

March 07, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8597-review.doc).

Title: Interferon-gamma And Other Inflammatory Mediators in Cardiomyocyte Signaling during Chagas disease Cardiomyopathy

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the reviewers' suggestions. Point-by-point replies to all the reviewers' comments are given below.

Reviewer 00646232:

Excellent review article covering the topic in a smooth and easy way. Very few comments

We would like to thank the reviewer for his (her) kind comments regarding our manuscript.

Reviewer 02522696:

This review analyzed the Chagas disease cardiomyopathy: a particularly aggressive inflammatory dilated cardiomyopathy that occurs decades after the initial infection with the obligate intracellular parasite *Trypanosoma cruzi* (T.cruzi) in 30% of infected individuals. The development of Chagas disease cardiomyopathy is associated with inflammation and activation of the immune system, with a local increased cardiac production of cytokines by the heart-infiltrating T cells and other mononuclear cells. The review is interesting and clearly written. We would like to thank the reviewer

We thank the reviewer for his (her) positive judgment of our work and for his (her) comments.

There are some minor suggestions: 1. The authors reported that "...Patients with Chagas disease cardiomyopathy have a progressive myocardial remodeling process with hypertrophy and fibrosis causing heart fiber damage, heart conduction abnormalities, arrhythmias, apical aneurysm, heart failure and sudden death...." The authors should explain the role of IFN-g and TNF-a in the clinical presentations of Chagas disease.

We have shown the molecular effects of IFN- γ and TNF- α in the section “IFN- γ in health and disease”. We described different work done with transgenic mice models to exemplify the effect of over expression of TNF- α and IFN- γ . These mice develop chronic active myocarditis, IFN- γ -mediated cardiotoxicity with left ventricular dilation and impaired systolic function, a true cardiomyopathy.

2. The authors should insert a “clinical implication paragraph”, where explaining the clinical treatment of these patients

We thank the reviewer’s suggestion and we included a new paragraph in the introduction section explaining the clinical treatment available for Chagas disease patients (as following):

“There are two drugs available to treat the acute phase of the disease, nifurtimox (nitrofurane) and benznidazole (nitroimidazole). The use of these drugs to treat the acute phase of the disease is widely accepted. However, their use in the treatment of the chronic phase is controversial. There is no specific treatment, against the parasite, that can benefit patients at the chronic stage of Chagas disease. The undesirable side effects of both drugs are a major drawback in their use, frequently forcing the physician to stop treatment. The treatment of chronic patients consists in controlling the symptoms and improving patient’s quality of life, by preventing cardiovascular complications following the guidelines to treat heart failure and arrhythmias.”

3. The authors should explain the role of diagnostic examinations (i.e. echocardiography, cardiac magnetic resonance). 4. What is the flow chart diagnostics aimed at preventing heart failure and sudden death?

We think this is beyond the scope of this paper which intends to review the basic mechanisms rather clinical/diagnostic/therapeutic themes. We preferred not to include these data in an already crowded review.

Reviewer 01954061

Introduction comment from article: “Regardless of the mechanisms underlying the initiation and maintenance of the myocarditis, the bulk of the evidence indicates that the inflammatory infiltrate is a significant effector of heart tissue damage.” Comment: This statement requires references in order to be believable. I can see that the ensuing text does attempt to do that. Perhaps the above statement could conclude by saying, “...as outlined in the following paragraphs.”

We thank the reviewer for his (her) careful review of our manuscript. We will include the sentence “as outlined in the following paragraphs” in the end of the statement “...the bulk of the evidence indicates that the inflammatory infiltrate is a significant effector of heart tissue damage”.

This section: “IFN- γ : A Yin-Yang mode of action in Chagas disease” “The concept of Yin and Yang is deeply rooted in Chinese philosophy. It describes the reach for balance and harmony. According to the Chinese philosophical concept, there are two opposing forces named Yin and Yang, which are constantly trying to gain the upper hand over each other [35]. IFN- γ and other cytokines as previously described for TNF- α [36] in the acute phase of infection by *T. cruzi* and other agents appears to follow this principle. A dual role for IFN- γ in pathogenesis and protection in chronic Chagas disease was also...” Comment: Introducing the “Yin-Yang” idea seems to me to be rather distractive. It would be adequate and keep the attention focused by simply talking about the “dual role...”. Documentation for this concept is well-referenced.

We agreed with the reviewer suggestion and we will remove the entire description of Yin-Yang concept from the paper.

The title is appropriate, attractive, anticipatory. But the manuscript body does not convincingly present the "facts" suggested by the title. What the reader wants to see is the actual patient evidence for the specific role of interferon-gamma as well as comparison to other cytokines. Overall, I think the manuscript would be improved by showing peripheral levels of the various cytokines and chemokines in actual patient data. Perhaps this could be accomplished by a suitable Table illustrating the levels as compared with non-Chaga control patients.

We will include the following table in the present manuscript:

Table I. Cytokine and chemokine expression in Chagas disease and animal models

<i>Cytokines/ Chemokines</i>	<i>Phase (acute / chronic / IND / severe / moderate CCC)</i>	<i>Host (mouse/human)</i>	<i>Organ/cell type</i>	<i>Reference</i>
IFN- γ	Severe CCC	human	Mononuclear cells	
IFN- γ	Severe CCC	human	myocardium	
IFN- γ	Severe CCC	human	Heart-infiltrating T cells	
IFN- γ	IND, Severe CCC	human	plasma	
TNF- α	Severe CCC	human	Mononuclear cells	
TNF- α	Severe CCC	human	Heart-infiltrating T cells	
TNF- α	Severe CCC	human	myocardium	
TNF- α	IND and Severe CCC	human	plasma	
IFN- γ	Acute/chronic	mouse	heart	
TNF- α	Acute/chronic	mouse	heart	
IL-6	Severe CCC	human	Heart-infiltrating T cells	
IL-2	Severe CCC	human	Heart-infiltrating T cells	
IL-4	Severe CCC	human	Heart-infiltrating T cells	
IL-10	Severe CCC	human	Heart-infiltrating T cells	
IL-7	Severe CCC	human	myocardium	
IL-15	Severe CCC	human	myocardium	
IL-12	Acute	mouse	Mononuclear cells	
IL-18	Acute	mouse	Mononuclear cells	
IL-10	Acute	mouse	Mononuclear cells	
TGF- β	Acute	mouse	Mononuclear cells	
IL-17	Chronic	mouse	Mononuclear cells	
CCL2, CXCL10,	Severe CCC	human	myocardium	

CXCL9 (mRNA)			
CCR2, CXCR3 (mRNA)	Severe CCC	human	myocardium
CCR5, CXCR3	Severe CCC, IND	human	Mononuclear cells
CCL5, CXCL9, CXCL10	Chronic	mouse	cardiomyocytes
CCR5	Chronic	mouse	heart
CCL5, CCL4, CXCR3 (mRNA)	Chronic	dog	heart

Reviewer 00646232:

I am very glad to review this paper because the subject is interesting. However, the following questions should be revised again. Firstly, the title should be corresponding with the text. The title contains interferon-gamm, other inflammatory mediators and CCC, however, we do not find "Other Inflammatory Mediators" which should describe in the paper. Secondly, in this paper, the Yin Yang theory of traditional Chinese medicine is not accurately described. The essence of yin-yang theory is opposition and unity. The viewpoint "IFN- γ : A Yin-Yang mode of action in Chagas disease" is farfetched. Thirdly, there are some problems with sentence and spelling. Maybe native English speaker should be warranted to help you in revising the MS.

We thank the reviewer for his (her) careful review of our manuscript. We agreed with the reviewer's comments and we removed the Yin-Yang comparison from the manuscript. An example of "other inflammatory mediators" that we described in the review are TNF- α , IL-7, IL-15, IL-12 and chemokines like RANTES/CCL5, MIP-2/CXCL2. Two native English-speaking researchers reviewed the manuscript and corrected it.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Cardiology*.

Sincerely yours,



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