Alternative studies: three-dimensional cell model to reduce animals for *Leptospira* virulence investigate

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Abstract

Leptospirosis is a serious public health threat in tropical and subtropical areas. The etiologic agents of leptospirosis are pathogenic spirochetes from the genus *Leptospira*. In severe cases, patients develop a pulmonary hemorrhage that is associated with high fatality rates of death. Several animal models were established for leptospirosis studies, such as, rodents, dogs, and monkeys. Although useful to study the relationship among *Leptospira* and its hosts, the animal models still exhibit economic and ethical limitations reasons and do not fully represent the human infection. As an attempt to bridge the gap between animal studies and clinical information from patients, we established a three-dimensional (3-D) human lung cell culture for *Leptospira* growth. We show that *Leptospira* is able to efficiently infect the cell lung spheroids and also to infiltrate in deeper areas of the aggregates. The ability to infect the 3-D cell lung aggregates was time-dependent. The 3-D spheroids infection occurred up to 120 hours in studies with two serovars, Canicola and Copenhageni. We standardized the number of bacteria in the initial inoculum for infection of the spheroids and we also propose two alternative culture media conditions. This new approach was validated by assessing the expression of three genes related to virulence and motility. The transcripts of these genes increased in both culture conditions, however, in higher rates and earlier times in the 3-D culture. We also assessed the production of chemokines by the 3-D spheroids before and after *Leptospira* infection. Two chemokines had expression confirmed in the 3-D spheroids. Importantly, chemokine CCL2 was expressed only in the 3-D cell culture, as previously observed in infected animal models. This new approach provides an opportunity to study the interaction of *Leptospira* with the human lung epithelium *in vitro*

Key world: Three-dimensional cell model, *Leptospira* virulence, host-pathogens interactions.

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