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EXPRESSION AND LIBERATION OF CHEMOATTRACTANT MOLECULES IN HUVECS INDUCED BY rSSP4 DERIVED FROM T. CRUZI AMASTIGOTES

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ABSTRACT
During the acute phase of infection, *Trypanosoma cruzi* replicates extensively and releases immunomodulatory molecules that delay parasite-specific responses mediated by effector T cells. This mechanism of evasion allows the parasite to spread in the host. Parasite molecules that regulate the host immune response during Chagas' disease have not been fully identified, particularly proteins of the amastigote stage. In this work we evaluated the role of the GPI anchored SSP4 protein of *T. cruzi* as an immunomodulatory molecule in human umbilical cord (HUVEC). rSSP4 protein was able to induced the expression of genes and production of molecules involved in the inflammatory process, such as, cytokines, chemokines, metalloproteinases (MMPs) and adhesion molecules (CAMs) determined by RT-PCR and ELISA. These results suggest that the amastigote SSP4 molecule could play a key role in the immunoregulatory process observed in the acute phase of infection with *T. cruzi*.

INTRODUCTION

Trypanosoma cruzi infects many cell types, including myocytes, fibroblast, vascular endothelial and smooth muscle cells. Vascular endothelial cells are one of the first types of cells to come in contact with *T. cruzi*, and a damaged endothelium generally leads to vascular dysfunction. Infection plays a significant role in the alteration of a variety of important pathways, resulting in the development of damage [1], and there is a fine balance between control of the replication of the parasite and the intensity of the inflammatory response, so that the host is unable to eliminate the parasite, resulting in the parasite persisting as a lifelong infection in most individuals [2].

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During infection with *T. cruzi*, it has been reported an increased expression of proinflammatory cytokines, chemokines, vascular adhesion molecules, nitric oxide synthase among other molecules; all of these factors promote inflammation and vascular injury [1]. The intracellular phase of the parasite has been poorly studied, and it is known that *T. cruzi* amastigote surface antigens induce an immune response [3]. However, few of such molecules have been thoroughly studied.

To analyze the effect of the protein rSSP4 on endothelial cells, a primary culture of human umbilical vein endothelial cells (HUVECs) was grown to model host cells, following previously published procedures [4]. The rSSP4 protein was obtained as previously described [5]. HUVECs were cultivated in Dulbecco's Modified Eagle (DMEM) medium supplemented with 20% (v/v) fetal calf serum (FCS). Cells were cultures separately in the

presence of 10µg/mL rSSP4 protein, medium alone or in the presence of a blocking activity of lipopolysaccharide (LPS) (Controls) [5]. Cells and culture supernatants were collected at different times; concentrations of cytokine and chemokine were quantified by ELISA assay (R&D System), the expression of genes for adhesion molecules, and metalloproteinases were determined by RT-PCR [6].

The interaction of the HUVECs with rSSP4 protein induces the production of IL-1β, TNF-α, IL-6 and chemokines CXCL-8, CCL-4, CCL-5, CXCL10, and CCL-11 (Fig 1). The MBP protein was used as a control, when cells were stimulated with this, they did not induce cytokine production. HUVECs stimulated produce TNF-α significantly from 24-48 h ($P < 0.05$ stimulated cells *Vs.* NS), IL-1 is produced incrementally from 24 to 96 h, and IL-6 from 24 to 96 h with an increase at 48 h ($P < 0.001$ stimulated cells *Vs.* NS) (Fig 1A), likewise, we observed a production of CCL4 and CCL5 from 48 to 96 h ($P < 0.001$ stimulated cells Vs. NS), a CXCL-10 production was observed from 48 to 96 h ($P < 0.001$ stimulated cells *Vs.* NS), CCL11 production was increased after 72 h of stimulus ($P < 0.001$ stimulated cells *Vs.* NS), and CXCL-8 production was observed only after 24 h of interaction ($P < 0.05$ stimulated cells *Vs.* NS) (Fig 1B).

We observed in HUVECs an increased expression of genes for MMP-2, ICAM-1 and VCAM-1 (12-24 h), an increase in the expression of genes for E-selectin (12 h) and MMP-9 (12-96 h) was also observed (Fig 2). There are reports in animal models, which show that inflammatory cytokines play a central role in acute *T. cruzi* infection, upon activation, such cells secrete pro-inflammatory cytokines and chemokines and are promptly released, furthermore activate other inflammatory cells. This pattern of expression has been observed in the inflammatory responses in cardiomyocytes during *T. cruzi* infection.

It was shown that heart tissue collected from *T. cruzi*-infected rats expressed IL-6, IL-1β, TNF-α, and *i*NOS, moreover, hearts of infected mice and cardiomyocytes express the same pattern of cytokines and chemokines [7,8]. Moreover, MMPs are associated with processes of tissue remodeling and are expressed in all infections with protozoan parasites, such as, *Plasmodium*, *T. cruzi*, *Leishmania*, and *Toxoplasma*. In all these infections, the balances between MMPs and endogenous MMP inhibitors are disturbed, mostly in favor of active proteolysis. When the infection is associated with leukocyte influx into specific organs, immunopathology and collateral tissue damage may occur. Destruction by these MMPs may be under the control of host cytokines and chemokines, as well as influenced by parasite product [9]. The participation of MMP-2 and MMP-9 could contribute to the damage induced by *T. cruzi*, by favoring the infiltration of immune cells and modulating the immune response, in addition, the accumulation of leukocytes at the site of local injury or infection of endothelial cells is dependent on the interaction of circulating leukocytes with vascular adhesion molecules such as E-selectin, VCAM-1 and ICAM-1 [10]. Finally, inflammatory response that follows the infection with *T. cruzi* is essential for host resistance to infection but is also responsible for the diverse pathology observed in Chagas disease [7]. Parasite persistence depends on a combination of factors, including release of molecules that interfere with the immune response. Therefore, suppression induced by parasite molecules is more relevant at the acute phase, when the concentration of such molecules can be fairly high. Although the amastigote stage is considered essentially as the stage of intracellular replication, this form of the parasite is present in the circulation during the acute phase of infection and can enter and develop in both phagocytic cells and in non-mammalian phagocytic [11].

Figure 1. Profile of cytokines and chemokines induced by rSSP4 in HUVECs. HUVECs were stimulated with the protein for 12-96 h, and (A) cytokines and (B) chemokines were measured in cells culture supernatants by ELISA. Graphs show values in pg/mL (means ± SD) of three experiments run in duplicate. MBP protein was used as control. NS (Non-stimulated).

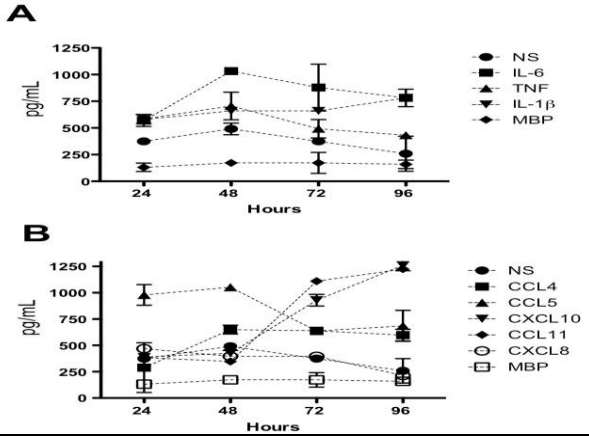
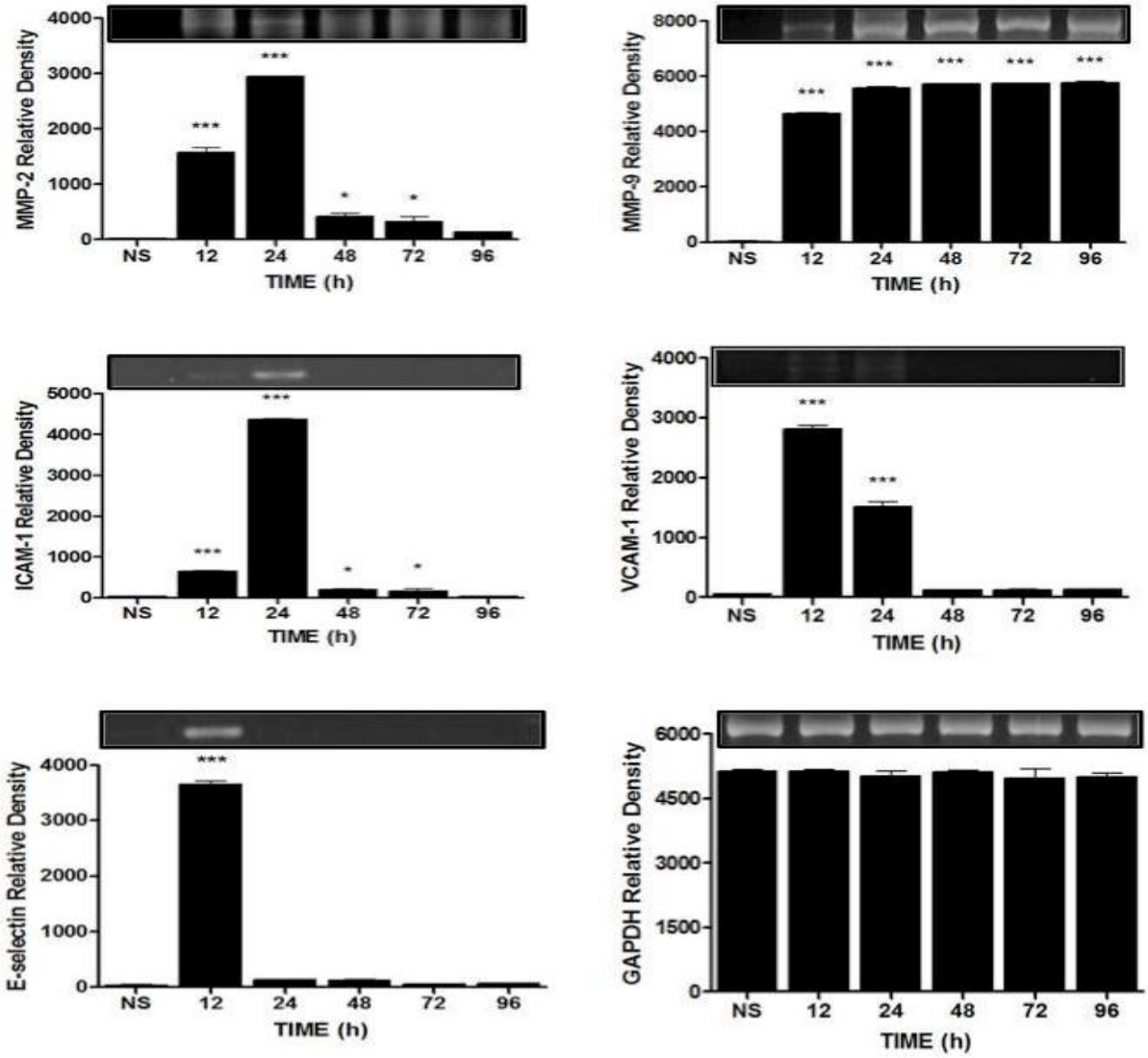


Figure 2. Effect of rSSP4 on gene expression of MMPs, and CAMs in HUVECs. RT-PCR analysis of MMPs, and CAMs mRNAs in HUVECs was performed as described. HUVECs were stimulated with the protein for 12-96 h, NS (Non-stimulated). The intensities of each band were quantified and plotted from the gels that are on top of each graph, corresponding to the expression of genes. GAPDH was used as control. *, **, * *P* < 0.05, 0.001, and 0.0001 respectively vs unstimulated cells.**



CONCLUSION

In conclusion, all these results suggest that the amastigote SSP4 molecule could play a key role in the inflammatory process, modulating the expression and production of inflammatory molecules which may represent a mechanism participating in the immunoregulatory processes carried out by *T. cruzi* during the development of the acute phase of Chagas' disease.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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