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Effects of *Histoplasma capsulatum* infection on activation and proliferation of hematopoietic stem cells

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Background

Hematopoietic stem cells (HSCs) are considered a multipotent population with high proliferative potential, and are widely used in the treatment of leukemias, multiple myeloma, and some lymphomas. In the context of infectious diseases, some microorganisms have been reported to induce changes in the expression of surface markers in HSCs by a direct effect or through the induction of cytokines. Systemic infections are characterized by inducing stress on the bone marrow, which is reflected in an increase or decrease in leukocytes and platelets in peripheral blood, a process known as "emergency hematopoiesis". 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 HSCs is unknown.

Objective

To evaluate, in vitro, the effects of *Histoplasma capsulatum* infection on activation and proliferation of HSCs.

Methods

HSCs were obtained from bone marrow of C57BL/6 male mice; after isolation and purification, they were characterized by flow cytometry. Later, the basal expression of toll-like receptor (TLR)-2, TLR4 and Dectin-1 was determined using flow cytometry. HSCs were infected with *H. capsulatum* yeasts 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 arginase-1 and iNOS were assessed.

Results

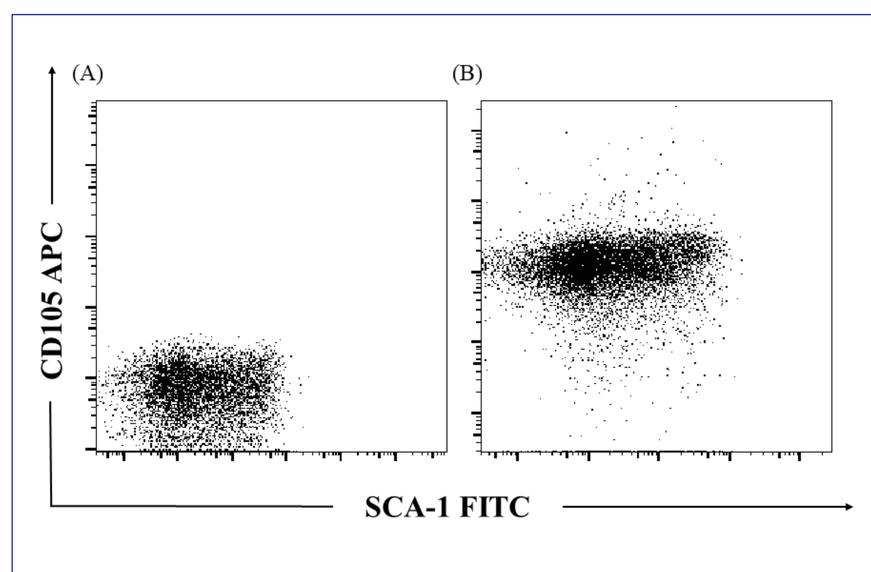

Figure 1. Characterization of the HSPC population. Expression of CD105+ and Sca-1+ surface antigens on mouse bone marrow long-term culture initiator cells (LT-CIC or LT-HSC). A) Control of Lin- cells without positive selection; B) CD105+ Sca-1+ cells corresponding to HSPC.

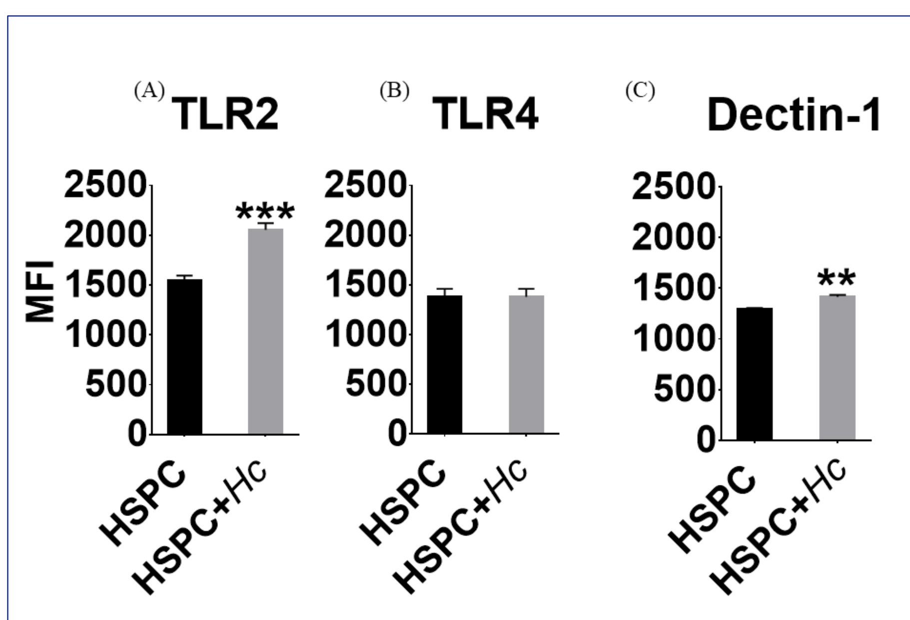

Figure 2. Expression of TLR2, TLR4 and Dectin-1 receptors in HSPC. A) TLR2 expression in unstimulated and stimulated HSPC with *H. capsulatum* yeasts; B) TLR4 expression in unstimulated and stimulated HSPC with *H. capsulatum* yeasts; C) Expression of Dectin-1 in unstimulated and stimulated HSPC with *H. capsulatum* yeasts. HSPCs were selected from the CD105+/Sca-1+ 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 < 0.01; *** P < 0.0001.

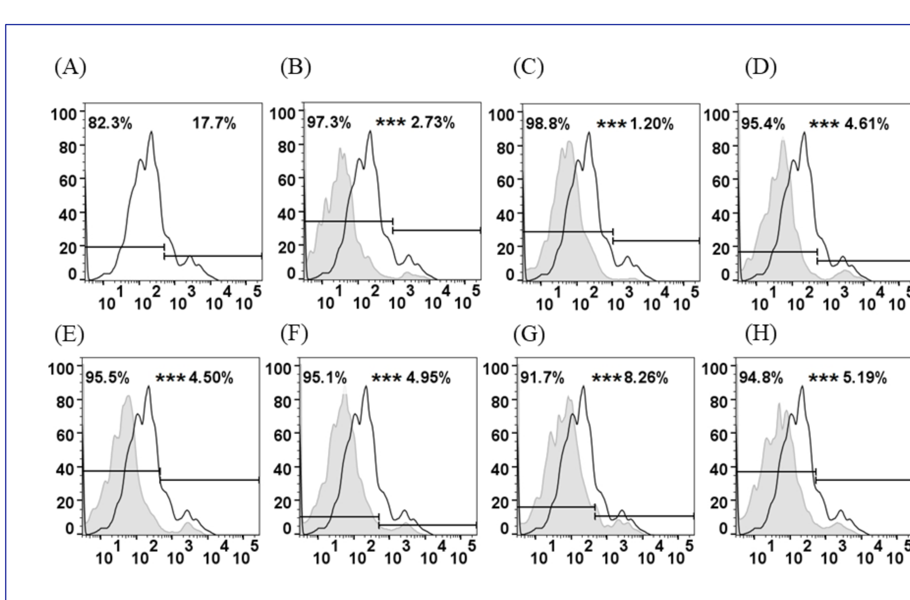

Figure 3. Phagocytosis of *H. capsulatum* yeasts by HSPC. Phagocytosis was analyzed by flow cytometry and the result is expressed as the percentage of FITC positive cells (% phagocytosis) of HSPC cells infected with H. capsulatum yeasts. A) Control, % of phagocytosis in HSPC co-cultured with *H. capsulatum* and without treatment; B) % of phagocytosis in HSPC treated with anti-TLR2; C) % of phagocytosis in HSPC treated with anti-TLR4; D) % of phagocytosis in HSPC treated with the peptide CLEC7A; E) % of phagocytosis in HSPC treated with the anti-TLR2/anti-TLR4 combination; F) % of phagocytosis in HSPC treated with the anti-TLR2/CLEC7A combination; G) % of phagocytosis in HSPC treated with the combination of anti-TLR4/CLEC7A, and H) % of phagocytosis in HSPC treated with the combination of anti-TLR2/anti-TLR4/CLEC7A. Data are representative from an experiment of three replicates; ***P<0.0001.

Results

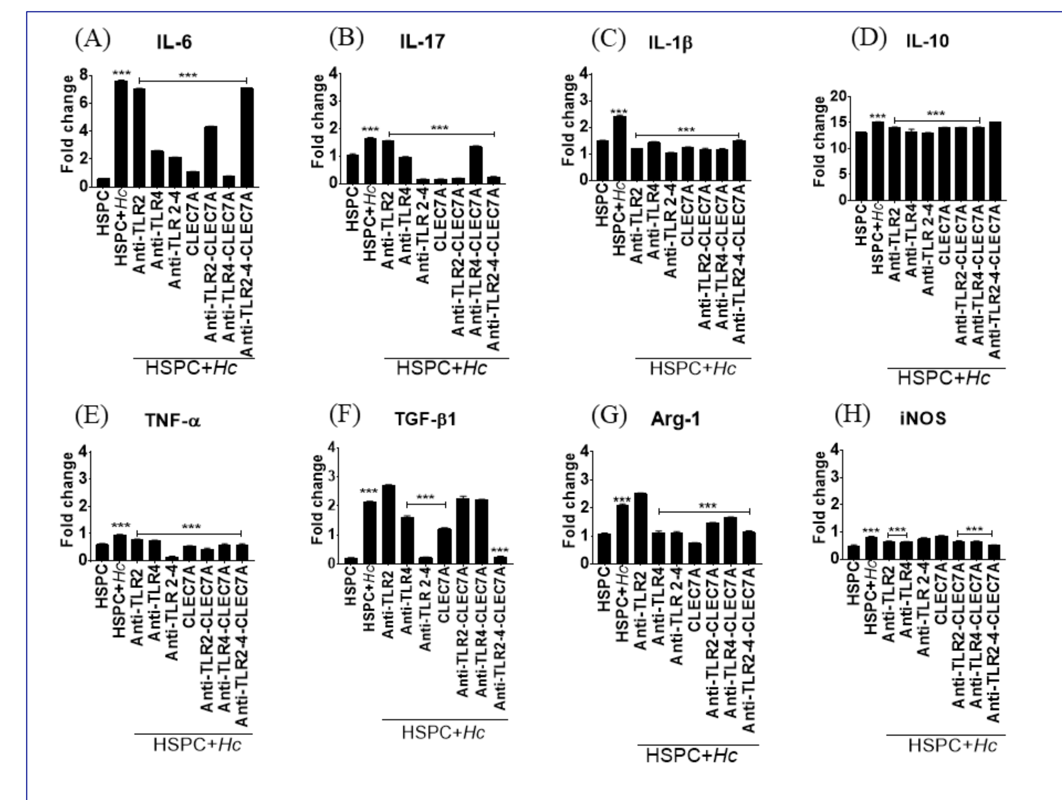

Figure 4. Expression of cytokines and inflammatory mediators in HSPC stimulated with H. capsulatum yeast. Analysis of mRNA expression of proinflammatory cytokines, arginase-1, and iNOS in HSPC stimulated or not stimulated with *H. capsulatum* yeast. A) IL-6; B) IL-17; C) IL-1β; D) IL-10; E) TNF-α; F) TGF-β1; G) Arg-1; and H) iNOS. HSPC, control, unstimulated cells; HSPC+Hc, cells stimulated with *H. capsulatum*; TLR, Toll-like receptor; CLEC7A, peptide blocker specific for Dectin-1. Results are expressed as means ± SD of pooled data from three independent experiments; ***P<0.0001. Comparisons were done between MSCs+Hc vs MSCs and MSCs+Hc vs MSCs+Hc plus the different treatments.

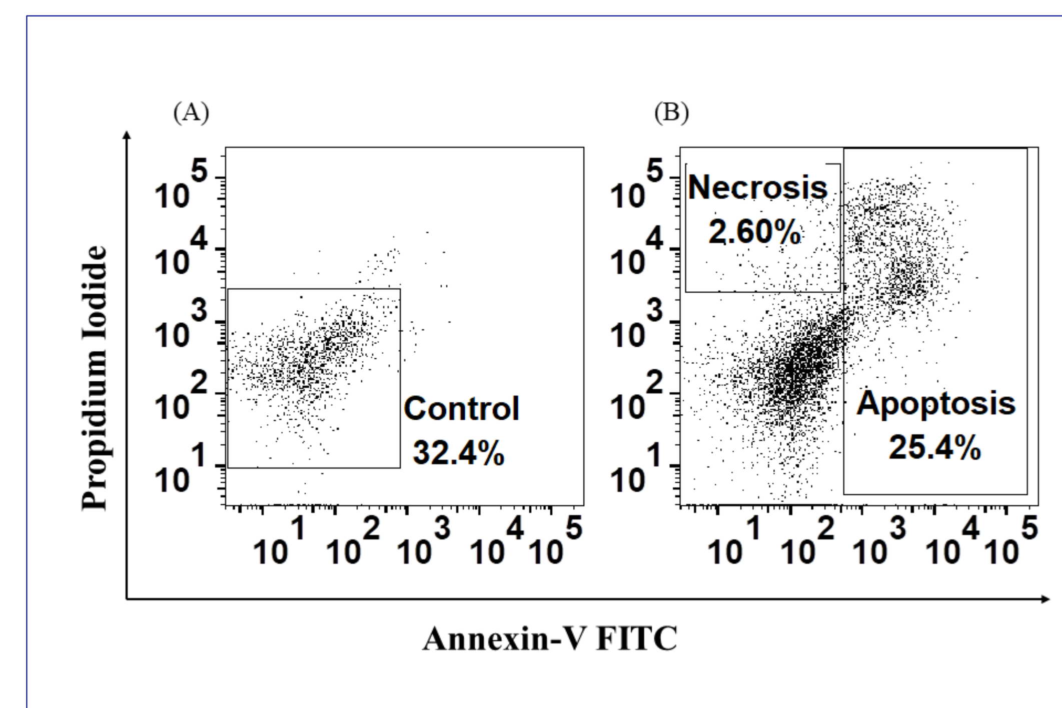

Figure 5. *Histoplasma capsulatum* induces apoptosis and necrosis in HSPC. HSPCs were treated with Annexin V-FITC and propidium iodide as described in materials and methods. A) Control, uninfected HSPC; B) HSPC stimulated with *H. capsulatum* yeasts. Percentages represent the number of cells positive for FITC and propidium iodide. Similar results were obtained from three independent experiments.

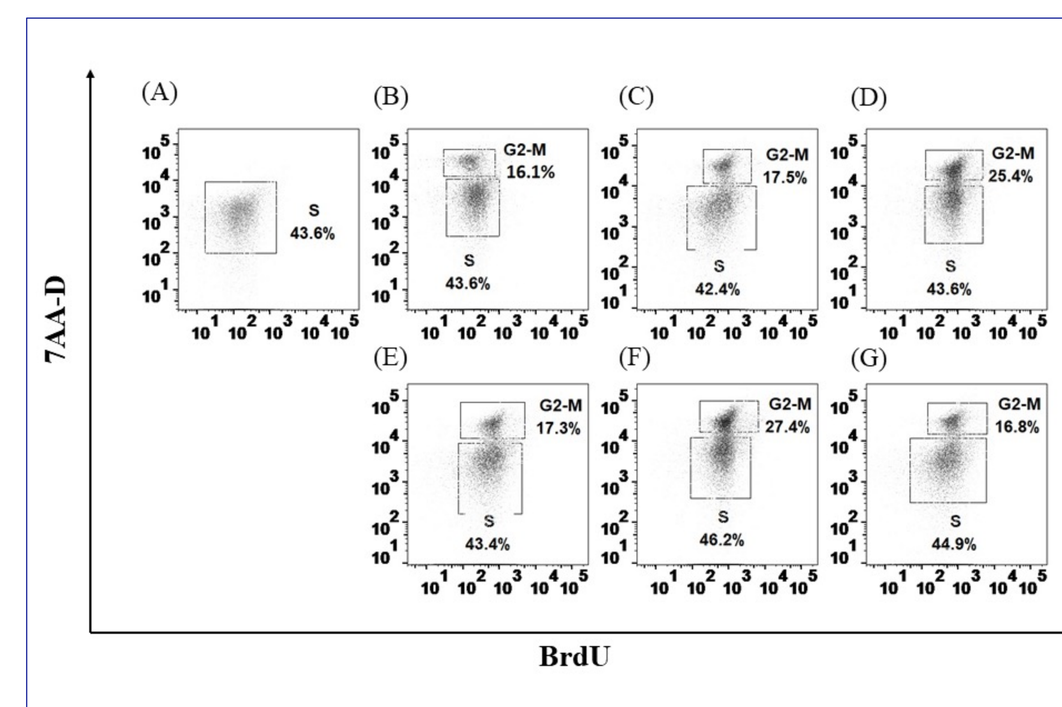

Figure 6. *H. capsulatum* yeasts affect HSPC proliferation. A) Control, unstimulated HSPC; B) HSPC + Pam3CysOH; C) HSPC + LPS; D) HSPC + β-glucan; E) HSPC + Pam3CysOH + *H. capsulatum*; F) HSPC + LPS + *H. capsulatum*; G) HSPC + β-glucan + *H. capsulatum*. Data represent the percentage of BrdU positive cells; results are from a representative experiment of three replicates. *P < 0.001 *** P< 0.0001.

Conclusions

These results indicate that HSCs are capable of phagocytosing *H. capsulatum* but do not affect its survival; moreover, this fungal pathogen could induce changes in the expression of pattern-recognition receptors (PRRs), especially TLR2 and Dectin-1, and could subsequently activate the HSCs leading to the expression of inflammatory mediators as well as affecting the viability of these stem cells. Altogether, these findings indicate that *H. capsulatum* could affect the hematopoiesis process as reflected in an increase or decrease in leukocytes, erythrocytes, and platelets as observed in patients with severe and disseminated disease, especially in those with dissemination to bone marrow.

Acknowledgements

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