Superoxide radical-dependent mechanisms in the control of *Trypanosoma cruzi* infection to macrophages

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Macrophages act in the first stages of *Trypanosoma cruzi* infection. NADPH oxidase activation (Nox2) and expression of inducible nitric oxide synthase leads to the formation of superoxide radical (O$_2^{-}$) and nitric oxide ('NO), as part of the defense mechanisms initiated during phagocytosis. The simultaneous production of O$_2^{-}$ and 'NO result in the formation of a strong oxidant species, peroxynitrite anion (ONOO$^-$). Our group demonstrated intraphagosomal formation of peroxynitrite and its cytotoxic role against *T.cruzi* through nitro-oxidative damage to the parasite1. While it is well established that O$_2^{-}$ and 'NO synergize oxidative parasite killing in the cellular model via peroxynitrite, *T.cruzi* infections in Nox2-/- mice resulted in similar parasitemias and tissue parasite load to that of wt animals2. Therefore, we further examined the oxidative mechanisms of parasite killing in bone marrow-derived macrophages of Nox2-/- or iNOS-/- mice to more thoroughly define the role of O$_2^{-}$ and/or 'NO on the success of the host cell response. Moreover, we made efforts to identify alternative O$_2^{-}$ sources that may take part in the control of infection in the Nox2-/- mice. First, we found that infection of Nox2-/- macrophages with trypomastigotes (CL-Brener) resulted in a poor control of infection compared with wt macrophages, supporting a cytotoxic role of O$_2^{-}$ and its dismutation product, hydrogen peroxide. These observations are in line with seminal reports in the area3. Nonetheless, we have also detected small amounts of O$_2^{-}$ (ca. 3% of wt cells) in macrophages Nox2-/- when pre-treated with LPS and infected with trypomastigotes. Furthermore, peroxynitrite was detected in immunostimulated Nox2-/- macrophages using a highly specific probe, the coumarin boronic acid. We have found that the Nox1 isoform of NADPH oxidase is expressed and induced, upon *T.cruzi* infection or LPS treatment. Thus, taken together, these results support that an isoform of NADPH oxidase other than Nox2 could be the alternative source of O$_2^{-}$ in Nox2-/- macrophages. Further studies will give insights into the role of the Nox enzymes in the mechanisms of macrophage-mediated oxidative signaling or damage during *T. cruzi* infection.