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Original article

Treatment of chronically *Trypanosoma cruzi*-infected mice with a CCR1/CCR5 antagonist (Met-RANTES) results in amelioration of cardiac tissue damage

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Abstract

The comprehension of the molecular mechanisms leading to *Trypanosoma cruzi*-elicited heart dysfunction might contribute to design novel therapeutic strategies aiming to ameliorate chronic Chagas disease cardiomyopathy. In C3H/He mice infected with the low virulence *T. cruzi* Colombian strain, the persistent cardiac inflammation composed mainly of $CCR5^+$ T lymphocytes parallels the expression of CC-chemokines in a pro-inflammatory IFN-γ and TNF-α milieu. The chronic myocarditis is accompanied by increased frequency of peripheral $CCR5^+LFA\text{-}1^+$ T lymphocytes. The treatment of chronically *T. cruzi*-infected mice with Met-RANTES, a selective CCR1/CCR5 antagonist, led to a 20–30% decrease in $CD4^+$ cell numbers as well as IL-10, IL-13 and TNF-α expression. Further, Met-RANTES administration impaired the re-compartmentalization of the activated $CD4^+CCR5^+$ lymphocytes. Importantly, Met-RANTES treatment resulted in significant reduction in parasite load and fibronectin deposition in the heart tissue. Moreover, Met-RANTES treatment significantly protected *T. cruzi*-infected mice against connexin 43 loss in heart tissue and CK-MB level enhancement, markers of heart dysfunction. Thus, our results corroborate that therapeutic strategies based on the modulation of CCR1/CCR5-mediated cell migration and/or effector function may contribute to cardiac tissue damage limitation during chronic Chagas disease.

Keywords: Chagas disease; *Trypanosoma cruzi*; myocarditis; heart disease; Met-RANTES

1. Introduction

Infection with the protozoan parasite *Trypanosoma cruzi* afflicts 15–16 million people in Latin America, nearly one-third of which present clinical manifestations of chronic Chagas disease [1]. Chronic chagasic cardiomyopathy (CCC), the most important clinical form of this disease, is mainly characterized by myocarditis associated with prominent fibrosis and organ dysfunction [2]. *T. cruzi* infection results in immunological attack of host tissues, and although autoimmunity has been raised to explain this, the most accepted conjecture is that cardiac injury results from unbalanced effector immune responses that are elicited by persistent parasites [3]. Identifying the molecular mechanisms responsible for inflammatory injury without interfering with parasite clearance is required to design effective therapies aiming to ameliorate heart fibrosis and dysfunction in CCC patients [4].

Abbreviations: CCC, chronic chagasic cardiomyopathy; Met-RANTES, N-terminal-methionylated RANTES.

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In the affected cardiac tissue there is a local production of inflammatory mediators, chiefly cytokines and chemokines, which might drive leukocyte migration contributing to CCC formation [5]. Chemokines, small (8–14 kDa) constitutive or inducible/inflammatory cytokines, comprise four protein subfamilies (CXC or α, CC or β, C or γ and CX_3C or δ) acting on seven trans-membrane spanning G-protein-coupled serpentine receptors expressed on the surface of several cell types, including leukocytes [6]. *T. cruzi*-infected macrophages and cardiomyocytes produce CC-chemokines CCL5, which stimulate infected cells to control *T. cruzi* growth in a nitric oxide (NO) dependent manner [7,8]. Conversely, elevated plasma concentrations of chemokines have been associated with heart failure severity [9] and chagasic cardiomyopathy [10]. Enhanced expression of CCR5 (receptor for CCL5/RANTES, CCL3/MIP-1α and CCL4/MIP-1β) was detected on leukocytes of CCC patients [11,12] and *T. cruzi*-infected mice [4,13]. Moreover, it has been described that the polymorphism at the CCR5-59029 G allele, related to lower expression of CCR5, is more frequent in asymptomatic compared to CCC patients [14]. These results led us to consider that CC-chemokines, especially CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES, acting on CC-chemokine receptors, particularly CCR5, could be involved in pathogenesis of *T. cruzi*-elicited cardiomyopathy [4]. To test this idea, we employed N-terminal-methionylated RANTES (Met-RANTES), a selective CCR1/CCR5 partial antagonist, to treat acutely *T. cruzi*-infected mice. Met-RANTES administration significantly inhibited heart inflammation in the absence of parasite burden, demonstrating that the massive influx of $CCR5^+$ inflammatory cells into cardiac tissue is not crucial for anti-*T. cruzi* immunity [4]. During the chronic phase of *T. cruzi* infection, most of the heart tissue remodeling and dysfunction is probably due to the unbalanced inflammation, and no efficient treatment is currently available [1–3]. In this context, the beneficial effect of Met-RANTES controlling fibronectin deposition and selectively modulating the cytokine profile in acutely infected animals [4] encouraged us to investigate its effects on myocarditis formation and heart dysfunction during the chronic phase of *T. cruzi* infection.

2. Materials and methods

2.1. Animals

All experiments were performed with 5- to 7-week-old female C3H/He ($H\text{-}2^K$) mice from our animal facilities (CECAL, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil). The animals were maintained under standard conditions and treated according to institutional guidelines regarding ethics of animal usage (CEUA-Fiocruz, protocol #161/03).

2.2. Parasites and experimental infection

Mice were infected intraperitoneally with 100 blood trypomastigote forms of the low virulence *T. cruzi* Colombian strain isolated from a cardiac chagasic patient and maintained by serial passages from mouse to mouse [4]. Parasitemia was estimated from 5 μl of tail vein blood and established as a parameter for acute and chronic phases [15].

2.3. Treatment of T. cruzi-infected mice with Met-RANTES

Groups of 8–10 mice were subcutaneously inoculated daily with 0.1 ml of in vivo injection-grade saline (BioManguinhos) or saline containing 10 μg of Met-RANTES from 150 to 180 day post-infection (dpi). The Met-RANTES was a kind gift from Dr Amanda Proudfoot (Serono Pharmaceuticals, Geneva, Switzerland). The parasitemia and survival rate were evaluated daily, and the animals were killed under anesthesia at 180 dpi.

2.4. Antibodies

The specific polyclonal antibody recognizing mouse fibronectin was purchased from Gibco-BRL (USA), the specific polyclonal antibody recognizing inducible nitric oxide synthase (iNOS) from mouse macrophages (RAW 264.7) from Cayman Chemical (USA), and the anti-connexin 43 antibody produced in rabbit from Sigma (#C6219, USA). The specific polyclonal antibody recognizing *T. cruzi* antigens was produced in our laboratory (LBI/IOC-Fiocruz, Brazil). Biotin- and FITC-conjugated anti-mouse CD8a (clone 53-6.7); APC- and biotin-conjugated anti-mouse CD4 (clone GK1.5); PE-conjugated anti-mouse CCR5 (clone C34-3448); FITC-conjugated anti-CD11a (LFA-1 or CD11a/CD18b, clone M17/4), the purified monoclonal antibody recognizing the conformational altered form of β1 integrin chain (CD29, clone K20); Cy5-chrome-streptavidin were purchased from PharMingen (USA). The anti-F4/80 polyclonal antibody recognizing macrophages was purchased from Caltag (USA). Hybridomas producing monoclonal antibodies anti-CD8α (clone 53-6.7) and anti-CD4 (clone GK 1.5) were obtained from the Cell Bank of the Federal University of Rio de Janeiro, expanded and the supernatants used for immunohistochemistry. The biotin-conjugated antibody recognizing rat immunoglobulins was purchased from DAKO (Denmark). Biotinylated anti-rabbit and peroxidase–streptavidin complex were purchased from Amersham (England). Appropriate controls were prepared by replacing primary antibodies with purified rat immunoglobulin or normal rabbit serum. All antibodies and reagents were utilized in compliance with the manufacturers' instructions.

2.5. Histological evaluation

Groups of 5–8 infected and 3–5 age-matched non-infected control mice were killed under anesthesia at various time points after infection. The heart tissue was processed and analyzed as previously described [15].

2.6. CC-chemokine and cytokine determination by ELISA

The concentrations of CC-chemokines and cytokines in the cardiac tissue were evaluated by enzyme-linked immunosorbent