Identification of new antigens for the diagnosis of visceral leishmaniasis

8. Impact paragraph

This thesis focused on one of the most important infectious disease challenges in Latin America, visceral leishmaniasis (VL). In addition to the existing socioeconomic challenges behind this disease, there are also scientific challenges to the successful control of leishmaniasis. The zoological character of this disease, in which dogs are seen as villains, means that control measures are not restricted to humans only. Therefore, this thesis focuses on improving human and especially canine diagnosis of the disease, which is one of the main disease control measures.

In this thesis, it is demonstrated that it is possible to improve the performance of human and canine serological diagnosis with the discovery of a new antigen that substantially increased the specificity both in ELISA assays and in immunochromatographic tests. Proudly, as an impact, we can say that the study presented in Chapter 2 of this thesis brought excellent results, resulting in a positive social impact by overcoming academic barriers, reaching the population that actually needs this service. This was demonstrated by the fact that in a short period since its discovery rKDDR-plus was patented (Figure 23), the patent was licensed and the antigen is currently being widely used in immunochromatographic tests for the diagnosis of the disease in Belo Horizonte, Minas Gerais/Brazil, showing good commercial but also societal impact (Figure 24). The discovery of rKDDR-plus was considered a milestone for this laboratory, being a reference for the search for more molecules with the same potential for the diagnosis of other infectious diseases.

Another positive impact generated by the thesis was the introduction of a new protein family to study to improve the diagnosis of CanL stabbed mainly in the identification of asymptomatic infections by *Leishmania*, which still lacks more targeted research. The rDyn-1 protein was able to identify 100% of cases of dogs without clinical signs of leishmaniasis. The population of dogs that goes unnoticed by most of the antigens used in the diagnosis of the disease has a great influence on the spread of the disease. It is also expected that rDyn1 will be introduced to epidemiological studies of VL, allowing the determination of the real prevalence and incidence rate, mainly in endemic regions. This could further improve understanding the disease and, in turn, controlling it with appropriate containment measures.

Following the current trend of using recombinant antigens as sensitizers, we show here the benefits generated by the systematic use of bioinformatics tools together with immunological techniques for faster and more efficient identification of these antigens. The use of bioinformatics tools was a facilitator and accelerator in this work, saving resources and mainly time, as it provides multiple filters before performing *in vitro* tests. We judge as another repercussion the importance of more meticulous studies directed to the identification of more specific portions of whole proteins responsible for peculiar characteristics of previous interest. This more targeted study made it possible to identify peptide portions with specific characteristics that, when mixed, proved capable of achieving performance equal to or greater than their individual use.

Thus, several premises were pioneered here, such as the use of a more specific antigen, the inclusion of a new family in diagnostic studies of asymptomatic dogs with leishmaniasis, and the screening of smaller portions of proteins with more

defined characteristics than whole proteins. The present work can contribute to the development of more efficient methodologies for the diagnosis of canine and human visceral leishmaniasis, which ultimately might positively influence disease control in Brazil. As a perspective, the development of new diagnostic platforms will allow control not only for VL but also serve as an example for creating tools for the diagnosis and containment of other neglected diseases.