Identification of evolutionary conserved DNA sequence and corresponding S21 ribosomal protein region for diagnostic purposes of all Borrelia spirochetes

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INTRODUCTION

It is still under investigation whether all Borrelia sp. causing Lyme borreliosis and other diseases are already identified and properly classified as human pathogens. For this reason, it is of great importance to develop a diagnostic ELISA test that detects all Borrelia sp. The aim of this study was to identify conserved DNA and protein regions present in all currently known Borrelia sp. In experimental studies 31 available Borrelia sp. genomes were aligned and screened for the presence of evolutionary conserved regions. As a result of bioinformatics analysis, one evolutionally conserved DNA region encoding a core fragment of the S21 ribosomal protein was identified. Both a couple of genus-specific PCR primers and the S21 protein B-cell epitope were designed for prospective diagnostic purposes.

Keywords: Borrelia sp., Lyme disease, diagnostic

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Abbreviations: ELISA, Enzyme-Linked Immunosorbent Assay; IgG, Immunoglobulin G; PCR, Polymerase Chain Reaction; PDB, Protein Data Bank

MATERIALS AND METHODS

In this study, 31 complete Borrelia sp. genomes were aligned using MAFFT v. 7.271 software (Katoh et al., 2002), and a DNA fragment encoding the most conserved protein within the entire Borrelia sp. proteome, S21, was identified. The conserved amino-acid sequence of the S21 protein was then compared with all protein sequences deposited at the Protein Data Bank of Japan. Next, we used RaptorX Structure Prediction server (http://raptorx.uchicago.edu/) to predict the 3D structure of the whole S21 ribosomal protein and its conserved fragment (Figs. 3A and 3B, respectively). The 5MMJ PDB protein structure from the small subunit of the chloroplast Spinacia oleracea ribosome was selected by the RaptorX online software as a template (Bič et al., 2017) after comparison with all available 3D protein structures from Protein Data Bank (PDB) (Källberget et al., 2012). B-cell epitopes present within the whole S21...
Figure 1. Multiple sequence alignment of the S21 ribosomal protein gene fragment for 31 genomic sequences deposited at the National Centre for Biotechnology Information (NCBI).

Bor_spF and Bor_spR are the degenerated primer sequences selected for PCR detection of all Borrelia sp.

Figure 2. Multiple sequence alignment of the S21 protein fragment of 29 Borrelia/Borreliella sp. amino acid sequences deposited in the Protein Bank of Japan.

Highlighted in grey is the conserved region characteristic for ribosomal S21 protein of Borrelia/Borreliella sp. The predicted B-cell epitope is distinguished on a black background.
Diagnostic of all Borrelia sp.

Ribosomal protein and the S21 conserved protein motif were predicted using B Cell Epitope Prediction Tools (http://tools.immuneepitope.org/main/bcell/), (Larsen et al., 2006).

RESULTS AND DISCUSSION

Multiple alignments of Borrelia sp. genomes allowed for the selection of the most conserved DNA protein coding region (Fig. 1). The identified DNA region was used to design slightly degenerate PCR primers: Bor_spF and Bor_spR specific to all Borrelia sp. Translation of the selected DNA region revealed, irrespective of point mutations in the DNA sequences from different genomes, an identical amino-acid chain among all of the analyzed Borrelia sp, suggesting that this core part of the ribosomal S21 protein is evolutionary conserved. All amino-acid sequences with 100% similarity identified using BLAST server at DDBJ belonged to the genus Borrelia/Borreliella (Fig. 2). The conserved part of S21 ribosomal protein may potentially serve as an intracellular antigen, indicating active human infection caused by all of the Borrelia spirochetes on the basis of ribosomal metabolism. The structure prediction analysis confirmed a similar 3D-conformation for both the entire S21 protein and for the conserved region only (Figs. 3A and 3B, respectively). Therefore, based on bioinformatics analysis, we can speculate that it will be possible to immunize animals for the production of specific antibodies using the S21 protein conserved region. Because the selected S21 protein fragment has a molecular weight below 5kDa, it may act as a hapten that is capable of inducing an immune response and recognizing specific antibodies only when attached to a large carrier such as a protein or assembled into one chimeric protein with other fragments of various antigens. This is why we suggest combining two genus-specific protein fragments, the S21 conserved peptide (Fig. 2) and, for example, a part of the FlaB protein: QIRGLSQASRNTSKAINFIQTTEGNL. Successful application of chimeric proteins, assembled using molecular biology techniques, in immunological assays for the diagnosis of viral, bacterial and parasitic diseases has already been demonstrated (Acaro et al., 2003; Holec-Gasior et al., 2012a, Holec-Gasior et al., 2012b; Drapala et al., 2014; Ferra et al., 2015). To date, only a few studies have shown the reactivity of this kind of proteins with specific IgG antibodies from human sera of individuals with Lyme borreliosis (Gomes-Solecki et al., 2000; Schreterova et al., 2017). However, chimeric proteins are a new generation of recombinant products which have the potential to replace the native antigen (e.i. crude fractions of sonicated cells of microorganisms). Furthermore, the construction of recombinant chimeras containing genes from several genospecies can allow generating one protein that confers antigenicity to multiple strains. In addition, the use of pure chimeric proteins as diagnostic antigens provides greater flexibility in adapting the test to different assay formats. Thus, the S21-FlaB fusion peptide proposed in this study appears to be a very promising antigen. The core region of the S21 protein contains the B-cell epitope (Figs. 3C and 3D), whereas FlaB, the major endoflagellar filament protein (Motaleb et al., 2006).
2000), acts as an antigen targeting the initial antibody response of the host (Aguero-Rosenfeld et al., 1993). The advantage of the chimeric antigen designed in this study is 100% specificity to all Borrelia sp. and the presence of two different antigens.

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LITERATURE

Aguero-Rosenfeld MF, Nowakowski J, McKenna DF, Carbonaro CA, Wormser GP (1993) Serodiagnosis in early Lyme disease. J Clin Microbiol 31: 3090–3095

Alocco MC, Peroni E, Rovero P, Papini AM (2003) Synthetic peptides in the diagnosis of HIV infection. Carr Proton Pept Sci 4: 285–290

Barstad B, Quarten H, Tveitnes D, Noraa S, Ask IS, Saeed M, Bosse F, Vimmerg H, Huber I, Øymar K (2018) Direct molecular detection and genotyping of Borrelia burgdorferi sensu lato in cerebrospinal fluid of children with Lyme neuroborreliosis. J Clin Microbiol 56. pii: e01868-17. doi: 10.1128/JCM.01868-17

Bieri P, Leibundgut M, Saurer M, Boehringer D, Ban N (2017) The expanding Lyme Borrelia complex - Expanding beyond Lyme borreliosis. Mol Cell Probes 34–39

Bieri P, Leibundgut M, Saurer M, Boehringer D, Ban N (2017) The complete structure of the chloroplast 70S ribosome in complex with translation factor pY. EMBO J 36: 475–486. doi:10.15252/embj.201695959

Cutler SJ, Ruiz-Salbije E, Potkonjak A (2017) Emerging borreliae - Expanding beyond Lyme borreliosis. Med Cell Proteins 31: 22–27. doi: 10.1016/j.mcp.2016.08.003

Drapala D, Holec-Gasior L, Kur J (2015) New recombinant chimeric antigens, P35-MAG1, MIC1-ROP1, and MAG1-ROP1, for the serodiagnosis of human toxoplasmosis. Diagn Microb Infect Dis 82: 34–39

Ferra B, Holec-Gasior L, Kur J (2015) A new Toxoplasma gondii chimeric antigen containing fragments of SAG2, GRA1, and ROP1 proteins-imact of immunodominant sequences size on its diagnostic usefulness. Parasitol Res 114: 3291–3299. doi:10.1007/s00436-015-4552-6

Gomes-Solecki MJ, Dunn J, Luft BJ, Castillo J, Dykhuaizen DE, Yang X, Glass JD, Dattwyler BJ (2000) Recombinant chimeric Borrelia proteins for diagnosis of Lyme disease. J Clin Microbiol 38: 2530–2535

Holec-Gasior L, Ferra B, Drapala D, Lautenbach D, Kur J (2012a) A new MIC1-MAG1 recombinant chimeric antigen can be used instead of the Toxoplasma gondii lysate antigen in serodiagnosis of human toxoplasmosis. Clin Vaccine Immunol 19: 57–63

Holec-Gasior L, Ferra B, Drapala D (2012b) MIC1-MAG1-SAG1 chimeric protein, a most effective antigen for detection of human toxoplasmosis. Clin Vaccine Immunol 19: 1977–1979

Jahfati S, Krawczyk A, Cojpan EC, Fonville M, Hovius JW, Sproong H, Takami K (2017) Enzootic origins for clinical manifestations of Lyme borreliosis. Infect Genet Evol 49: 48–54. doi: 10.1016/j.meegid.2016.12.030

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 35: 3059–3066

Källberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, Xu J (2012) Template-based protein structure modeling using the RaptorX web server. Nat Protoc 7: 1511–1522. doi:10.1038/nprot.2012.085

Kodym P, Kurzovoz Z, Benenovoz D, Picha D, Smitkozovoz D, Moravcozovoz L, Malý M (2018) Serological diagnostics of Lyme borreliosis: comparison of universal and Borrelia species-specific tests based on whole-cell and recombinant antigens. J Clin Microbiol 56. pii: e00601-18. doi: 10.1128/JCM.00601-18

Larsen JE, Land O, Nielsen M (2006) Improved method for predicting linear B-cell epitopes. ImmunoRes 2: 2. doi:10.1186/1745-7802-2-2

Motahe MA, Corum L, Bono JJ, Elías AF, Rosa P, Samuels DA, Charon NW (2000) Borrelia burgdorferi periplasmic flagella have both skeletal and motility functions. Proc Natl Acad Sci USA 97: 10899–10904. doi: 10.1073/pnas.200221797

Ni XB, Jia N, Jiang BG, Sun T, Zheng YC, Liu K, Ma L, Zhao QM, Yang H, Wang X, Jiang JF, Cao WC (2014) Lyme borreliosis caused by diverse genospecies of Borrelia burgdorferi sensu lato in northeastern China. Clin Microbiol Infect 20: 808–814. doi: 10.101111/1469-0691.12532

Parola P, Diatta G, Socolovschi C, Mediniokovoz O, Tall A, Bassen H, Trape JF, Raoult D (2011) Tick-borne relapsing fever borreliosis, rural Senegal. Emerg Infect Dis 17: 883–885. doi: 10.3201/eid1705.100573

Reed KD (2002) Laboratory testing for Lyme disease: Possibilities and practicalities. J Clin Microbiol 40: 319–324

Rudenko N, Golovchenko M, Rüdecz P, Piskunova N, Mallatöv N, Grubhoffer L (2008) Detection of Borrelia bissetii in cardiac valve tissue of a patient with endocarditis and aortic valve stenosis in the Czech Republic. J Clin Microbiol 46: 840–845. doi:10.1128/JCM.01032-08

Schneider BS, Schriefer ME, Dietrich G, Dolan MC, Morshed MG, Zeidner NS (2008) Borrelia bissetti isolates induce pathology in a murine model of disease. Vector-Borne Zoonotic Dis 8: 625–633. doi:10.1128/Vbz.00076-08

Stanek G, Reiter M (2011) The expanding Lyme Borrelia complex - clinical significance of genomic species. Clin Microb Infect 17: 487–493. doi:10.1111/j.1469-0691.2011.03492.x

Wodecka B, Leototska A, Skotarczak B. (2010) A comparative analysis of molecular markers for the detection and identification of Borrelia spirochaetes in Ixodes ricinus. J Med Microbiol 59: 309–314. doi: 10.1099/jmm.0.013508-0