INFECTIOUS DISEASES

An mRNA-based anti-tick vaccine catches ticks red-handed

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An anti-tick mRNA cocktail vaccine promotes tick detachment and prevents transmission of tick-borne infection in guinea pigs (Sajid *et al.*).

Ticks are blood-sucking ectoparasites and vectors of a wide range of pathogens that cause severe infectious diseases in humans as well as in domestic and wild animals. Unlike blood-feeding insects, ticks feed on their host for several days. Such a prolonged association with their host offers an opportunity to impair the tick's capacity to engorge and transmit tick-borne pathogens, such as the pathogen that causes Lyme disease, *Borrelia burgdorferi*. As an environmentally friendly alternative to the commonly used repellents and acaricides, scientific teams around the globe have been seeking to develop an efficient anti-tick vaccine that would limit attachment of ticks to their hosts and prevent transmission of tick-borne pathogens. This endeavor has involved a variety of strategies ranging from the identification of individual molecules playing an essential role in tick physiology and pathogen transmission to high-throughput "vaccinomics" approaches (*1*). Traditionally, attempts have been made to block tick salivary and midgut proteins using antibodies raised against corresponding recombinant antigens. The global use of mRNA vaccines and their remarkable success in limiting the coronavirus 2019 (COVID-19) pandemic has raised the logical question of whether mRNA vaccines could also be developed against ticks and tick-borne pathogens. In this issue of *Science Translational Medicine,* Sajid *et al.* (*2*) report that an mRNA vaccine encoding tick salivary proteins could limit the duration of tick nymph feeding on guinea pigs as model animals and prevent subsequent transmission of *B. burgdorferi* spirochetes.

TICK SALIVARY PROTEINS AS VACCINE TARGETS

Ticks salivate while feeding on their hosts. They modulate host defense responses by secreting a salivary pharmacopeia containing hundreds of bioactive proteins and lipids into their feeding site (the tick-host interface). Myriad biological functions have been attributed to tick salivary components, including suppression of the host immune response, inflammation, pain, blood clotting, and vasoconstriction. Together, these tick salivary molecules allow the parasite to feed undisturbed on the host for an extended period of time (*3*).

The 80-year-old observation that some mammalian hosts can acquire resistance to repeated tick infestations (*4*) has become the basis for intense research on antigenic molecules present in tick saliva that could elicit host defense responses and lead to tick rejection (*5*). Tick salivary proteins, including blood clotting inhibitors, metalloproteases, vasodilators, and complement inhibitors, play a crucial role at the tick-host interface. These proteins are represented by multi-gene families whose expression waxes and wanes during the course of tick feeding. This complicates any attempt to mimic acquired resistance by vaccination using a single recombinant antigen, no matter how promising. Therefore, the concept of mixing antigens into multi-target vaccine "cocktails" has been proposed as a solution toward increasing vaccine efficacy (*6*). Following this hypothesis, several research groups have tested different vaccine cocktails against ticks. The vaccine cocktails were prepared by mixing up to five recombinant antigens or by generating chimeric antigens through the fusion of two or more antigenic epitopes. However, vaccine efficacy seldom fulfilled expectations that were based on the simple summing of the benefits of individual antigen components (*7*).

In their new work, Sajid *et al.* (*2*) present a pioneering study that uses an mRNA vaccine targeting multiple tick salivary proteins. The vaccine technology platform, based on nucleoside-modified mRNAs encapsulated in lipid nanoparticles (LNP) (*8*), allowed the authors to combine mRNAs encoding 19 salivary proteins from the black-legged tick, *Ixodes scapularis*, the most important vector of Lyme disease in the United States. The specific mRNAs were rationally selected based on previously published studies by this group and others demonstrating the roles played by the respective salivary proteins in eliciting an inflammatory reaction in the host and, in some cases, in limiting the transmission of the Lyme disease pathogenic agent, *B. burgdorferi*. The cocktail mRNA vaccine, called 19ISP (19 Ixodes salivary proteins), was used for prophylactic intradermal vaccination of guinea pigs, a mammalian host known to gradually develop resistance to repeated tick infestations.

First, the authors validated the antigenicity of individual salivary protein components. To do so, all mRNA-encoded proteins in the vaccine were prepared as recombinant proteins and tested in an enzyme-linked immunosorbent assay (ELISA) against serum samples from vaccinated guinea pigs. The ELISA results showed that at least 10 out of 19 antigens were immunogenic and led to production of antibodies. Immunization of guinea pigs with 19ISP generated acquired immunity against ticks, which manifested as redness or erythema surrounding tick bite sites as early as 18 hours after tick attachment. No such reaction was observed in the guinea pigs immunized with a control vaccine containing an mRNA that encoded luciferase. The authors also performed differential transcriptomic mapping analyses and observed differences in humoral and cellular immune responses in 19ISP-vaccinated guinea pigs associated with acquired tick resistance.

As a consequence of the inflammatory reaction in vaccinated guinea pigs, the feeding of *I. scapularis* nymphs was impaired. The authors observed that nymphs dropped off of the vaccinated hosts earlier than they did in control animals, and most nymphs did not succeed in becoming fully engorged. In

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contrast, this effect was not observed in vaccinated mice, the natural hosts of *I. scapularis* nymphs, which do not develop acquired tick immunity. Humans, as non-natural hosts of *Ixodes* sp., also display features of acquired resistance to repeated tick feeding, which reportedly reduces the likelihood of contracting Lyme disease. Therefore, a logical question, further addressed in the Sajid *et al.* study (*2*), was whether mimicking acquired tick resistance by immunization with 19ISP would also protect guinea pigs against the transmission of *Borrelia* spirochetes from infected tick nymphs. In contrast to the swift transmission of tick-borne viruses, infection of a naïve host with the bacterial spirochete *B. burgdorferi* is a time-dependent process that usually requires about 36 hours of nymphs feeding on the host (*9*). In accordance with this, *Borrelia* spirochetes were not detected in skin biopsies from 19ISP-immunized guinea pigs when the infected nymphs were removed by investigators at the time that erythema was observed. Protection against *Borrelia* transmission was not as effective when ticks were allowed to feed until repletion. Thus, the authors demonstrated that early tick removal, together with the 19ISP-driven inflammatory reaction around the tick bite, is a key step toward the development of a vaccine for preventing Lyme disease transmission (Fig. 1).

THE THORNY ROAD TO A LYME DISEASE VACCINE

Lyme disease (borreliosis) is the most common vector-borne infection in the USA and Europe. With the actual incidence of Lyme disease estimated to be up to 10 times higher than reported cases, this disease is a true silent epidemic. Since the discovery of *B. burgdorferi* as the causative agent of Lyme disease in 1982, considerable research efforts have been dedicated to identifying suitable targets for a protective vaccine. The initial simple idea of finding a universal molecule on the surface of *Borrelia* to be used as the basis for a new vaccine turned out not to be straightforward. *Borrelia* bacteria alternate between two completely different environments in their life cycle: the lumen of the tick gut and tissues of the vertebrate host. *Borrelia* spirochetes entirely change the expression of their surface antigens during tick feeding, which is a time-consuming process essential for successful host colonization. Thus, *Borrelia* in a tick and *Borrelia* in the host are hardly recognizable as the same bacterial pathogen. The situation is further complicated by the broad spectrum of *Borrelia* species and the associated high interspecies variability of surface molecules. Overcoming such a formidable competitor and identifying reliable vaccine targets on the surface of highly variable *Borrelia* is a challenge. The outer surface protein A (OspA) that spirochetes express while dwelling in the tick midgut formed the basis of the first licensed vaccine launched in 1998 under the brand name LYMErix; this eventually turned out to be commercially unsuccessful. However, the second generation of OspA-based vaccines is still in the development pipelines of several pharmaceutical companies (*10*).

The pioneering proof-of-concept study of Sajid *et al.* (*2*) using an mRNA vaccine cocktail is the most recent example of a strategy for preventing transmission of *Borrelia* by immunizing the host with tick vector–derived antigens. This approach may be a useful alternative or, perhaps, a complementary strategy to vaccines using pathogen antigens. Grooming has been recognized as the first line of defense against ectoparasites; therefore, the early awareness of a tick bite, induced by the vaccination with the 19ISP mRNA cocktail, would substantially reduce the incidence of Lyme disease in the human population. Given the capability and versatility of mRNA vaccine technology, one can anticipate that refined and optimized mRNA vaccine cocktail recipes will be developed combining tick salivary antigens eliciting resistance to ticks with pathogen-related antigens such as OspA. This could lead to the formulation of the long-desired Lyme disease vaccine for humans. Thus, mRNA vaccine technology has initiated a new era in the development of efficient and commercially appealing vaccines for the effective control of ticks and tick-borne diseases.

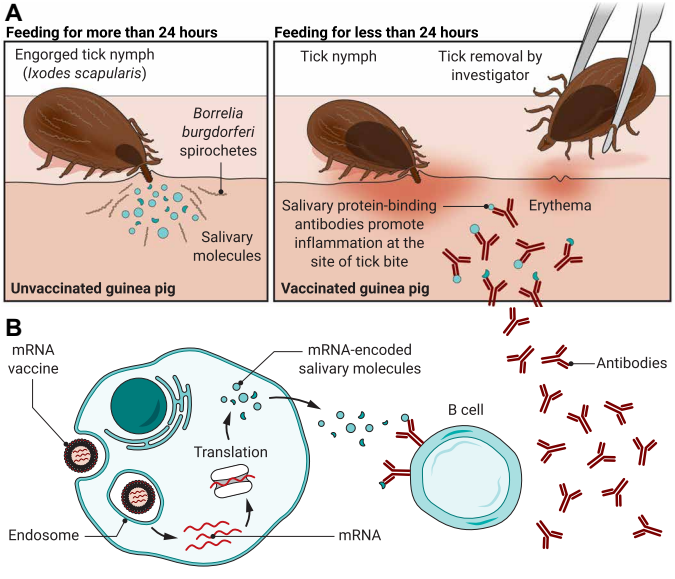

Fig. 1. Catching ticks in the act. Sajid *et al*. demonstrate that a vaccine cocktail of mRNAs encoding tick salivary proteins promotes redness at the site of tick bites and prevents transmission of *Borrelia burgdorferi*, the pathogenic agent of Lyme disease, in a guinea pig model (*2*). (**A**) Nymphs of the tick *Ixodes scapularis*, the vector that transmits *B. burgdorferi*, take several days to feed on their mammalian hosts. *B. burgdorferi* requires at least one day of tick feeding to change its surface coat and become infectious to the vertebrate host. Acquired resistance to ticks, where antibodies induce immune activation and redness (erythema) at the site of the tick bite, promotes awareness of tick presence. This makes it possible to remove the tick quickly, thus preventing transmission of *B. burgdorferi*. (**B**) In the Sajid *et al*. study (*2*), the authors vaccinated guinea pigs with a vaccine cocktail of 19 mRNAs encoding tick salivary proteins packaged in lipid nanoparticles. This mRNA "anti-tick" vaccine promoted antibody responses against tick salivary proteins in the guinea pigs, resulting in swelling and redness at tick bite sites. Such an inflammatory response could help to alert hosts quickly to attached tick nymphs and prevent *Borrelia* transmission.

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Abstract

One-sentence summary: An anti-tick mRNA cocktail vaccine promotes tick detachment and prevents transmission of tick-borne infection in guinea pigs (Sajid *et al.*).