Communicative & Integrative Biology 3:4, 390-391; July/August 2010; © 2010 Landes Bioscience

Intracellular cryptococci suppress Fc-mediated cyclin D1 elevation

Yong Luo[1,†] and Arturo Casadevall[1,2,*]

[1]Department of Microbiology and Immunology; [2]Department of Medicine; Albert Einstein College of Medicine; Bronx, NY USA

[†]Current Address: Department of Medicine; Long Island Jewish Medical Center; New York, NY USA

Cryptococcus neoformans (Cn) is the only encapsulated fungal pathogen pathogenic to humans and macrophages play a vital role in the Cn infection pathway. Previously we documented that phagocytosis or crosslinking of FcγR on macrophage cell surface promoted macrophage cell cycle progression via the activation of MAPK pathway. However, we seldom observed sustained macrophage growth after they phagocytosed live Cn. In fact, many macrophages were finally observed to undergo apoptotic changes after phagocytosis of live Cn. Here we report that cyclin D1 elevation in macrophages promoted by phagocytosis was suppressed by intracellular Cn cells. This suggests a novel cytotoxic mechanism for host cells of intracellular cryptococci.

Key words: *Cryptococcus neoformans*, macrophage, Fc-mediated phagocytosis, cyclin D1, cell cycle

Abbreviations: Cn, *Cryptococcus neoformans*; mAb, monoclonal antibody

Submitted: 04/13/10

Accepted: 04/13/10

Previously published online: www.landesbioscience.com/journals/cib/article/12090

*Correspondence to: Arturo Casadevall; Email: arturo.casadevall@einstein.yu.edu, casadeva@aecom.yu.edu

Addendum to: Luo Y, Pollard JW, Casadevall A. Fcγ receptor cross-linking stimulates cell proliferation of macrophages via the ERK pathway. J Biol Chem 2010; 285:4232–42; PMID: 19996316, DOI: 10.1074/jbc.M109.037168.

Cryptococcus neoformans (Cn) is the only encapsulated fungal pathogen pathogenic to human and a major cause of life-threatening infections such as pulmonary cryptococosis and meningoencephalitis in patients with impaired immunity.[1,2] Macrophages play a vital role in the Cn infection pathway.[3-9] In previous studies using macrophage—Cn interaction as a model, we described that phagocytosis or crosslinking of FcγR on macrophage cell surface promoted macrophage cell cycle progression. Furthermore, we showed that the molecular mechanism underlying this phenomenon involved the activation of MAPK pathway in macrophages leading to the activation of major cell cycle machinery such as cyclin D1 expression.[10,11] This phenomenon was commonly observed following phagocytosis of both dead Cn cells as well as inert polystyrene particles. However, we seldom observed sustained macrophage growth after they phagocytosed live Cn, even though transient mitotic figures in macrophages were observed after phagocytosis of live Cn. In fact, many macrophages were observed to undergo apoptotic changes after phagocytosis of live Cn after a relatively long period of co-incubation.

Given the fact that previous reports documented toxic effect of intracellular live Cn on macrophages,[3,4,12,13] we suspected that there might be a underlying mechanism by which live Cn could induce the cytotoxic changes of macrophages intracellularly. To explore the mechanism for the relative paucity of cell cycle progression after phagocytosis live Cn, we probed cyclin D1 expression levels in bone marrow macrophages after Fc-mediated phagocytosis assay by western blots (**Fig. 1**). Bone marrow macrophages were obtained from mice and cultured with M-CSF. Before the phagocytosis assay, the cells were deprived of M-CSF for 2 d and the cells were synchronized in G_1 phase. Monoclonal antibody (mAb) 18B7 was used as opsonin for Fc-mediated phagocytosis as previously described.[10,11] For comparison, we incubated macrophages with mAb 18B7 alone, live Cn opsonized with mAb 18B7 or polystyrene beads opsonized with mAb 18B7 at an Effector: Target (E:T) ratio of 1:1. In the control group, mAb 18B7 was added without the presence of live Cn or polystyrene beads. The cyclin D1 expression was not detectable after phagocytosis until 4 h post stimulation when a transient increase of cyclin D1 expression was noted. This might be due to spontaneous crosslinking of FcγR on macrophages through mAb

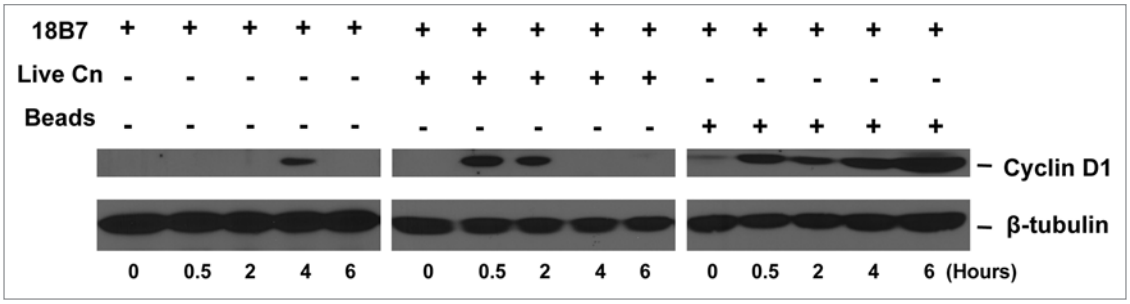

Figure 1. Western blots of Cyclin D1 in bone marrow macrophage cell extract after Fc-mediated phagocytosis of live Cn or polystyrene beads. Bone marrow macrophage cells were challenged with mAb 18B7-opsonized live Cn or polystyrene beads at an E:T ratio of 1:1. In the negative control group, mAb 18B7 without the presence of particles was added. Macrophage cell contents were extracted after 0, 0.5, 2, 4 and 6 h post challenge. Cyclin D1 expression levels in the cell extracts after phagocytosis assay were probed by western blots. The same loading amount for each sample was confirmed by β-tubulin western blots. The experiment has been independently repeated three times and produced similar results.

18B7. However, in the group that live Cn were phagocytosed, cyclin D1 expression increased as early as 0.5 h with a strong signal. These results were consistent with our previous findings that phagocytosis or crosslinking of FcγR on macrophage cell surface promoted macrophage cell cycle progression.[10,11] However cyclin D1 levels decreased 6 h post ingestion of live Cn cells, possibly reflecting a fungal-mediated toxic effect on macrophage cell cycle. To test this possibility, phagocytosis assay was performed with inert polystyrene beads opsonized by mAb 18B7. Cyclin D1 expression was consistently increased after 0.5 h post stimulation and high levels were maintained at least until 6 h. No decrease of cyclin D1 expression was observed after 6 h. This result suggested that phagocytosed live Cn suppressed macrophage growth by decrease cyclin D1 expression which is vital in cell cycle progression of macrophages. This finding in turn suggests a novel cytotoxic mechanism for host cells of intracellular cryptococci. At this time we do not know if the suppression in cyclin D1 expression is a specific mechanism of fungal damage or a consequence of generalized cytotoxity mediated by intracellular cryptococci. The phenomenon is worthwhile to further explore given the importance of macrophage growth in host defense.

Acknowledgement

A. Casadevall is supported by NIH grants HL059842, AI033774, AI033142, and AI052733.

References

1. Casadevall A, Perfect JR. *Cryptococcus neoformans*. Washington DC: Am Soc Microbiol Press 1998.
2. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS—100 years after the discovery of *Cryptococcus neoformans*. Clin Microbiol Rev 1995; 8:515-48.
3. Feldmesser M, Tucker S, Casadevall A. Intracellular parasitism of macrophages by *Cryptococcus neoformans*. Trends Microbiol 2001; 9:273-8.
4. Tucker SC, Casadevall A. Replication of *Cryptococcus neoformans* in macrophages is accompanied by phagosomal permeabilization and accumulation of vesicles containing polysaccharide in the cytoplasm. Proc Natl Acad Sci USA 2002; 99:3165-70.
5. Levitz SM. Receptor-mediated recognition of *Cryptococcus neoformans*. Nihon Ishinkin Gakkai zasshi 2002; 43:133-6.
6. Mukherjee S, Feldmesser M, Casadevall A. J774 murine macrophage-like cell interactions with *Cryptococcus neoformans* in the presence and absence of opsonins. J Infect Dis 1996; 173:1222-31.
7. Alvarez M, Burn T, Luo Y, Pirofski LA, Casadevall A. The outcome of *Cryptococcus neoformans* intracellular pathogenesis in human monocytes. BMC Microbiol 2009; 9:51.
8. Alvarez M, Casadevall A. Phagosome extrusion and host-cell survival after *Cryptococcus neoformans* phagocytosis by macrophages. Curr Biol 2006; 16:2161-5.
9. Alvarez M, Casadevall A. Cell-to-cell spread and massive vacuole formation after *Cryptococcus neoformans* infection of murine macrophages. BMC Immunol 2007; 8:16.
10. Luo Y, Pollard JW, Casadevall A. Fcgamma receptor cross-linking stimulates cell proliferation of macrophages via the ERK pathway. J Biol Chem 2010; 285:4232-42.
11. Luo Y, Tucker SC, Casadevall A. Fc- and complement-receptor activation stimulates cell cycle progression of macrophage cells from G_1 to S. J Immunol 2005; 174:7226-33.
12. Kozel TR, Cazin J. Nonencapsulated Variant of *Cryptococcus neoformans* I. Virulence Studies and Characterization of Soluble Polysaccharide. Infect Immun 1971; 3:287-94.
13. Idnurm A, Bahn YS, Nielsen K, Lin X, Fraser JA, Heitman J. Deciphering the model pathogenic fungus *Cryptococcus neoformans*. Nat Rev Microbiol 2005; 3:753-64.