

## Novel nanoparticle vaccines for Listeriosis

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**I**n recent years, nanomedicine has transformed many areas of traditional medicine, and enabled fresh insights into the prevention of previously difficult to treat diseases. An example of the transformative power of nanomedicine is a recent nano-vaccine against listeriosis, a serious bacterial infection affecting not only pregnant women and their neonates, but also immune-compromised patients with neoplastic or chronic autoimmune diseases. There is a major unmet need for an effective and safe vaccine against listeriosis, with the challenge that an effective vaccine needs to generate protective T cell immunity, a hitherto difficult to achieve objective. Now utilizing a gold nanoparticle antigen delivery approach together with a novel polysaccharide nanoparticulate adjuvant, an effective T-cell vaccine has been developed that provides robust protection in animal models of listeriosis, raising the hope that one day this nanovaccine technology may protect immune-compromised humans against this serious opportunistic infection.

Listeriosis is caused by *Listeria monocytogenes* (*Listeria*), a bacterium that causes opportunistic infections of high-risk individuals including immune-compromised patients, pregnant women, new-borns and the elderly, resulting in high mortality even with timely antibiotic treatment. The most common listeriosis-related morbidities are abortions, premature birth, foetal malformations, meningitis, meningoen- cephalitis and septicaemia by virtue of *Listeria's* ability to target to the central nervous system<sup>1</sup> and fetus. However, even

individuals lacking risk factors can be affected by *Listeria* infection since it is a common food borne pathogen and a faecal carrier in 1–10% of the population.

Listeriosis occurs sporadically and in outbreaks, mainly through the ingestion of contaminated food or vertical mother-fetus transmission. In Europe there has been an increase in the number of listeriosis cases since 2008,<sup>2–4</sup> with the last occurring in summer 2014 in Northern Spain with the cause being ingestion of contaminated food.<sup>2</sup> An increasing number of individuals within the population are susceptible to infection due to the increasing use of new biological immune-suppressive therapies for patients with chronic autoimmune disease, the aging of the population and hence the increasing number of elderly and immune-compromised patients.<sup>4</sup> The detailed analysis of *Listeria* strains isolated from the last outbreak in northern Spain, showed a 10-fold increase in bacterial growth inside dendritic cells, compared to strains isolated in Spain 20 years ago<sup>5</sup> and suggested that the character of human listeriosis might have undergone some modifications over this time. Unfortunately *Listeria* is not yet included on the Mandatory Notification System of microbes,<sup>6</sup> making comprehensive studies of its epidemiology difficult. Nevertheless, the increasing number of serious cases of listeriosis urges the development of a vaccine to help in its control and prevention.

Conventional vaccination strategies against listeriosis using antigens or killed bacteria have been abandoned as they failed to induce significant T-cell responses required for protection. *Listeria*

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live vector vaccines are also not an alternative since they represent a serious risk in immune-compromised individuals. Another important issue concerning listeriosis vaccines is that they need to induce simultaneous CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses and be effective across the broad range of HLA haplotypes possessed by individuals at risk of listeriosis. While cellular dendritic cell (DC) vaccines loaded *ex vivo* with the *Listeria* GAPDH<sub>1-22</sub> peptide from glyceraldehyde-3-phosphate-dehydrogenase induced significant T-cell immunity and wide protection<sup>7-9</sup> they are highly impractical and expensive to make, and thereby not suitable for routine human prophylactic vaccination. Hence better options are needed if a human vaccine against listeriosis is ever to be achieved.

Nanomedicine offers versatile tools for infectious diseases with nanomaterials having been used to develop vaccines<sup>10,11</sup> and antibacterial agents.<sup>12,13</sup> Multivalent glycoconjugates based on gold glyconanoparticles (GNP) which allow multiple interactions between their sugars and cell surface receptors<sup>14,15</sup> represent an appealing vaccine delivery technology. Major GNP advantages are their easy handling, resistance to enzymatic degradation, water solubility, convenient antigen loading, immune cell targeting, lack of toxicity and small size around 2 nanometers.<sup>16</sup> They were therefore used to generate a *Listeria* based GNP nanovaccine by covalent linking of glucose and a peptide representing the *Listeria* T-cell epitope (LLO<sub>91-99</sub>) to the GNP metallic core thereby creating GNP-LLO<sub>91-99</sub> nanoparticles.<sup>17</sup> The aim was to improve upon the effectiveness previously seen with a DC vaccine loaded with LLO<sub>91-99</sub> peptide that induced CD8 T-cell responses and IFN- $\gamma$  levels<sup>6,9,18</sup> but only modest *Listeria* protection. The GNP-LLO<sub>91-99</sub> nanovaccine was shown to be free of cytotoxicity, effectively delivered the *Listeria* epitope to DC *in vitro* and *in vivo* and induced effective CD8<sup>+</sup> T-cell immunity. Unfortunately, listeriosis protection with the LLO<sub>91-99</sub> nanovaccine was only partial and inferior to that obtained with a DC vaccine loaded with GNP-LLO<sub>91-99</sub>. Consequently, the GNP-LLO<sub>91-99</sub> nanovaccine was combined with the novel polysaccharide nanoparticle

adjuvant, Advax<sup>TM</sup>, an adjuvant derived from delta inulin microparticles that has previously been shown to be effective and safe in human trials of influenza and hepatitis B vaccines<sup>19,20</sup> and has been shown to induce robust CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses.<sup>21,22</sup> GNP-LLO<sub>91-99</sub> nanovaccines formulated with Advax<sup>TM</sup> had markedly enhanced T-cell immunogenicity and conferred listeriosis protection to levels previously only achieved with *in vitro* DC loaded vaccines. Notably, the frequency of activated DC doubled when Advax<sup>TM</sup> adjuvant was added to the nanovaccine, together with significantly increased IL-12 production. Furthermore, Advax<sup>TM</sup> incorporation resulted in robust *Listeria*-specific CD4 and CD8 T-cell responses after a *Listeria* challenge. Thus, Advax<sup>TM</sup> induced epitope spreading to the GNP-LLO<sub>91-99</sub> nanovaccine resulting post-*Listeria* challenge in T-cell recognition of other *Listeria* epitopes not present in the vaccine.

In summary, a GNP-LLO<sub>91-99</sub> nanovaccine combined with the novel polysaccharide adjuvant provided unique *in vivo* DC targeting and induction of robust T-cell responses. This translated into robust protection against *Listeria* challenge not previously able to be achieved except with a complex and expensive *in vitro* DC loaded vaccine approach. This offers the first opportunity to produce a cheap and reliable *Listeria* vaccine for use as a conventional prophylactic vaccine for all individuals at risk of listeriosis. It could also be potentially useful as an immune potentiating vaccine for already infected patients, although this use must await studies of its effectiveness in already infected models. This novel nanovaccine shows the potential for nanomedicine to transform vaccine development and help solve longstanding problems including the need for an effective vaccine platform able to generate protective T-cell responses.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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