Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Early serodiagnosis of *Pneumocystis* infection using gold nanoparticles and amyloid-based nanofibrils for signal detection and amplification

Synopsis: *Pneumocystis* pneumonia (PcP) is an opportunistic infection caused by the atypical fungus *Pneumocystis jirovecii* that remains a major public health problem globally, with high morbidity (500 000 cases annually) and mortality (up to 30%) among immunosuppressed patients. As *P. jirovecii* cannot be cultured, current diagnosis relies on its microscopic visualization or DNA detection in respiratory specimens. These techniques, mainly performed on induced sputum or bronchoalveolar lavage, are time-consuming, and dependent on specialized personnel and equipment, which makes them unsuitable for global application, particularly in technology-deprived settings. Thus, this project aims to develop a time and cost-competitive point-of-care alternative method for PcP diagnosis.
Considering this, we will work with previously developed newly recombinant synthetic (multi-epitope) antigens (RSA) of *P. jirovecii*, applying them as antigenic tools to detect specific anti-*P. jirovecii* antibodies in human sera. Previous results indicate that RSA are excellent antigenic candidates to apply in the detection of specific anti-*P. jirovecii* antibodies [1-2]. A proof-of-concept supporting the application of these RSA conjugated with gold nanoparticles (AuNP) as signal reporting tools (in an immunochromatographic format) validates the approach [3].
A major innovative feature of this proposal consists in the combination of the two previously developed technologies (*P. jirovecii*'s RSA production and conjugation with AuNP) with a new technology based on customized amyloidogenic fibrils, specifically developed for signal detection and/or amplification in immunoassays. These fibrils (which can be further optimized) act as a flexible support that anchors several relevant biomolecules (*e.g.*, RSA-labelled AuNP), improving the detection limit of a given immunoassay. Considering this, the combination of *P. jirovecii*'s RSA with the exceptional properties of AuNP and short amyloidogenic fibrils is an INNOVATIVE and PROMISSING strategy to achieve project's aim. Thus, the specific objectives of this project are to:
1. Express and purify the RSA of *P. jirovecii*.
2. Produce and characterize streptavidin(SA)-labelled bioconjugates composed of AuNP-RSA (hereafter referred to AuNP-RSA-SA).
3. Produce and characterize biotin-derivatized amyloid-based nanofibrils.
4. Functionalize biotin-derivatized amyloid-based nanofibrils with AuNP-RSA-SA conjugates.
5. Assess functionalized nanofibrils' ability to specifically interact with anti-*P. jirovecii* antibodies.

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3 - Tomás, A.L. et al. Front. Microbiol. 10 (2019): 507739. DOI: 10.3389/fmicb.2019.02917