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Pathogenic *Leptospira* spp induce higher superoxide anion production by HL 60-derived neutrophils

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The superoxide anion ($O^{\cdot}_2^-$) is the first free radical produced in phagocytes which enables pathogen killing and also generates other microbicidal reactive oxygen species (ROS).The induction of high $O^{\cdot}_2^-$ production by phagocytes as a virulence factor for pathogenic *Leptospira* is not known. The objective of this study was to assess the induced $O^{\cdot}_2^-$ production by human neutrophils exposed *Leptospira* and compare the differences in the level of $O^{\cdot}_2^-$ produced by HL-60 derived neutrophils interacted with pathogenic and saprophytic *Leptospira spp.* HL-60 cells were treated with dimethyl sulfoxide to differentiate in to mature neutrophils. Saprophytic *Leptospira biflexa* serovar Patoc and pathogenic *L. Interrogans* serovar Pyrogenes were grown in Ellinghausen-McCullough-Johnson-Harris media containing 10% heat inactivated rabbit sera. Intracellular $O^{\cdot}_2^-$ production of neutrophils interacted with bacteria in the presence of sera from leptospirosis patients (n=30/group of severe and mild leptospirosis patients) and healthy subjects (n=20) was assessed using quantitative nitroblue tetrazolium assay. Both anti-leptospiral IgM and IgG levels were measured in test sera prior to the assay. In the presence of normal and also patients' sera, neutrophils interacted with *L. Interrogans* Pyrogenes produced significantly high $O^{\cdot}_2^-$ levels than that of *L. biflexa* ($p<0.05$). When *Leptospira* density was varied for neutrophil-bacteria interactions, low *Leptospira* densities induced higher $O^{\cdot}_2^-$ productions compared to the high concentrations ($p<0.03$). Moreover, analysis of $O^{\cdot}_2^-$ production by neutrophils in the presence of patients' sera showed that, opsonization of both saprophytic and pathogenic *Leptospira* by IgM or IgG had not induced a significant difference in the level of $O^{\cdot}_2^-$ produced ($p = 0.012$). These results suggest that, the increased $O^{\cdot}_2^-$ production induced by *L. Interrogans* Pyrogenes could be a virulent factor in pathogenic *Leptospira spp* which may contribute to the pathogenesis of severe leptospirosis.

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