Generation of *Borrelia burgdorferi* attenuated strains as potential vaccine candidates against Lyme disease

Andrea Aran

Borrelia burgdorferi is the causal agent of the Lyme disease or Lyme borreliosis, which is the most common tick-borne disease in North America and Europe. Nowadays, there is not an effective vaccine to prevent the infection. Without using antibiotics and even with them, sometimes the spirochetes can spread into the body and cause a late phase where organs as brain, eyes, articulations, among others, can be affected.
In this project it is described a method for the generation of B. burgdorferi attenuated strains for using as potential vaccine candidates.

Objectives

To design a method to develop an attenuated *B. burgdorferi* strain for being used as a vaccine against the Lyme disease.

Benefits

- The development of a vaccine against the Lyme borreliosis is, for itself, a benefit since there is not an effective vaccine.
- Attenuated vaccines can frequently produce a better immune responses.
- It has been considered the type of immune response produced by the attenuated mutants.

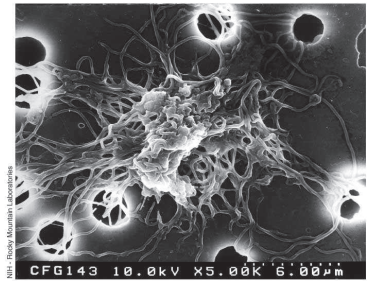

FIG. 1. BSK II cultured *B. burgdorferi* processed by the scanning electron microscopy. Magnification approximately 7-10, 000x.

Materials and methods

Transposon mutagenesis

The suicide vector pMarGent (Fig.2.) used by Stewart et al. [1] can be used in this project. In this vector, the transposase *Himar1* and a hyperactive C9 transposase (to increase the frequency of transposition) are under control of the borrelial promoter *flgBp*, which is fused with a gentamicin marker (*flgBp:aacC1*).

Thanks to transpositions in different sites at the genome of *Borrelia*, some of the mutants obtained will have deleted essential genes for virulence.

As it is shown in Figure 3, the strains can be transformed by electroporation and then the transformants should be plated in presence of gentamicin.
The presence of the vector in the *B. burgdorferi* colonies grown in selective media can be confirmed by PCR of the aacC1 cassette region.
Those positive colonies should be cultured and then it would be necessary to identify the precise insertion sites of the transposon in different mutants to identify the missing genes.

It would be interesting to analyze if isolated strains after transposon mutagenesis have lost some plasmids required for full infectivity. Those mutants which have lost some of these plasmids will be discarded for the next phases of the project.

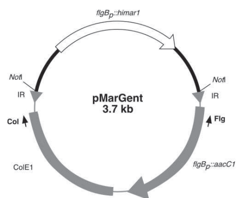

FIG. 2. Plasmid map of pMarGent. The transposase, *Himar1*, is under the control of the borrelial promoter, *flgBp*. The region in grey represents the transposable element and is bounded by inverted repeats (IR), denoted by triangles. The arrows (Flg and Col) indicate oligonucleotides used for subsequent sequencing after rescue in *E. coli*. ColE1, *E. coli* origin of replication; *flgBp::aacC1*, the gentamicin resistance marker fused to the *flgB* promoter.
Reproduced from [1].

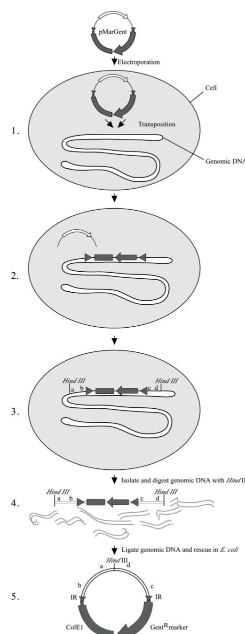

FIG. 3. Transposon mutagenesis system for *B. burgdorferi*. Suicide vector pMarGent is electroporated into competent *B. burgdorferi* cells allowing transient expression of the *Himar1* transposase (1). After transposition, the *Himar1* gene remains on a DNA fragment that is presumably degraded by intracellular nucleases (2). Mutants are selected in the presence of gentamicin, and those with desired phenotypes were transferred to liquid culture for DNA isolation. The *B. burgdorferi* DNA flanking the transposon insertion site is recovered by digestion with HindIII, an enzyme that does not cut within the transposon (3 and 4); self-ligation; and transformation into *E. coli* (5). Purified plasmid DNA was then isolated from *E. coli* clones and sequenced. IR, inverted repeat.
Reproduced from [1].

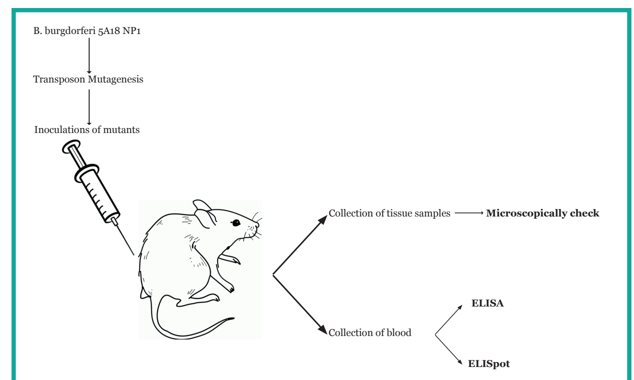

FIG. 4. General procedures for the generation of the attenuated strains. After the injection in mice, tissue and blood samples are collected for the evaluation. The attenuation of the strains is evaluated with a microscopically check of tissues, the presence of antibodies is evaluated with an ELISA and finally, an ELISpot assay has been choosen to know which type of cytokines are being synthesized.

B. burgdorferi strain

Borrelia has some plasmids which interfere with transformation by cleaving incoming DNA, such as the described genes BBE02 and BBQ67, encoded on lp25 and lp56, respectively [2].

Botkin et al. developed a strain containing a mutation in BBE02; 5A18 NP1 [2], in which are also missing the plasmids lp56 and lp28-4. Thus, the transformation frequency with a shuttle vector increased [3].
This strain, derived from the B. burgdorferi B31, has been chosen for the project since it retained full infectivity in mice after modifications in its genome.

Infectivity study

Mice can be infected intradermally with the mutants to investigate the significance of the missing genes and then sacrificed between 2 or 3 weeks postinoculation.

Tissue samples from ear, heart, joint and urinary bladder should be collected and cultured and incubated and monitored to microscopically check for the presence of spirochetes in the medium.

The transposon mutant clones can be divided into different infectivity phenotypes according to spirochete isolation from the tissues examined.

ELISA

The presence or absence of antibodies in blood mice can be evaluated with an enzyme-liked immunosorbent assay (ELISA).

To prepare the ELISA, an immunoreactive protein from *Borrelia* must be used, such as OspC, FlaB or VisE.

ELISpot

To evaluate the generated immune responses, it has been considered the possibility of doing a cytokine ELISpot assay for knowing which response is predominating.

It is supposed that IL-4 plays a beneficial role by launching a type 2 T cell response that directs immunoglobulin class switching to IgG1 and IgE [4, 5]. These antibodies seem to contribute to a protective immunity against the infection [6]. Moreover, other studies showed a relation of the production of IFN-γ and IL-17 with the Lyme arthritis and other symptoms of the Lyme disease [7, 8].

Expected results

	✓	✗
Infectivity study	Attenuated: Present in 0/4 or in1/4 tissues	Infectious: Mutants present in 2/4, 3/4 or 4/4 tissues
ELISA	Presence of antibodies in blood mice	Absence of antibodies in blood mice
ELISpot	Predomination of the type 2 cytokine IL-4	Predomination of cytokines IL-17 and IFN-γ.

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