CCL20, INHBA, VEGFA and IL23A, which are overexpressed 3- to 15-fold compared with control (Figure 1). Chemokines, which coordinate the recruitment of leucocytes involved in homeostasis and in innate and adaptive immune responses, play a crucial role in the regulation of immunity against Leishmania. In addition, CCL3 and CCL20 have been shown to display antiparasitic activity against the promastigote form of the Leishmania mexicana parasite by acting directly on plasma membranes on Leishmania parasites, causing their lysis. Evidence of an increase in phagocytosis and NO-mediated destruction of Leishmania by chemokines (including CCL3 and CCL4) was reported a decade ago. Moreover, we show here that IL-23A levels are induced by GSNO. Murray et al. showed that IL-23 displayed an antileishmanial effect, especially during late-stage parasitic infection. Thus, GSNO may be implicated in a cellular signalling pathway leading to macrophage activation and exhibiting immunostimulatory effects.

Our results indicate that GSNO not only exhibits microcidal activity but also acts as a macrophage activator. This activation was possible with the involvement of at least three chemokines (CCL4, CCL3 and CCL20) and one cytokine (IL23A). All of them are known to have antiparasitic activity, and thus could be effective against Leishmania infection. In conclusion, our results may be relevant to investigators working on GSNO antiparasitic activity in vitro and in vivo.

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**Transparency declarations**

None to declare.

**References**

1 Costa ISF, de Souza GFP, de Oliveira MG et al. S-nitrosoglutathione (GSNO) is cytotoxic to intracellular amastigotes and promotes healing of topically treated Leishmania major or Leishmania braziliensis skin lesions. J Antimicrob Chemother 2013; 68: 2561 – 8.


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**S-nitrosoglutathione (GSNO) is cytotoxic to intracellular amastigotes and promotes healing of topically treated Leishmania major or Leishmania braziliensis skin lesions—authors’ response**

Inez Silva Fernandes Costa1, Gabriela Freitas Pereira de Souza2, Marcelo Ganzarolli de Oliveira2 and Ises de Almeida Abrahamsohn1*

1Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, USP, 05508-900 São Paulo, SP, Brazil;
2Departamento de Físico-Química, Instituto de Química, Universidade Estadual de Campinas, UNICAMP, 13083-970, CP 6154, Campinas, SP, Brazil

*Corresponding author. Tel: +55-11-3091-7327; Fax: +55-11-3091-7224; E-mail: labraham@usp.br

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Sir,

Thank you to Ronzani et al. for calling attention to our paper. The results reported by Ronzani et al. are certainly interesting as they indicate that expression of chemokines and cytokines in a human monocyte cell line (THP-1) can be up-regulated by S-nitrosoglutathione (GSNO). Although most studies have reported down-regulation of the synthesis of most proteins, up-regulation also occurs and the net result of GSNO action appears to be much dependent on the levels of nitrosoglutathione reductase expressed by each cell type. In addition, NO and GSNO also act post-transcriptonally and directly inhibit the phosphorylation of transcription factors such as STAT-3. Regarding the activities of chemokines and cytokines on Leishmania infection, these molecules are secreted by several host cells of the innate or acquired immunity systems. They are secreted to the extracellular milieu and act by recruiting and/or activating the respective cell targets, e.g. neutrophils, lymphocytes and macrophages (after interaction with target-cell membrane receptors). Thus, chemokines and cytokines are recognized as important regulators of the immune and inflammatory responses and because of this many participate in the control of Leishmania parasitism in the vertebrate host.

Regarding a possible direct cytotoxic activity of chemokines/ cytokines against Leishmania, it should be recalled that the study by Söbirk et al. described this cytotoxicity to occur against free promastigotes of Leishmania, at micromolar concentrations of these mediators in contrast to the nanomolar levels normally found in the inflammatory microenvironment.

However, in the cultures of Leishmania-infected THP-1 cells described in our paper, the parasites were amastigotes lying inside parasitophorous vacuoles in the cytoplasm. Therefore,
chemokines or cytokines synthesized by the host cells in response to GSNO (should they still be secreted as biologically active molecules) would have first to interact at physiological levels with their respective receptors on the host cell plasma membrane. Hence, it is difficult to envisage a direct microbicidal effect by GSNO-induced chemokines or cytokines on Leishmania amastigotes. Nevertheless, the GSNO-induced up-regulation of chemokine and cytokine expression in macrophages, as reported by Ronzani et al., may be important in the context of topically applied GSNO as one of the mechanisms underlying the in vivo ulcer-healing effects in cutaneous leishmaniasis and in other skin ailments.

In addition, our findings in a rat model of periodontitis showed that intragingival applications of GSNO (25 or 100 nmol) significantly reduced local inflammation and bone loss; these effects were associated with reduction of IL-1β and TNF-α levels, metallo-proteinases MMP-1 and -8, NF-κB and inducible NO synthase activity, when compared with control groups. However, these effects are lost at the higher GSNO dose (500 nmol). Therefore, the observed biological effects of GSNO appear to be critically dependent on optimal dosage. It is possible that GSNO doses above a certain threshold may activate distinct pathways that would abrogate the anti-inflammatory effects of lower GSNO concentrations, or even favour a pro-inflammatory effect.

Transparency declarations
None to declare.

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2 Costa ISF, de Souza GFP, de Oliveira MG et al. S-nitrosoglutathione (GSNO) is cytotoxic to intracellular amastigotes and promotes healing of topically treated Leishmania major or Leishmania braziliensis skin lesions. J Antimicrob Chemother 2013; 68: 2561–8.