	Project title		
	Development of a prophylactic vaccine and diagnostic markers to prevent and diagnose Lyme borreliosis specific to Europe and North America		
Instrument	Co-operative research projects	Acronym	BOVAC
Thematic priority	Horizontal research activities involving SMEs	Project no.:	512598

Publishable Final Activity Report – BOVAC 512598

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1 PROJECT OBJECTIVES AND MAJOR ACHIEVEMENTS

The BOVAC project addressed as main goals the identification of vaccine candidates to develop a prophylactic vaccine against Lyme borreliosis and the identification of novel diagnostic markers enabling the early and reliant diagnosis of disease to allow proper treatment of patients. The achievement of these goals has a large benefit for the health of people in Europe and beyond. It also has been beneficial for the participating SMEs and advanced the science of *Borrelia* and its pathogenesis. Importantly, the networking between SMEs and RTDs pursuing these goals are strengthened and has been further extended beyond the present consortium. In order to reach these goals, a number of clearly defined objectives were specifically pursued during the proposed project.

- Collect and characterise several hundred human sera from pre-existing collections obtained from patients with various clinical manifestations of Lyme borreliosis. Identify sera with high antibody titres suitable for antigen screening and purify immunoglobulins.
- Prepare genomic libraries from *Borrelia* for bacterial surface display. Apply these libraries using the state-of-the-art genome-wide screening method developed at IC for identification of antigenic proteins from *Borrelia*.
- Determine the genomic sequence of *Borrelia afzelii* and provide an annotation to enable the selection of common candidate antigens from the three *Borrelia* genospecies causing Lyme borreliosis in Europe as well as diagnostic markers by bioinformatics.
- Validate the identified and predicted antigens in order to select potential vaccine candidates to be tested in animal models of infection and purify recombinant proteins.
- Select protective antigens against Lyme borreliosis by experimental animal models using recombinant proteins in order to develop a prophylactic vaccine. Provide first toxicology data for the protective antigens.
- Identify candidate diagnostic markers specific for Lyme borreliosis by serological screening of antigens with human sera obtained during the epidemiological study as well as with existing serum collections.
- Define possible functions of protective antigens in pathogenesis of *Borrelia* to support any further development of vaccine candidates.

2 EXECUTIVE SUMMARY

The BOVAC consortium was assembled as a team of 3 SMEs and 3 RTDs that is highly knowledgeable and experienced to reach the objectives of the BOVAC project. The main goal of the project was the identification of candidate antigens suitable for the development of a prophylactic vaccine to prevent Lyme borreliosis and for the development of diagnostic markers to diagnose infections caused by *Borrelia*. Therefore, one of the first objectives was delivered by the collection of more than 1,400 serum samples from patients with clinical manifestations of Lyme borreliosis as well as from individuals frequently exposed to ticks carrying one of the three pathogenic *Borrelia* genospecies causing Lyme borreliosis. The samples were obtained from the individual collections of the consortium partners at the Medical University of Vienna (MUW), the National Institute of Public Health (NIPH) in Prague and Intercell (IC) in Vienna. The individual sera were subsequently analysed for high levels of *Borrelia*-specific antibodies by a suitable ELISA assay allowing the comparison of all sera and the selection of individual sera for antigen identification. This characterisation was performed by IC and the data were compared and correlated to the previously obtained data at the respective individual institutions. It was thus possible to generate four distinct serum pools, consisting each of three to five sera from patients diagnosed with Erythema migrans, Neuroborreliosis, Lyme arthritis, Acrodermatitis chronica atrophicans (ACA) and two pools were generated from patients showing diverse manifestations of Lyme borreliosis. The second objective encompassed the construction of genomic libraries for bacterial surface display of most, if not all possible peptides encoded by the genome of *Borrelia*. Several libraries were constructed at IC, while the genomic DNA was prepared at Umeå University (UmU). Due to the prevalence of *B. afzelii* in Europe, it was decided to include libraries of this genospecies for antigen identification, although the genomic sequence was not available at the time. In addition, libraries were constructed from the *B. burgdorferi* sensu stricto (s.s.) strain B31, as it constitutes the only *Borrelia* species for which the genomic sequence was available at the start of the BOVAC project. The choice of the *B. afzelii* strain was determined by its virulence in humans and animals. Although *B. afzelii* VS461 was initially used, a natural human isolate, K78 was obtained from the MUW and shown to be infectious in gerbils. It was therefore chosen to be used for library construction, antigen identification as well as for genomic sequencing.

The identification of novel antigenic proteins from *Borrelia* was performed at IC using its proprietary antigenome technology. Initially, antigen screens were performed using the *B. burgdorferi* s.s. B31 libraries, but due to the high abundance of the VlsE antigen in these libraries, the main work towards antigen identification was performed with the *B. afzelii* K78 libraries. The completed screens identified more than 130 novel antigenic

proteins from *Borrelia*, of which a large number are conserved among the three relevant pathogenic genospecies of *Borrelia*. The entirety of the identified antigens, the antigenome, constitutes a major milestone of the project, as it builds the basis for the selection of the most promising candidates for vaccine as well as for diagnostic marker development.
In parallel to the identification of antigens from *Borrelia*, MWG Biotech pursued the determination of the genomic sequence of the *B. afzelii* strain K78 as another important objective of the BOVAC project. The *B. afzelii* genospecies was chosen for genomic sequencing, as its sequence was not available, whereas the *B. burgdorferi* s.s. B31 was publicly available and the *B. garinii* genome sequence expected to be submitted to public databases within the year 2004. The genomic sequencing of *B. afzelii* K78 would thus enable a comparison of all three pathogenic *Borrelia* delivering a list of genes common to all three pathogens. The generation of *Borrelia* libraries for sequencing and the assembly of the many plasmids of the genome caused substantial problems. At the end the linear chromosome could be assembled into six contigs and three circular and 15 linear plasmids could be assembled. A list of annotated genes is now available and common genes have been identified. This constitutes another important milestone of the project, as now all genes selected by bioinformatic analyses can be subjected to the validation procedure at IC in order to focus on the most promising vaccine candidates. The validation procedure at IC included 1) Peptide ELISA; peptides were designed covering the identified epitopes and tested in ELIA against a panel of human sera for reactivity 2) Gene distribution; the presence of the identified antigens was assessed in a wide variety of *Borrelia* strains with PCR using oligonucleotides designed to amplify the identified genes 3) *In vitro* surface exposure; surface exposure of the antigens on the *B. afzelii* strain K78 was tested by flow cytometry using mouse sera generated by injecting mice with bacterial surface display clones 4) Intellectual property survey. The results from the *in vitro* validation enabled us to make a list of the 25 most promising vaccine candidates. IC has cloned and expressed 24 of the 25 selected antigens in *E. coli*. In consideration of the known challenge to express *Borrelia* proteins in a heterologous host several different expression systems were tested to identify the optimal conditions. The work on the recombinant expression of proteins was supported by UmU, which provided amongst others, recombinant OspA protein to be used as positive control in animal experiments.

The experimental animal models were supposed to be set-up and performed by Biotest in the Czech Republic, in close collaboration with the NIPH, UmU and IC. Towards the objective to set-up the relevant model for *Borrelia* in mice, several experiments were performed at Biotest and UmU. The results showed that the selected *B. afzelii* K78 strain was not as virulent in mice as initially suggested by the experimental data from gerbils. Thus, the model had to be set-up with an alternate virulent strain, the *B. burgdorferi* s.s.

strain N40, which has previously been reported to be virulent in mice. As a result of these initial experiments and other factors it was decided to perform the further animal model experiments not at Biotest, but at UmU. Because of the problematic cloning and expression of the *Borrelia* antigens, 15 out of 24 antigens could be prepared and tested in the animal model until the end of the BOVAC project. An important result of the BOVAC project is the observation that two of the first 15 antigens tested in the animal model showed partial protection, reduced bacterial load, against the heterologous challenge with the *B. burgdorferi* s.s. strain N40. The remaining proteins were purified before the end of the BOVAC project and are now tested in the animal model as a continuation of the BOVAC project in collaboration between IC and UmU.
The work on the epidemiological analysis of Lyme borreliosis in the Czech Republic (National Institute of Public Health) and Austria (Medical University of Vienna) has been completed, so that the incidence of Lyme borreliosis as well of infected ticks could be assessed in Austria and the Czech Republic. While Lyme borreliosis is a notifiable disease in the Czech Republic, it is not in Austria. Therefore a questionnaire had to be prepared and distributed to medical doctors throughout Austria to obtain a representative picture of Lyme borreliosis in Austria. However, the response to the questionnaire was too low to allow a statistically significant evaluation. The epidemiological work further involved the collection of ticks in their natural habitat to evaluate the distribution of *Borrelia* and other tick-borne pathogens in their natural habitats. Initially a DNA array approach was tested for determination of pathogens in infected ticks and the last host they fed on. However, this approach could not be realised, because it was problematic to identify suitable probes, especially for the vertebrate hosts. Therefore, MWG Biotech designed a nested PCR approach for detection of *Borrelia* and other tick-borne pathogens which was used by MUW to identify pathogens in ticks collected throughout Austria. The infection rate of *Borrelia* in ticks collected from the nine different states of Austria could be determined. The serological analysis of identified antigens with human sera from the epidemiological studies will support the selection of suitable diagnostic markers and is ongoing and will continue beyond the BOVAC project as collaboration between IC and MUW.
An important consideration for any antigen to be used in a vaccine is the understanding of its role for the virulence of the pathogen. It was therefore an objective of the BOVAC project to characterize selected antigens for their possible functions by various approaches, including knock-out deletions and biochemical studies. UmU has therefore established the knock-out technique by specifically deleting two genes in *B. burgdorferi* sensu lato and is currently engaged to knock-out the two antigens that showed partial protection in the animal model. These knock-out mutants are expected to be available shortly after the end of the BOVAC project for testing of virulence and biochemical characterisation. Shuttle vectors for the plasmid directed expression of genes have

already been generated successfully for all three genospecies so that the knock-out mutants can be complemented via a plasmid-borne gene.

The BOVAC consortium has presented the project on many occasions and has created a website (www.bovac.org) describing the project, the consortium and any news regarding the project during its entire duration. A patent has been filed securing the intellectual property of the identified antigens. Further patents will be filed during 2007 and 2008 regarding vaccine antigens and diagnostic markers. First peer-reviewed publications of results are therefore anticipated to be submitted during 2007, while several additional publications are planned for 2008.
The aim of the BOVAC project was the development of a new vaccine to prevent Lyme borreliosis and significant progress was achieved towards this goal. The currently available assessment of the identified antigens in animal models justifies the assumption that protective candidates were and will be selected and thus provided by this project for further preclinical and clinical development. A successful development of a novel *Borrelia* vaccine will have a large impact on the success of the involved SMEs, but more importantly will provide a means to eliminate the disease burden from many patients suffering from the symptoms of Lyme borreliosis. The identified antigens are also being evaluated for the development of novel markers for the early and reliant diagnosis of Lyme borreliosis, so that specific treatment can be initiated when and only when it is necessary. This will impact on the successful treatment of patients and will in addition have a positive impact on the financial burden of the health care system.

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