Interaction between hematopoietic stem cells and mesenchymal stromal cells and the dimorphic fungal pathogen *Histoplasma capsulatum*: new immunological insights

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Background

Adult stem cells, including hematopoietic stem cells (HSC) and mesenchymal stromal cells (MSC), are characterized not only by their regenerative and immunomodulatory capacity but also by their therapeutic potential in various pathologies. 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, including the bone marrow, and is associated with anemia and pancytopenia. So far, the effect of a possible interaction of *Histoplasma* with stem cells present in the bone marrow is unknown.

Aim

To determine whether *H. capsulatum* exerts an immunomodulatory effect on either HSC and/or MSC.

Methods

MSCs and HSCs were obtained from bone marrow of C57BL/6 male mice; after isolation and purification, they were characterized by flow cytometry. In addition, MSCs were induced to mesodermal lineages. Later, the basal expression of toll-like receptor (TLR)-2, TLR4 and Dectin-1 was determined using flow cytometry. MSCs and HSCs 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.

Results

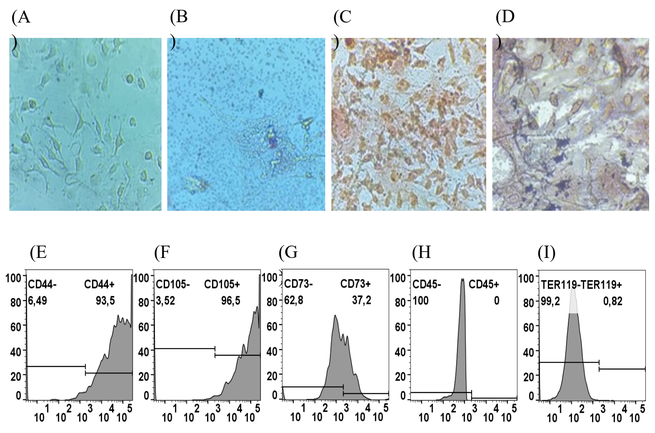

Figure 1.
Morphological and phenotypic characterization of MSC. A) Unstained cell control; B) Adipogenesis, staining with the Oil Red dye; C) Chondrogenesis, staining with the dye Safranin; D) Osteogenesis, staining with Von Kossa dye contrasted with Safranin.
(E-I) Expression of surface markers.

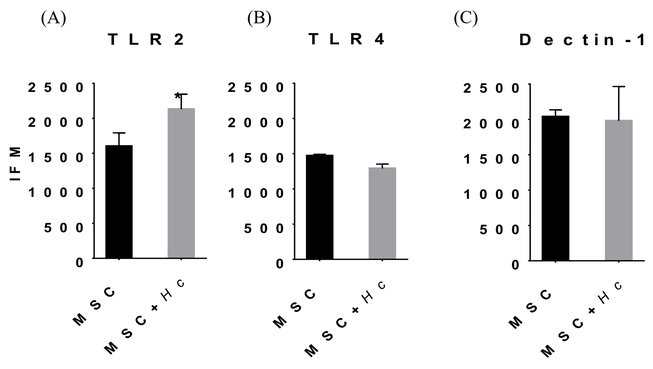

Figure 2.
Infection of MSC with *Histoplasma capsulatum yeast* induces a higher expression of TLR2.

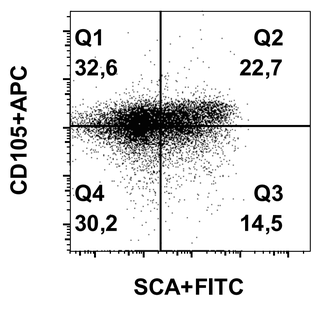

Figure 3.
Phenotypic characterization of HSC. Positive cells for CD105 and Stem Cell Antigen 1 (SCA)

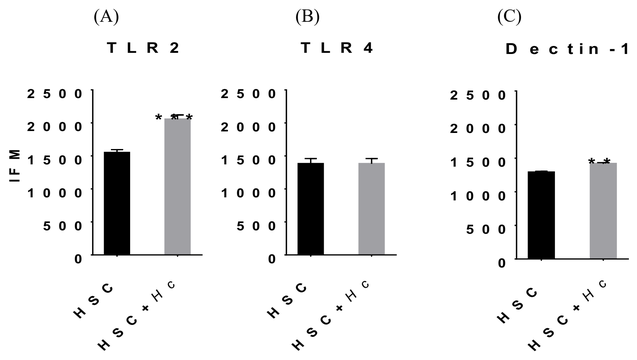

Figure 4.
Infection of HSC with *Histoplasma capsulatum yeast* induces a higher expression of TLR2 and Dectin-1

Results

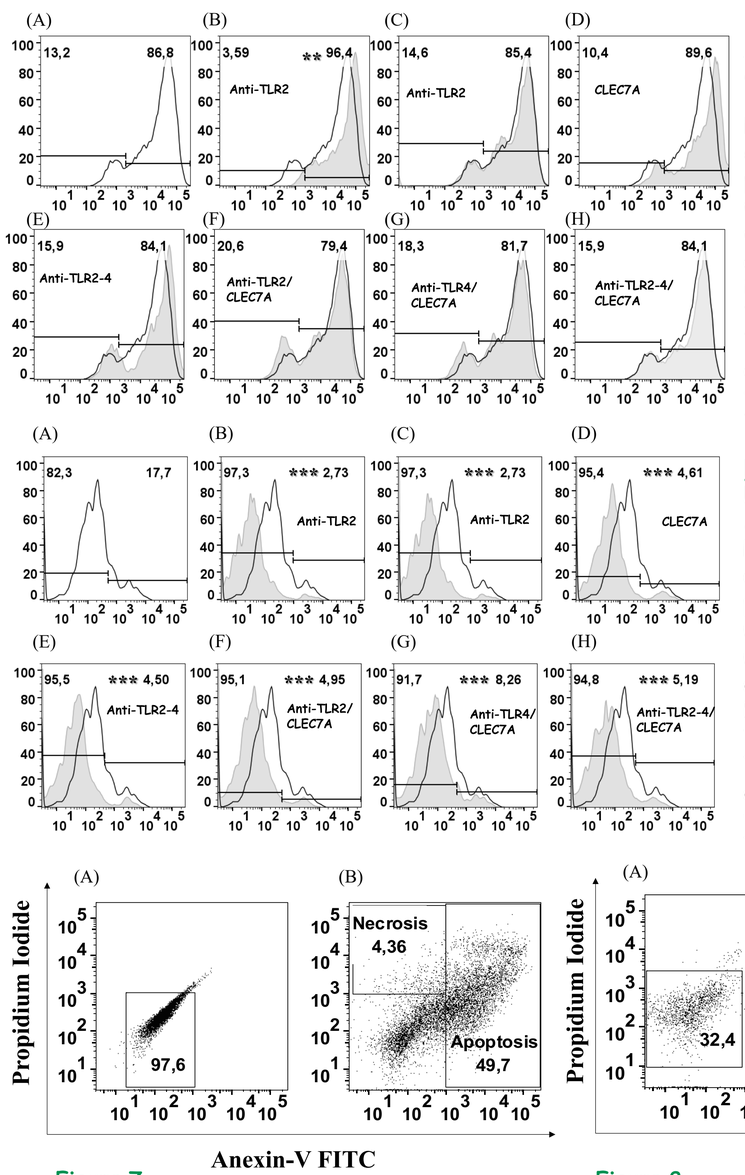

Figure 5.
MSC are able to phagocytose *H. capsulatum* yeas cells. Phagocytosis was expressed as % of FITC positive cells. A) % phagocytosis in infected MSC without treatment; B) treated with anti-TLR2; C) treated with anti-TLR4; D) treated CLEC7A; E) treated with anti-TLR2/anti-TLR4; F) treated with the anti-TLR2/CLEC7A; G) treated with anti-TLR4/CLEC7A, and H) treated with the anti-TLR2/anti-TLR4/CLEC7A. **P<0.01.

Figure 6.
HSC are able to phagocytose *H. capsulatum* yeas cells. Phagocytosis was expressed as % of FITC positive cells. A) % phagocytosis in infected MSC without treatment; B) treated with anti-TLR2; C) treated with anti-TLR4; D) treated CLEC7A; E) treated with anti-TLR2/anti-TLR4; F) treated with the anti-TLR2/CLEC7A; G) treated with anti-TLR4/CLEC7A, and H) treated with the anti-TLR2/anti-TLR4/CLEC7A. **P<0.01.

Figure 7.
Histoplasma capsulatum induces apoptosis and necrosis in MSC.

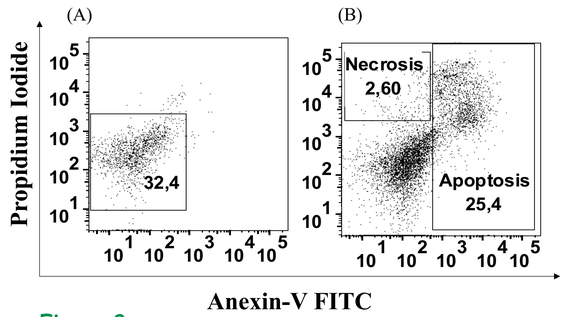

Figure 8.
Histoplasma capsulatum induces apoptosis and necrosis in HSC.

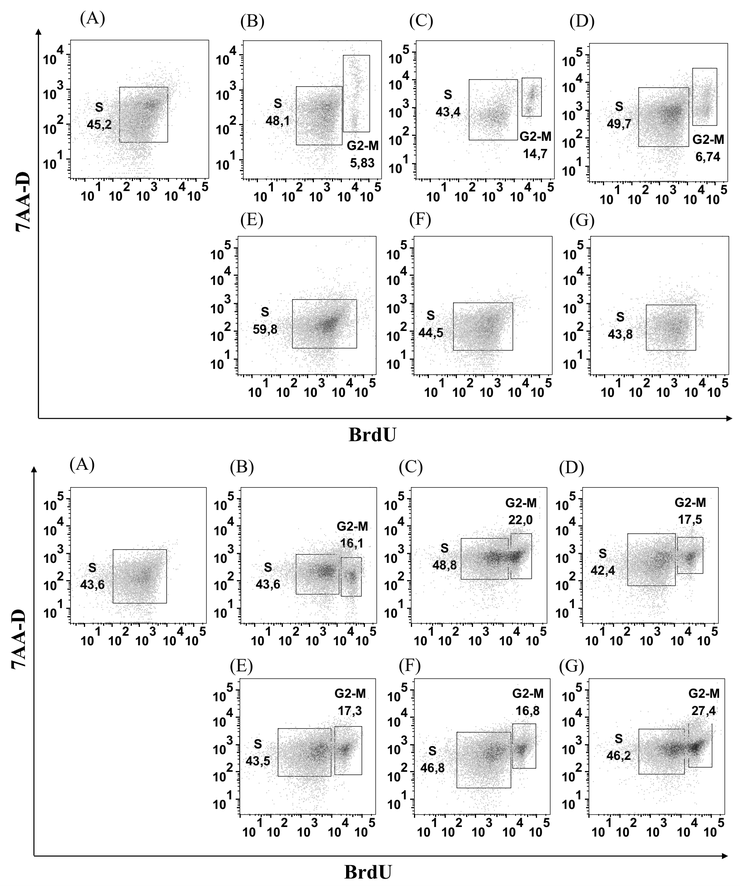

Figure 9.
Histoplasma capsulatum inhibits MSC proliferation.
A) Control, uninfected MSC; B) MSC + Pam3CysOH; C) MSC + LPS; D) MSC + β-glucan; E) MSC + Pam3CysOH + *H. capsulatum*; F) MSC + LPS + *H. capsulatum*; G) MSC + β-glucan + *H. capsulatum*.

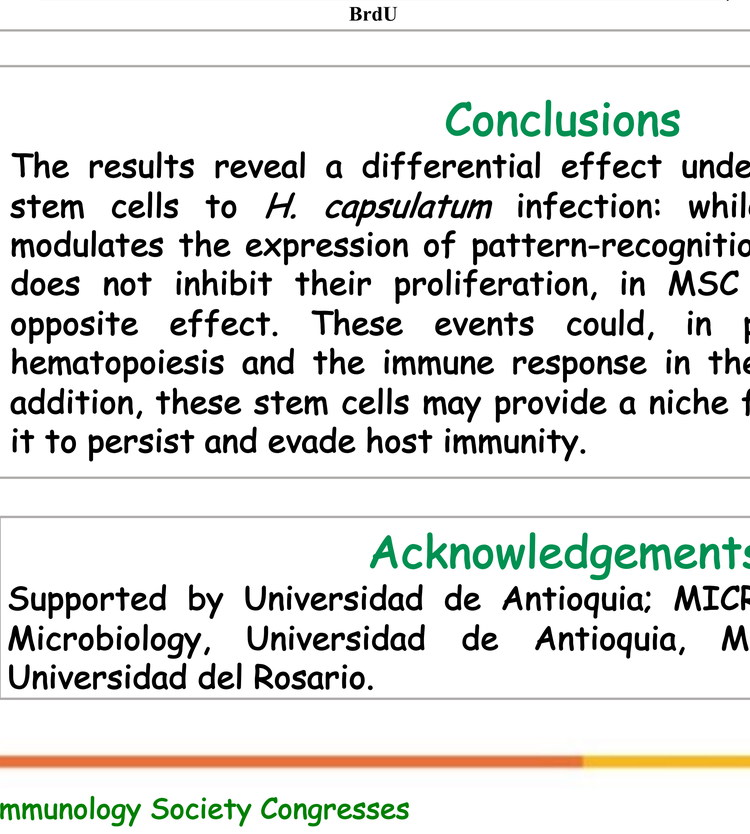

Figure 10.
Histoplasma capsulatum does not inhibit HSC proliferation.
A) Control, uninfected MSC; B) MSC + Pam3CysOH; C) MSC + LPS; D) MSC + β-glucan; E) MSC + Pam3CysOH + *H. capsulatum*; F) MSC + LPS + *H. capsulatum*; G) MSC + β-glucan + *H. capsulatum*.

Conclusions

The results reveal a differential effect underlying the response of stem cells to *H. capsulatum* infection: while in HSC the fungus modulates the expression of pattern-recognition receptors (PRRs) and does not inhibit their proliferation, in MSC the fungus exerts an opposite effect. 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.

References

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- Kobayashi H, et al. How hematopoietic stem/progenitors and their niche sense and respond to infectious stress. Exp Hematol. 2016; 44: 92–100.
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