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New perspectives for the detection of Anaplasma phagocytophilum infection using aptamers.

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Introduction

Anaplasma phagocytophilum is a zoonotic and tick-borne bacterium, responsible for granulocytic anaplasmosis, threatening human and animal health. This bacterium is particularly difficult to isolate and cultivate because of its strict intracellular nature, its short life span in blood samples and the lack of specific tools, such as monoclonal antibodies, currently unavailable. Current methods (microscopic observation of blood smears, *in vitro* cultivation and PCR detection) are not very effective (1): microscopic observation of blood smears considerably lacks sensitivity, as this method depends on the relative amount of target cells, bacterial load, and infection status. PCR tests are sensitive. However, the bacterial load is often low in blood for *A. phagocytophilum*, which compromises detection by PCR, as well as *in vitro* culture, also resulting in great difficulty in obtaining complete genome sequences of *A. phagocytophilum* . Cases of human and bovine granulocytic anaplasmosis are consequently underestimated and the real impact of *A. phagocytophilum*, particularly its link with bovine abortions, is difficult to assess. Therefore, new tools for diagnostic and therapeutic purposes need to be developed. Aptamers are powerful tools in infectious diseases, as these single-stranded DNA or RNA sequences are able to bind with high affinity and specificity to a variety of cells (eukaryotic, prokaryotic), molecules (proteins, lipids,…) or ions (2). Since their discovery in 1990, aptamers have aroused great interest with an increasing number of applications for the diagnostic and treatments of infectious diseases in human/veterinary medicine (3). Considered as chemical antibodies, aptamers are a good alternative to monoclonal antibodies, with significant advantages in terms of stability, non-toxicity and synthesis. Therefore, our team is working on the selection of DNA aptamers targeting *A. phagocytophilum* and/or molecules expressed during the infection. Nevertheless, the selection of aptamers against strict intracellular bacteria is particularly challenging and has been poorly described in the literature.

Method and results

Aptamers are selected *in vitro* following a process named SELEX for Systematic Evolution of Ligands by Exponential Enrichment, consisting of series of incubation with ssDNA library and a target, PCR amplification of the bound sequences, and generation of ssDNA (5). The first step was to set up and optimize the SELEX method, against *A. phagocytophilum*-enriched suspensions. After 12 rounds of selection and NGS sequencing, we obtained a rapid and specific enrichment of three aptamers, further used for binding and specificity studies through confocal microscopy and flow cytometry analysis. We demonstrated that these aptamers are able to recognize, with high affinity an intracellular target of cells infected by *A. phagocytophilum.*

Conclusion

The first results suggest that certain aptamers could be specific to bacterial proteins expressed during the infection or to *A. phagocytophilum*. The aptamers specific to *A. phagocytophilum* are a real opportunity to develop a capture method, which will allow purifying bacteria directly from blood samples for in-depth genomic studies and for diagnostic purposes in animals and humans.