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Original article

The involvement of $CD4^+CD25^+$ T cells in the acute phase of *Trypanosoma cruzi* infection

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Abstract

The infection with *Trypanosoma cruzi* leads to a vigorous and apparently uncontrolled inflammatory response in the heart. Although the parasites trigger specific immune response, the infection is not completely cleared out, a phenomenon that in other parasitic infections has been attributed to $CD4^+CD25^+$ T cells (Tregs). Then, we examined the role of natural Tregs and its signaling through CD25 and GITR in the resistance against infection with *T. cruzi*. Mice were treated with mAb against CD25 and GITR and the parasitemia, mortality and heart pathology analyzed. First, we demonstrated that $CD4^+CD25^+GITR^+Foxp3^+$ T cells migrate to the heart of infected mice. The treatment with anti-CD25 or anti-GITR resulted in increased mortality of these infected animals. Moreover, the treatment with anti-GITR enhanced the myocarditis, with increased migration of $CD4^+$, $CD8^+$, and $CCR5^+$ leukocytes, TNF-α production, and tissue parasitism, although it did not change the systemic nitric oxide synthesis. These data showed a limited role for CD25 signaling in controlling the inflammatory response during this protozoan infection. Also, the data suggested that signaling through GITR is determinant to control of the heart inflammation, parasite replication, and host resistance against the infection.

Keywords: *Trypanosoma cruzi*; Treg Cells; Myocarditis; Chagas cardiomyopathy

1. Introduction

Trypanosoma cruzi is the intracellular protozoan parasite which causes Chagas' disease, an important public health concern in Latin America [1]. Intense parasitemia, cardiac and skeletal muscular parasitism, and high mortality are hallmarks of acute infection in mice. The systemic dissemination of this pathogen is accompanied by an intense immune response, which allows the parasitemia control but also leads to massive infiltration of mononuclear cells in the myocardium, leading to local and systemic production of cytokines, chemokines and other mediators, such as nitric oxide (NO) [2]. A predominant Th1 reaction is central to the parasitism control and protective response against *T. cruzi*. $CD4^+$ T cells secrete TNF-α and IL-12, which stimulate the production of IFN-γ by NK, $CD4^+$ and $CD8^+$ T cells [3]. On the other hand, control of acute inflammation and avoidance of secondary tissue damage may occur by anti-inflammatory (Th2) cytokines like IL-4, IL-10 and TGF-β. These mediators can also down-regulate the intracellular control of parasite, the activity of Th1 cells and inhibit NO release, culminating in incomplete parasite elimination. However, in most cases, inflammatory damage predominates and there is no consensus about the role of Th1/Th2 balance in the immune response in Chagas' disease. Therefore, we believe that other cell populations might be involved in the counter-regulatory response necessary to prevent heart disease progression.

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In spite of this intense investigation, the mechanism by which *T. cruzi* induces localized heart damage remains unknown [1]. The vigorous and apparently uncontrolled response that is not capable to completely clear the infection could be explained by an altered immune regulatory mechanism, perhaps involving $CD4^+$ $CD25^+$ regulatory T cells (Tregs). These cells modulate the immune response, but are also involved in maintaining relapsing infections. They suppress the proliferation of co-cultured $CD4^+CD25^-$ T cells and inhibit both the induction and effector function of autoreactive T cells, representing a unique lineage of immune-regulatory cells [4]. In fact, parasite persistence in host tissues is related to an endogenous population of Tregs that, while it is important for the protection against a chronic self-wasting inflammatory reaction, suppresses the immune response, allowing parasite survival [4,5]. In humans infected by protozoan and fungus, the active Tregs in the lesions were associated with pathogen survival and disease persistence [6,7].

Natural Tregs represent 5–10% of the total peripheral $CD4^+$ T cells in healthy adult mice (2–4% in humans), and is responsible for maintaining self-tolerance. The main phenotype markers for this T-cell population are CD25, the transcription factor Foxp3, CD103, GITR, $CD45Rb^{low}$, and the negative co-receptor CTLA-4 [4,5,8]. The latter molecule is overexpressed during *T. cruzi* infection and is involved in the parasite persistence and suppression of the immune response [9]. To become suppressive (i.e. regulatory), $CD4^+CD25^+$ cells require activation through T-cell receptor [10]. Subsequently, the suppressor activity of Tregs is estimated to be mostly dependent on contact. However, it can also be induced by production of soluble factors such as TGF-β and IL-10 [11], both produced during *T. cruzi* infection [12,13].

The selective expression of chemokine receptors is a major determinant to drive effector cells (and Tregs) to the inflammatory site. Indeed, the infection with *T. cruzi* triggers the production of adhesion molecules [14] as well as chemokines [15], which potentiate the migration of leukocytes and the trypanocidal activity of macrophages [16,17]. Tregs express CCR5 and CCR4, and a selective response to CCL4 [4,18]. *T. cruzi* induces production of CCL3, CCL4, and CCL5 [17] and, consequently, the accumulation of $CCR5^+$ cells in the cardiac tissue of infected mice [19]. These data suggest that Tregs are able to migrate to the site of cardiac inflammatory lesion triggered by *T. cruzi*. Although a recent report using anti-CD25 blocking antibody in mice infected with *T. cruzi* suggested that Tregs do not suppress the effector function of CD8 T cells during infection [20], the role of GITR signaling remains unknown.

Here we investigated the involvement of $CD4^+CD25^+$ T cells in the pathogenesis of *T. cruzi* induced myocarditis, using monoclonal antibodies which are known to inhibit Treg functions through the blockade of CD25 or stimulation with agonistic anti-GITR antibody [8,20,21]. Our results indicate that treatment with anti-GITR but not anti-CD25 resulted in increased parasitism and cardiac inflammation, associated with increased production of the cytokine TNF-α, while production of TGF-β and IL-10 were reduced.

2. Materials and methods

2.1. Mice and infection

Female 6–8-week-old BALB/c (ten per group) were bred at the animal bounding facility of our institution, and all procedures had prior approval from the local ethics committee for animal care and research. The Y strain of *T. cruzi* was used in all experiments. Mice were infected intraperitoneally (i.p.) with 10^3 blood-derived trypomastigote forms, obtained from previously infected mice.

2.2. Antibodies and treatment

The anti-CD25 (PC61) and anti-GITR (DTA-1) hybridomas were grown i.p. in mineral oil-injected nude mice. The antibodies were purified from ascites by precipitation using ammonium sulfate (45%, w/v), and subsequently purified by G protein column (Amersham Biosciences USA, Piscataway, NJ). Protein was quantified by bicinchoninic method. The blockage of CD25 molecules was performed by i.p. injecting 500 μg/mouse purified mAb each 72 h, during 2 weeks before the infection. The anti-GITR was injected 24 h before infection (500 μg/mouse; single dose). Controls received 500 μg of normal rat IgG diluted in PBS.

2.3. Parasitemia and mortality

The amount of circulating parasites was monitored by periodic microscopic analysis of 5 μl blood samples drawn from the tail vein during the first 3 weeks of infection. Survival rates were determined in independent groups of animals.

2.4. Histological evaluation

Three mice from each experimental group were euthanized at several time points after infection. The hearts were removed, fixed in neutral 10% formalin, embedded in paraffin, sectioned (5 μm), hematoxylin–eosin stained, and examined by light microscopy.

2.5. Immunohistochemical analysis

Heart fragments of three mice from each experimental group were frozen in O.C.T. compound (Sakura Finetek, Torrance, CA). Immunohistochemistry was performed using the avidin–biotin peroxidase method with rat anti-mouse CD8 (H-35, at 1:100 in PBS), IFN-γ (XMG1.2 1:200 in PBS 0.01% saponin), and goat anti-mouse CCR5 (8B10 1:50 in PBS 0.01% saponin) (Santa Cruz Biotechnology, Santa Cruz, CA). After overnight incubation the slides were incubated with biotinylated rabbit anti-rat or donkey anti-goat antibodies. The staining was developed with 3,3′-diaminobenzidine (DAB) (Vector Laboratories, Burlingame, CA) and counterstained with Mayer hematoxylin. Controls were performed by incubating slides with IgG isotype control instead of primary antibodies and proceeding as described