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Oral Lyme disease vaccine

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Abstract

The present invention relates to a Lyme disease vaccine, a genetic construct, recombinant protein, method for genetic construct design, method for vaccine delivery, method for recombinant proteins delivery, use of recombinant proteins in the production of Lyme disease vaccine. In particular, the method concerns the use of TROSPA and TROSPA- Salpl 5 recombinant proteins derived from castor bean tick (*Ixodes riccinus*) as a component of Lyme disease vaccine for animals. The antibodies present in blood of an immunized vertebrate directed against the TROSPA proteins considerably reduce the chance of infecting new ticks by blocking or hindering the interaction of TROSPA protein with OspA protein of Borrelia burgdorferi sensu lato. The interaction is crucial in the process of the spirochete entering a tick. The antibodies directed against the TROSPA- Salpl5 protein protect vertebrates from infection on the stage of Borellia diffusion by destroying their protective coating formed at the surface as a result of the interaction between the Salpl5 tick protein and OspC spirochete protein. The vaccine based on TROSPA tick proteins and TROSPA-Salpl5 proteins may be used independently or together with the OspA recombinant proteins and OspC protein of *Borrelia burgdorferi sensu lato*.

State of the art

Ticks are external parasites living on vertebrates' blood. During their life-cycle, ticks feed on several hosts, which creates an opportunity to transmit different pathogenic microorganisms between the hosts. An example of such microorganism is *Borrelia burdgorferi* spirochete that causes Lyme disease (Franke et al., 2013). The spirochete enters a tick at the larval or nymphal stage, during their feeding on an infected vertebrate. A group of animals that are competent hosts for the spirochete (i.e., animals for which the infection remains permanent) constitute a natural reservoir of *B. burgdorferi*. This group includes rodents and other small mammals and birds, while humans and livestock are occasional hosts for *B. burgdorferi*. For the past decade, there has been a rapid increase of Lyme disease incidence rate in Poland and Europe. According to the data of the Department of Epidemiology of the National Institute of Public Health, the situation in Poland is alarming as they show a tenfold increase of the incidence rate during the past decade, from around 9 to 36 cases of infection per 100 000 people. Similar situation has been observed for other European countries.

Lyme disease is not typically a lethal disease; however, the quality of life of the infected individuals is considerably lower than in case of diabetes, heart disease, depression, arthrosis or rheumatoid disease patients. Furthermore, Lyme disease has a destructive influence on domestic animals and livestock, which causes losses in agriculture. As the number of infections with *B. burgdorferi* increases, it is necessary to prevent further spread of the dangerous pathogen. Currently, the most common method of prevention used worldwide, and the only one used in Poland, is education of those exposed

to contact with ticks. In general, it consists in encouraging wearing protective clothing or use tick deterrents and instructing on how to act in case of a tick bite. However, according to the data from the USA, the effectiveness of wearing protective clothing is 40%, and in case of tick deterrents use it is 20% (Vasquez et al., 2008). Vaccination for humans and animals could be an effective method for Lyme disease prevention. Attempts to develop a vaccine are being made in Europe and USA. The only vaccine allowed for the 3rd phase of clinical trial and, consequently, approved by the FDA was Lymerix – that entered the U.S. market in 1998. An active ingredient of the vaccine was the OspA surface recombinant protein *B. burgdorferi sensu stricto*. The antibodies directed against OspA neutralize the bacteria present in the tick gut, which prevents human infection. Nevertheless, the vaccine was withdrawn by the manufacturer in 2002 due to poor demand, high price and rheumatological side effects (*arthralgia*) that had appeared for a few individuals after using Lymerix, as officially stated. The possible side effects were associated with the fact that the OspA protein includes an antigen homologous to human LFA-1 antigen (*Lymphocyte function-associated antigen 1*), which, in case of immunization with OspA protein, may result in autoimmune response of an organism. A vaccine based on OspA administered in three doses showed a considerably high effectiveness of 79% at the 3rd phase of clinical trial (Abbott, 2006). In the USA, the vaccines based on OspA recombinant protein for domestic animals are still available.

A more comprehensive approach to Lyme disease prevention could involve the introduction of prophylactic vaccination of wild animals that constitute a reservoir of *Borrelia*. This would decrease the number of infections with *B. burgdorferi.* One of the research groups in the USA is working on an OspA protein-based vaccine for wild animals that applies the *Vaccinia* virus, similarly as in case of rabies vaccine. A product has been prepared in the form of feed that included a harmless, modified *Vaccinia* virus carrying in its DNA a gene coding for OspA. An expression of the virus genes and OspA protein has been shown for the mice treated with the product. The presence of OspA in mouse organism resulted in the development of anti-OspA antibodies to the level that conferred effective protection against infection with *B. burgdorferi* in laboratory conditions (Bhattacharyaa et al., 2011).

In Europe numerous *Borrelia burgdorferi* strains occur responsible for Lyme disease in people. These species are clustered into one phylogenetic group called *Borrelia burgdorferi sensu lato* (*s. l.*) or Lyme borreliosis group (LB). This diversity of Borrelia species cause Lyme disease prevention in Europe even more complicated than in North America, as the vaccine against *B. burgdorferi sensu stricto* (*s. s.*) manufactured in the USA, based on the surface protein of the single Borrelia species, is ineffective. This is a consequence of serological disparities among the numerous bacteria strains occurring in Europe, which is due to the fact that the *B. burgdorferi sensu lato* surface proteins are encoded by plasmid DNA that is highly variable (Franke et al., 2013). Therefore, intensive studies that aim at the development of a vaccine against the infection with *B. burgdorferi* for humans are still being carried out. These studies aim at the production of OspA-derivatives based vaccine or rely on the searching of the other Borrelia derived proteins which could be applied as a vaccine.

Still, the serologic differences occurring among the bacteria strains of various geographical regions, that result from the fact that *B. burgdorferi s. l.* surface proteins are coded by highly variable plasmid DNA, are the cause of a continuous search for an alternative to the vaccines based on surface proteins of *B. burgdorferi*. An interesting paper has been published that presents protective features of TROSPA and Salp15 *Ixodes scapularis* proteins. The TROSPA protein occurs on the surface of *Ixodes scapularis* gut (Pal et al., 2004; Urbanowicz et al., 2013). This protein participates in the process of *B. burgdorferi s. s.* entering the vector via interaction with OspA protein of the spirochete. Another protein, called Salp15 is present in *Ixodes scapularis* saliva. It interacts with a surface OspC protein of *B. burgdorferi s. s.* and forms a protective coating on the bacteria surface that inhibits bacteria recognition by immunological system of an infected vertebrate (Lewandowski et al., 2012). Even though both TROSPA and Salp15 proteins come from a tick (*Ixodes scapularis*), they play key role in two different phases of *B. burgdorferi s. s.* life cycle. It was also showed, that the immunization of *B. burgdorferi s. s.*-infected animals through intraperitoneal or subcutaneous injection of recombinant TROSPA protein from *I. scapularis* results with the decreasing of the percentage of the next ticks colonized by

B. burgdorferi s. s. The laboratory animals injected with recombinant Salp15 protein revealed considerable immunity to the infection with *B. burgdorferi s. s.* Moreover, it was showed that enrichment of the previously produced vaccines based on recombinant surface OspA and OspC proteins with Salp15 protein significantly increased the effectiveness of protection against the infection with a spirochete. Taking these findings to account, TROSPA and Salp15 proteins from *I. scapularis* were suggested to be good candidates for the vaccine limiting the incidence of *B. burgdorferi s. s.* in its natural reservoir – wild living animals. This reservoir vaccination strategy should be effective in North America since *I. scapularis* is endemic for this region and is a vector for the single species *Borrelia burgdorferi s. s.*

In Europe, tick vector for *Borrelia*, *I. ricinus* is found. The present invention enable obtaining a vaccine against Lyme disease that comprises recombinant TROSPA and TROSPA-Salp15 proteins produced in bacterial system inducing the response of the immunological system after an oral administration. The aim of the invention is to provide an oral vaccine protecting animals from *Borrelia burgdorferi s. l.* infection. The oral vaccine comprises TROSPA and TROSPA-Salp15 proteins from *I. ricinus* found in Europe. The oral route of administration of the vaccine is appropriate for the *Borrelia* reservoir vaccinations. The reservoir vaccinations via injection are unfeasible task. The reservoirs are both the wild living animals and the animal farm livestock, where the populations of *Ixodes* ticks occur. After the oral immunization of animals with the presented vaccine, the antibodies against TROSPA and TROSPA-Salp15 proteins will be produced by their immunological system. The antibodies present in blood of an immunized vertebrate directed against TROSPA proteins significantly lower the chance of infecting new ticks by blocking or inhibiting the interaction of TROSPA and OspA from at least three *Borrelia* species found in Europe: *B. burgdorferi sensu stricto, B. garinii and B. afzelii*. This reduces the natural reservoir of bacteria, which results in lower incidence of Lyme disease in humans and animals. Furthermore, the antibodies present in blood of an immunized vertebrate directed against Salp15 antigen of TROSPA-Salp15 fusion protein protect an animal against infection on the spirochetes entering stage through destroying the protective coating on the bacteria surface formed by the Salp15 and OspC interaction. The vaccine based on TROSPA protein and TROSPA-Salp15 protein may be used separately or combined with OspA and OspC proteins of *Borrelia burgdorferi sensu lato.*

Claims

1) Oral Lyme disease vaccine based on TROSPA tick protein characterized in that it contains recombinant TROSPA protein defined by SEQ. ID No. 3
2) Oral Lyme disease vaccine based on TROSPA-Salp15 tick protein characterized in that it contains recombinant TROSPA-Salp15 protein defined by SEQ. ID No. 4.
3) The oral vaccine, according to claim 1, characterized in that it contains a protein obtained from the expression of the TROSPA genetic construct defined by SEQ. ID No. 1 in *E. coli*
4) The oral vaccine, according to claim 2, characterized in that it contains a protein obtained from the expression of the TROSPA-Salp15 genetic construct defined by SEQ. ID No 2.
5) The use of a protein, according to claim 1, where the preparation containing the purified recombinant protein identified by SEQ. No. 3 and recombinant OspA and OspC protein Bo*rrelia burgdorferi sensu lato* is administered orally.
6) The use of a protein, according to claim 2, where the preparation containing the purified recombinant protein identified by SEQ. No. 4 and recombinant OspA and OspC protein Bo*rrelia burgdorferi sensu lato* is administered orally.

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