). These mice remained free of parasites in the blood as assessed both by microscopic examination and by injection of their potentially infected blood into immunosuppressed mice. With the use of blind passages, this procedure can detect a single parasite (23). The mice pretreated with the optimal dose of anti-CD25 Ab remained without any apparent disease up to the end of the observation period (day 90) (Fig. 2). As assessed by levels of parasitemia and survival, pretreatment of infected mice with lower amounts (15 μg) of anti-CD25 Ab yielded partial protection (Figs. 1E and 2), and with even lower amounts, i.e., 3 or 1 μg of anti-CD25 Ab, no significant effects (Figs. 1F and 2). Analysis by FACS of spleen cells from optimally protected mice at day 7 postinfection indicated, in contrast to the infected control mice, an absence of CD25high T cells (Fig. 3). They had, however, retained overall ~30% of the CD4⁺CD25⁺Foxp³⁺ T cells (p < 0.05), expressing CD25 at a relatively low level (Figs. 3 and 4).

Treatment with the optimal dose of anti-CD25 Ab before infection results in an increase of early cytokine release
We examined the kinetics of various responses of infected mice either pretreated with rat IgG or with the optimal amount of the anti-CD25 Ab. We determined the production of IFN-γ, TNF-α, MCP-1, IL-6, IL-10, and NO in the spleen cell culture supernatants of infected mice previously (10, 12).

FIGURE 5. Anti-CD25 treatment before infection alters the profiles of cytokines and NO in mice infected with T. congolense TC13. Groups of BALB/c mice were treated i.v. with 45 μg of anti-CD25 mAb PC61 or rat IgG control on day −2. They were then infected s.c. with 10⁶ TC13 in a hind footpad on day 0. Spleen cell cultures were conducted on different days after infection. Cytokines in the spleen cell culture supernatants were determined by ELISA. Open bar, control group; Filled bar, anti-CD25 treated group. Note: On day 5 postinfection, all cytokines (except IL-10) tested and NO were significantly higher in the anti-CD25-treated group than in the control group (*, p = 0.05; **, p = 0.005).
Parasitemia was monitored after infection by examining the effect of anti-IFN-γ Ab on the production of NO. We tested a prediction of this hypothesis that inhibition of CD25+ regulatory T cells (Tregs) in normal conditions. We then tested the effect of a specific inhibitor specific for inducible NO synthase, L-NIL, on mice treated with anti-CD25 Ab. CD122, the IL-15 receptor, is a marker for NK T cells. In fact, IL-15 is a pivotal cytokine for the development and survival of NK T cells.

The completely protective effect of anti-CD25 Ab treatment is not reversed by anti-IFN-γ Ab but is reversed by treatment with an inhibitor specific for inducible NO synthase. IFN-γ-induced NO has been shown to be a major mechanism for controlling T. congolense infections (24, 25). Because our data showed that IFN-γ and NO were significantly enhanced in the principal group at day 5 (Fig. 5, A and F), it was reasonable to hypothesize that some cells are not activated to produce NO in control infections, and that in the infected pretreated group, a subset of T cells produce IFN-γ that mediates the activation of macrophages to produce NO. We tested a prediction of this hypothesis by examining the effect of anti-IFN-γ Ab treatment on mice given anti-CD25 Ab 2 days before infection with 10^5 TC13. The anti-IFN-γ Ab (200 μg/day) was administered i.p. 0, 2, and 4 days after infection. This treatment did not reverse the protective effect of the anti-CD25 Ab treatment (data not shown), indicating that IFN-γ was not a major mediator of parasite control under these experimental conditions. We then tested the effect of a specific inhibitor of inducible NO synthase, L-NIL, on mice treated with anti-CD25 Ab 2 days before infection with 10^5 TC13. Mice were given L-NIL on days 0, 1, 2, 3, 4, and 5 postinfection. This treatment led to lethal infections (Fig. 6). These results lead us to suggest that the generation of NO, able to mediate parasite control (25), is somehow inhibited by CD25^high regulatory T cells (Tregs) in normal T. congolense infections.

Treatment of susceptible BALB/c mice with the optimal dose of anti-CD25 Ab results in an early elevation of CD8^+CD122^+ T cells in the spleen cell population. Because it seemed likely that the production of NO by macrophages was a result of macrophage activation by a subset of T cells, we examined the level of different T cell subsets in the spleen of mice at a time corresponding to 3 days posttreatment, i.e., 1 day postinfection. It can be seen from the observations recorded in Fig. 7 that anti-CD25 Ab-treated mice, whether uninfected or infected, have greatly elevated (p ≤ 0.05) CD8^+CD122^+ T cells at day 3 posttreatment. It appeared plausible that some of these CD8^+CD122^+ T cells might be responsible for the envisaged activation of macrophages to produce NO and to control the T. congolense infection in the mice pretreated with optimal amounts of anti-CD25 Ab. CD122, the β chain of the IL-2 as well as of the IL-15 receptor, is a marker for NK T cells. In fact, IL-15 is a pivotal cytokine for the development and survival of NK T cells.

FIGURE 6. The completely protective effect of anti-CD25 treatment in T. congolense infection was reversed by treatment with an inhibitor specific for inducible NO synthase. Groups of five to six BALB/c mice were treated with/without anti-CD25 mAb PC61 (45 μg/mouse) on day −2. On day 0, the mice were infected s.c. with 10^5 T. congolense in a hind footpad. Groups C and D were treated i.p. with 200 μg of L-NIL per mouse daily from day 0 to day 5. Parasitemia was monitored after infection.

FIGURE 7. Treatment of BALB/c mice with anti-CD25 mAb PC61 results in an early elevation of CD8^+CD122^+ cells in the spleen cell population. A group of BALB/c mice were treated with 45 μg of mAb PC61 on day −2. They were infected s.c. with 10^5 TC13 in a hind footpad on day 0. On day 1 postinfection, spleen cells were stained for FACS analysis. The results are representative of three different experiments. Note: The CD8^+CD122^+ cells were significantly increased (p ≤ 0.05) in anti-CD25-treated mice, both uninfected and infected.
The completely protective effect of anti-CD25 Ab treatment is reversed by treatment with anti-CD8 Ab

It has been shown that CD4$^+$CD25$^+$ regulatory T cells can control the synthesis of IFN-γ and other functions of NKT cells (28). A potentially protective role of NKT cells in trypanosome infections seems plausible as the immune response of mice immunized with the purified membrane form of VSG of *T. brucei*, which contains GPI, depends on CD1d-restricted NKT cells (29). There are different subsets (CD4$^+$, CD8$^+$, or CD4$^+$CD8$^+$) of NKT cells in the spleen of mice, that differ in their functions (30). Considering the observed change in the CD8$^+$ NKT cell populations, we analyzed the potential role of CD8$^+$ T cells. Mice were treated i.v. with the optimal dose of anti-CD25 Ab and with 150 µg of anti-CD8 Ab (mAb TIB 210) 2 days before infection with 10⁴ *T. congolense* in a hind footpad. Parasitemia was monitored after infection.

The completely protective effect of anti-CD25 Ab treatment is reversed by treatment with anti-CD8 Ab

Infections (Fig. 8). These observations demonstrate that a subpopulation of CD8$^+$ T cells is present in anti-CD25 Ab-treated mice and is essential for the control of the trypanosomes. Our observations suggest the activity of these CD8$^+$ T cells are held in check by CD25high Treg cells in normal BALB/c mice.

The protective effect of treatment with the optimal dose of anti-CD25 Ab is CD1d-dependent

It is known that NKT cells are CD1d-restricted in mice (31). We therefore investigated the potential role of CD1d-restricted T cells in this early protective response. We infected CD1d$^{-/-}$ BALB/c mice either exposed to optimal amounts of anti-CD25 Ab or to rat control IgG. Whereas infected wild-type (wt) mice did not control parasitemia (Fig. 9A) and had a mean survival time of 10 ± 1 days,
infected CD1d−/− mice yielded a heterogeneous result, with some mice controlling the early parasitemia while others died with fulminating parasitemia (Fig. 9C). The anti-CD25 Ab-treated, infected wt mice did not develop any parasitemia (Fig. 9B). Eighty percent of the anti-CD25 Ab-treated CD1d−/− mice developed parasitemia but did control subsequent waves of parasitemia (Fig. 9D) and survived for 34.5 ± 1.5 days. The development of parasitemia in the anti-CD25 Ab-treated CD1d−/− mice would support the notion that the protective, NO-inducing T cells are a CD1d-restricted NKT cell population.

Discussion

Although it is well established that activated Foxp3+ Tregs have a high expression of CD25, the use of anti-CD25 Ab to remove Foxp3+ Tregs is problematic because other T cell populations express CD25 (18). Thus, we considered it absolutely crucial to test the effects of giving different doses of anti-CD25 Ab. In our experimental design, the administration of a single dose of 45 μg of anti-CD25 Ab appeared optimal, in that it had removed all CD25high (Fig. 3) and ~70% of Foxp3+ T cells (Fig. 4) by 7 days of infection and completely prevented infection of the blood (Fig. 1) as well as disease (Fig. 2). The dramatic nature of this result allowed us to readily analyze the defense mechanisms involved.

Analysis of the kinetics of production of cytokines and NO by spleen cell cultures (Fig. 5) pointed to a T cell-mediated defense at day 5 and earlier. Treatment with a specific inhibitor of inducible NO synthase (Fig. 6) shows that NO controls trypanosomes in infected BALB/c mice previously treated with the optimal dose of anti-CD25 Ab. We conclude that the control of trypanosomes in infected BALB/c mice pretreated with the optimal dose of anti-CD25 is mediated by CD8+ NKT cells activating macrophages to produce NO. We come to this conclusion for several additional reasons. Firstly, T. congolense are susceptible to NO in vitro (24) and in vivo (25) (Fig. 6). Secondly, purified membrane form of VSG of T. brucei has been shown to trigger a CD1d-dependent NKT cell response (29). Thirdly, the anti-CD25 Ab treatment enhances a population of CD8+ T cells expressing high levels of CD122 (Fig. 7), which is a marker of NKT cells (27). Fourthly, treatment with anti-CD8 Ab reverses the beneficial effect of anti-CD25 Ab treatment (Fig. 8). Finally, whereas wt mice treated with anti-CD25 Ab show no parasitemia (Fig. 9A), CD1d−/− mice treated with anti-CD25 Ab do develop parasitemia (Fig. 9D). The mechanism by which the CD8+ NKT cells might induce NO is presently unknown. The results of the treatment with anti-IFN-γ (see above) suggest that it is not mediated by IFN-γ.

The control of an existing parasitemia is mediated by Abs to the VSG of the parasites (6). Anti-CD25/anti-CD8 Ab-treated wt mice did not control the first wave of parasitemia (Fig. 8). In contrast, infected CD1d−/− mice pretreated with anti-CD25 Ab did control several subsequent parasitemias (Fig. 9C). The observation of control of parasitemia in the group of anti-CD25 Ab-treated CD1d−/− mice indicates reduced suppression of synthesis of anti-VSG Abs as compared with anti-CD8 Ab-treated mice. How can one explain this difference? These different results could be accounted for if another CD1d-restricted NKT cell population, different from the trypanosome-controlling CD8+ NKT cells, is contributing to expansion of Tregs. In fact, a subset of CD4+ NKT cells, but not CD4+ NKT cells, has been reported to secrete abundant IL-2 that, in turn, promotes proliferation of Tregs (32). Thus, we conclude that the anti-CD8-treated wt mice lack a CD8+ NKT cell population that induces early control of the trypanosomes but has a CD8− NKT cell population that contributes to the expansion of Tregs. The CD1d−/− mice treated with anti-CD25 Ab would contain a reduced population of Foxp3+ Tregs (Figs. 3 and 4) which, however, is not expanded by the hypothetical CD8− NKT cell population. The proposed NKT cell-mediated contribution to immunosuppression via causing the proliferation of the remaining Foxp3+ T cells (Figs. 3 and 4), would be missing in the anti-CD25 Ab-treated CD1d−/− mice. Such a condition would allow some production of anti-VSG Abs leading to the control of the subsequent waves of parasitemia (Fig. 9D). These observations and interpretations are in agreement with a view on cross-regulations of NKT cells and CD4+CD25+ Treg in maintaining immune tolerance (33).

Thus, based on our observations, we propose a hypothesis, i.e., there is a cross-regulation of NKT cells and regulatory T cells in trypanosomal infections: A CD8− NKT cell population is envisaged to contribute to the expansion of trypanosome-specific Tregs. By a negative feedback loop, NKT cells are subsequently downregulated by Tregs during progressive infections. An early CD8+ NKT cell response has the potential to control the parasites via macrophage-dependent production of NO, but, in regular infections, this response is held in check by Tregs.

Although it is apparent that NKT cells as well as Tregs influence the early kinetics of immune responses in trypanosomal infections, many questions remain. Can the elevated population of CD8+CD122+ T cells, present at day 3 in optimally treated mice, prevent a full blown infection of T. congolense when transferred to normal mice at the time of infection? Can our prediction be confirmed that a CD8− subset of NKT cells promotes proliferation of Tregs in trypanosome infections? Are the regulatory loops between different cell types of the immune system that we are uncovering in susceptible mice pertinent to what transpires in relatively resistant C57BL/6 mice and in different parasite infections? Apart from NKT cells, what parasite-derived factors and what parasite-mediated mechanisms drive the early activation/expansion of Tregs rather than the expansion of Th1 and/or Th2 cells in trypanosomal infections?

After completing this manuscript, we discovered a study on treatment with anti-CD25 Abs of relatively resistant C57BL/6 mice infected with T. congolense (34). The authors report findings and conclusions that differ from ours. We had recently speculated that Tregs would, via IL-10, down-regulate the immunopathology in infected relatively resistant C57BL/6 mice (14). Guilliams et al. (34) have now provided good evidence that Tregs control the immunopathology in T. congolense-infected C57BL/6 mice, with IL-10 being a major mediator. Although they also used administration of anti-CD25 Abs before infection, they did not, as we did, observe an early elimination of the parasites as an effect of the treatment. How can we explain this discrepancy? There are three major differences in their and our experimental designs: 1) Guilliams et al. (34) used a much higher dose of anti-CD25 Abs. In our hands, higher doses of anti-CD25 Abs did not lead to elimination of infection (Figs. 1 and 2). 2) Guilliams et al. (34), although they do not specifically indicate the route of infection, presumably infected the mice i.p. In the past, we also used the i.p. route of infection. We, however, altered our experimental route of infection in this study by injecting T. congolense s.c. into the footpad. We reasoned that the s.c. route of infection might more closely resemble the natural infection by the tsetse fly. In our hands, the dramatic effect of administration of anti-CD25 Abs is not obtained when mice are infected i.p. We have evidence that BALB/c mice infected i.p. with 105 T. congolense TC13 will die early with fulminating parasitemia, whereas infection i.p. results in the absence of detectable parasitemia and in survival (G. Wei and H. Tabel, unpublished observations). Such result would indicate that the local immune response to T. congolense infection significantly differs from the systemic immune response. 3) Although it is known that
BALB/c mice differ from C57BL/6 mice in their susceptibility to T. congolense infections by at least five genes located on chromosomes 17, 5, and 1 (35, 36), the genes involved are yet unknown. It is conceivable that there might be differences in BALB/c and C57BL/6 mice regarding early presentations of trypanosomal Ags to NKT cells and/or Tregs. We believe that the different outcomes of the two different experimental designs do not exclude each other. They simply show that the host-parasite interactions are very complex and still insufficiently understood. The complexity of the host-parasite relationship is demonstrated by multiple effects of IFN-γ as well as IL-10 in African trypanosomiasis. IFN-γ is mediating mortality in susceptible BALB/c mice infected with T. congolense (12, 37) and immunopathology in infected, relatively resistant C57BL/6 mice (34) but is also required for survival (25). IL-10 down-regulates production of IFN-γ and the immunopathology in trypanosome-infected mice (12, 34, 38) but also suppresses protective immune responses (39).

In summary, we show that an optimal amount of anti-CD25 Ab can eliminate most of the CD4+CD25+Foxp3+ regulatory T cells, leading to a complete control of infection of the extracellular parasite T. congolense in susceptible mice. We conclude that, in normal infections, Treg cells prevent the activation of CD8+ NKT cells which could activate macrophages to produce NO that kills T. congolense. Our observations and those in the literature have led us to propose a hypothetical model of cross-regulation of NKT cells and Tregs in trypanosome infections.

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Disclosures
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