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Histoplasma capsulatum modulates the immune response exerted by mesenchymal stromal cells

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Background

Mesenchymal stromal cells (MSCs) have become a tool not only for tissue regeneration but also for the treatment of inflammatory diseases. Several studies have demonstrated the therapeutic potential of MSCs for the treatment of noninfectious inflammatory diseases; however, they appear to play a dual role in infectious diseases. Histoplasmosis is a systemic mycosis caused by *Histoplasma* spp., which occurs mainly in immunosuppressed individuals; this mycosis can present a severe clinical picture with dissemination to various organs, and is associated with an exacerbated inflammatory response and with anemia and pancytopenia if bone marrow is affected. So far, the effect of a possible interaction of *Histoplasma* with stem cells present in the bone marrow is unknown.

Objective

To examine, in vitro, the immunomodulatory effects of MSCs in response to *Histoplasma capsulatum* infection.

Methods

MSCs were obtained from bone marrow of C57BL/6 male mice; after isolation and purification, they were induced to mesodermal lineages and characterized by flow cytometry. Later, the basal expression of toll-like receptor (TLR)-2, TLR4 and Dectin-1 was determined using flow cytometry. MSCs were infected with *H. capsulatum* yeasts (isolate CIB 1980) in a multiplicity of infection (MOI) of 5 and incubated for 24 h. In addition, some of the co-cultures were previously treated with specific blocking antibodies for TLR2 and TLR4 or with a blocking peptide specific for Dectin-1 (CLEC7A). Furthermore, phagocytosis, microbicidal and cell proliferation assays were done, and the expression of the genes encoding the cytokines IL-1α, IL-6, IL-10, IL-17, TNF-α and TGF-β as well as of those for arginase-1 and iNOS were assessed.

Results

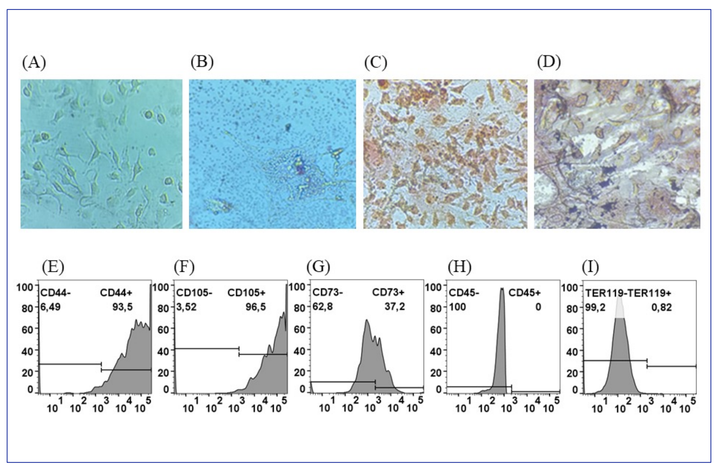

Figure 1.
Morphological and phenotypic characterization of MSC. A) Unstained cell control; B) Adipogenesis, day 7 of culture, staining with the Oil Red dye; C) Chondrogenesis, day 14 of culture, staining with Safranin dye; D) Osteogenesis, day 21 of culture, staining with Von Kossa dye contrasted with Safranin. Expression of surface markers by flow cytometry: (E) PECy5-CD44, (F) APC-CD105, (G) PE-CD73, (H) FITC-CD45, and (I) APC-TER119.

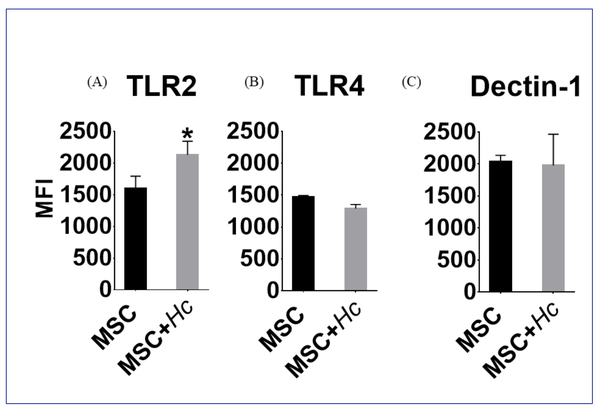

Figure 2.
Expression of TLR2, TLR4 and Dectin-1 receptors in MSC. A) TLR2 expression in uninfected and *H. capsulatum* yeast-infected MSCs; B) TLR4 expression in uninfected and H. capsulatum yeast-infected MSCs; C) Expression of Dectin-1 in uninfected MSCs and those infected with *H. capsulatum* yeasts. MSCs were selected from the CD45 negative population and the number of receptors is expressed as mean fluorescence intensity (MFI). Results are expressed as means ± SD of pooled data from three independent experiments; *P < 0.05.

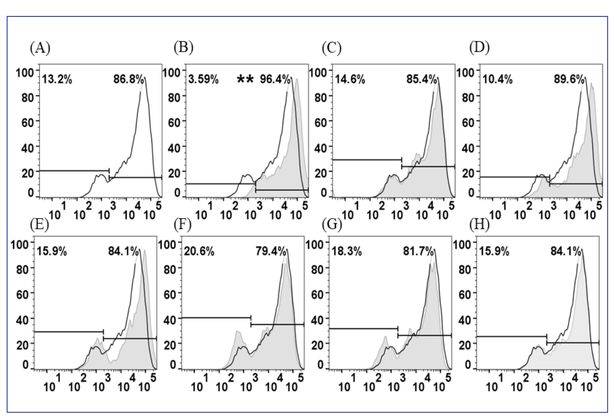

Figure 3.
Phagocytosis of *H. capsulatum* yeasts by MSC. Phagocytosis was analyzed by flow cytometry and the results are expressed as percentage of FITC positive cells (% phagocytosis). A) Control, % of phagocytosis in infected MSCs without treatment; B) % of phagocytosis in MSC treated with anti-TLR2; C) % of phagocytosis in MSC treated with anti-TLR4; D) % of phagocytosis in MSC treated with the peptide CLEC7A; E) % of phagocytosis in MSC treated with the combination of anti-TLR2/anti-TLR4; F) % of phagocytosis in MSCs treated with the anti-TLR2/CLEC7A combination; G) % of phagocytosis in MSCs treated with the combination of anti-TLR4/CLEC7A, and H) % of phagocytosis in MSCs treated with the combination of anti-TLR2/anti-TLR4/CLEC7A. Data correspond to a representative experiment of three experiments triplicated; **P < 0.01.

Results

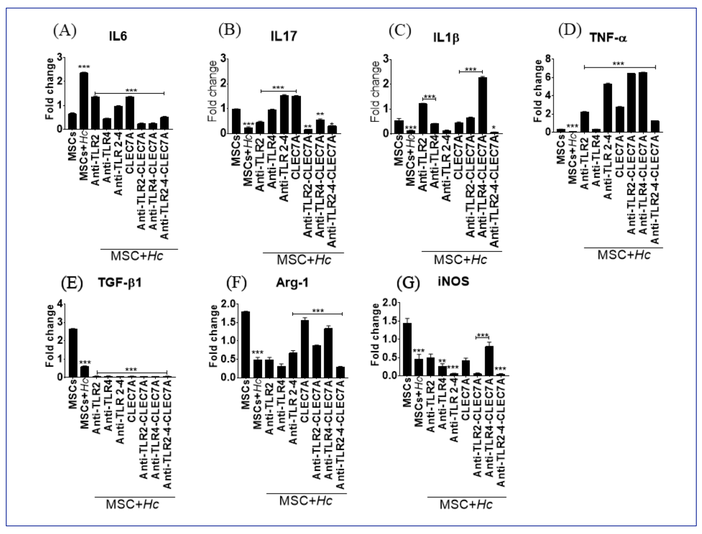

Figure 4.
Expression of inflammatory mediators in MSC infected with *H. capsulatum*. Analysis of mRNA expression of proinflammatory cytokines, arginase-1, and iNOS was performed in MSCs infected or uninfected with *H. capsulatum* yeasts. A) IL-6; B) IL-17; C) IL-1β; D) TNF-α; E) TGF-β1; F) Arg-1, and G) iNOS. MSC, control, uninfected cells; MSCs+Hc, cells infected with *H. capsulatum*; TLR, Toll-like receptor; CLEC7A, peptide blocker specific for Dectin-1. Results are expressed as the mean ± SD of pooled data from three independent experiments; *P < 0.05; **P < 0.01; ***P < 0.001. Comparisons were done between MSCs+Hc vs MSCs and MSCs+Hc vs MSCs+Hc plus the different treatments.

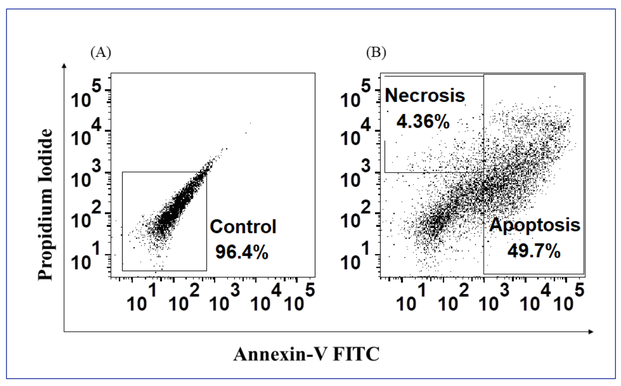

Figure 5.
Histoplasma capsulatum induces apoptosis and necrosis in MSCs. MSCs were treated with Annexin V-FITC and propidium iodide. A) Control, uninfected MSCs; B) MSc infected with *H. capsulatum* yeasts. The percentages represent the number of cells positive for FITC and propidium iodide. Data correspond to a representative experiment of three experiments triplicated.

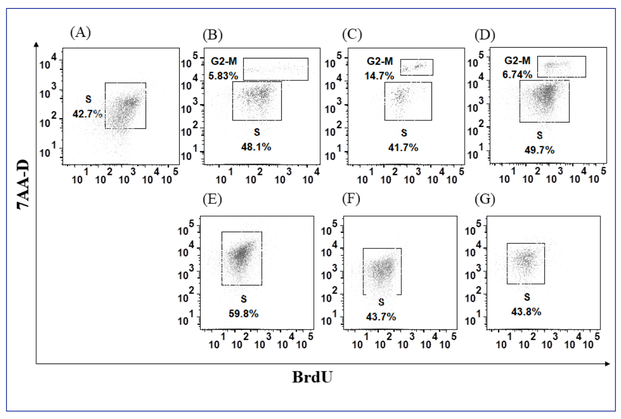

Figure 6.
Histoplasma capsulatum infection affects the proliferation of MSCs. A) Control, uninfected MSCs; B) MSC + Pam3CysOH; C) MSC + LPS; D) MSC + β-glucan; E) MSC + Pam3CysOH + *H. capsulatum*; F) MSC + LPS + *H. capsulatum*; G) MSC + β-glucan + *H. capsulatum*. Data represent the percentage of BrdU positive cells and correspond to a representative experiment of three experiments triplicated.

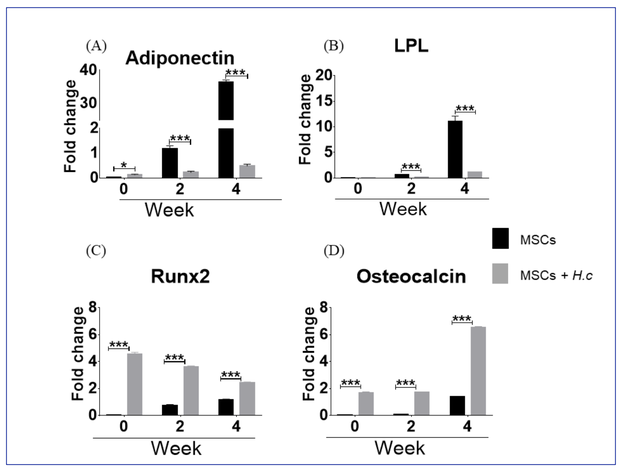

Figure 7.
Expression of genes involved in the osteogenic and adipogenic differentiation of MSCs infected with *H. capsulatum*. Expression analysis of genes associated with differentiation to mesodermal lineages in MSCs infected with *H. capsulatum* yeasts was performed. Genes associated with adipogenic differentiation: A) adiponectin (early expressed); B) LPL (late expression). Genes associated with osteogenic differentiation: C) Runx2 (early expression); D) Osteocalcin (late expression). LPL, Lipoprotein Lipase; Runx2, Run2 transcription factor. Results are expressed as the mean ± SD of pooled data from three independent experiments; *P < 0.05; **P < 0.01; ***P < 0.001.

Conclusions

The above results indicate that MSCs do not exert a notable antifungal effect against *H. capsulatum*; on the contrary, this fungal pathogen not only modulates the expression of inflammatory mediators in MSCs, by a mechanism dependent on TLR2, TLR4, and Dectin-1, but also affects their viability and their ability to differentiate into a different type of specialized cells. These events could, in principle, affect both hematopoiesis and the immune response in the infected host, and in addition, these stem cells may provide a niche for this fungus, allowing it to persist and evade host immunity.

Acknowledgements

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References

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- Mittal J, et al. *Histoplasma Capsulatum*: Mechanisms for Pathogenesis. Curr Top Microbiol Immunol. 2019;422:157-191.