Journal of Controlled Release 243 (2016) 146–159

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Non-invasive epicutaneous vaccine against Respiratory Syncytial Virus: Preclinical proof of concept

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ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form 3 October 2016
Accepted 4 October 2016
Available online 6 October 2016

Keywords:
Respiratory Syncytial Virus
Cutaneous vaccine
Viaskin® patch
N-nanoring nanoparticle

ABSTRACT

To put a Respiratory Syncytial Virus (RSV) vaccine onto the market, new vaccination strategies combining scientific and technical innovations need to be explored. Such a vaccine would also need to be adapted to the vaccination of young children that are the principal victims of acute RSV infection. In the present project, we describe the development and the preclinical evaluation of an original epicutaneous RSV vaccine that combines two technologies: Viaskin® epicutaneous patches as a delivery platform and RSV N-nanorings (N) as a subunit antigen. Such a needle-free vaccine may have a better acceptability for the vaccination of sensible population such as infants since it does not require any skin preparation. Moreover, this self-applicative vaccine would overcome some issues associated to injectable vaccines such as the requirement of sterile medical devices, the need of skilled health-care professionals and the necessity of stringent store conditions. Here, we demonstrate that Viaskin® patches loaded with a formulation containing N-nanorings (Viaskin®-N) are highly immunogenic in mice and promotes a Th1/Th17 oriented immune response. More importantly, Viaskin®-N epicutaneous vaccine confers a high level of protection against viral replication upon RSV challenge in mice, without exacerbating clinical symptoms. In swine, which provides the best experimental model for the transcutaneous passage of drug/antigen in human skin, we have shown that GFP fluorescent N-nanorings, delivered epicutaneously with Viaskin® patches, are taken up by epidermal Langerhans cells. We have also demonstrated that Viaskin®-N induced a significant RSV N-specific T-cell response in pig. In conclusion, Viaskin®-N epicutaneous vaccine seems efficient to protect against RSV infection in animal model.

1. Introduction

The development of a safe and effective RSV vaccine for infants in the first six months of life is a public health challenge for reducing the severe burden of RSV-associated respiratory diseases, especially bronchiolitis and hospitalizations. Globally, it is estimated that RSV causes >30 million lower respiratory tract infections each year resulting in >3 million hospitalizations, making it the most common cause of hospitalizations in children under 5 [1]. Moreover, severe RSV incidence is highest in infants younger than 5 months.

Development of RSV vaccines has been complicated by the dramatic outcome of the first clinical trial in the 60′s, which examined the efficacy of a formalin-inactivated virus vaccine (FI-RSV) in infants and young children. Indeed, this vaccine formulation exacerbated clinical symptoms upon RSV infection and led to the hospitalization of almost 80% of the vaccinated children [2].

To date, there is no available vaccine against RSV. A large array of alternative vaccination strategies (antigen candidates and routes of administration) are being explored, but without providing satisfactory solutions [3–5]. There are indeed major challenges unique to RSV related to i) the young age of infection, ii) the failure of natural infection to induce immunity that prevents re-infection, and iii) the risk of immune-mediated disease exacerbation. Furthermore, due to the young age of the target population, a non-invasive and painless vaccine approach would be highly desirable.

In the present study, we evaluated a novel vaccination strategy against RSV that combines two original technologies: Viaskin®

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http://dx.doi.org/10.1016/j.jconrel.2016.10.003

epicutaneous patches as a delivery platform (patent WO/2011/128430) [6] and RSV nucleoprotein nanorings (N-nanorings) as a subunit antigen (patents WO/2007/119011 and WO/2006/117456) [7,8]. Purified recombinant RSV N assembles as soluble nanorings of 15 nm diameter, composed of 10 to 11 N protomers bound to a bacterial RNA of 70 bases [9,10]. These N-nanorings, when administered intranasally, induce potent local and systemic immune responses in mice and calves and confer protection against an RSV challenge [11,12]. Protection afforded by N-nanorings in mice correlates with the presence of potent N-specific CD4 and CD8 T-cell responses [11]. N-nanorings are also able to induce a strong anti-N humoral response. However, these antibodies are non-neutralizing and passive transfer experiments performed in mice suggested that they possess no effective role in protection [11].

Skin has been recently explored as an original route for immunization, especially for the induction of a potent mucosal immune response against respiratory, gastro-intestinal or sexually transmissible pathogens [13]. Several vaccine strategies have been explored for the transcutaneous delivery of antigens [14]. However, due to the difficulty to get through the skin's top dead layer (*stratum corneum*), the vast majority of these approaches requires a preparation of the skin such as mechanical or chemical disruption, or the use of microneedles [15]. A few years ago, a novel epicutaneous delivery system was designed by DBV Technologies for food allergy desensitization [16]. This system is composed of patches (Viaskin® technology) that form an occlusive condensation chamber where allergen is solubilized by skin perspiration (skin hydration) and delivered across the stratum corneum to the epidermis without any skin preparation (see Graphical abstract) [16–22]. More recently, DBV Technologies got the proof-of-concept that Viaskin® patches can also be used as an efficient vaccine delivery platform in a murine model, in the context of *Bordetella pertussis* (*B. pertussis*) booster vaccine development [23]. Indeed, a single application of Viaskin® patch loaded with genetically-detoxified pertussis toxin (rPT), in the absence of adjuvant, was able to efficiently recall memory vaccine-induced immunity against *B. pertussis*.

The skin barrier is composed of a dense network of antigen presenting cells (APCs), including dendritic cells (DC) such as Langerhans cells (LC) that resides in the epidermis layer [24]. These DC provide immune-surveillance by "sensing" pathogens passing through stratum corneum and play a central role to activate the adaptive immunity. Our group has pioneered the characterization of DC subsets within the skin of pig and shown that it shares many similarities with human skin DC [25]. In fact, pig skin histology is very similar to the human one's and is thus recognized as one of the most appropriate model to study delivery of pharmaceutical compounds *via* skin [25–27]. Thus we investigated in piglets whether fluorescent N-nanorings fused to GFP would reach skin immune cells when administered with the Viaskin® platform. We observed both an internalization of N-GFP antigen by skin Langerhans cells and the induction of antigen-specific cellular immune responses in spleen.

The immunization procedure with N-nanorings-loaded Viaskin® patches (Viaskin®-N) was further investigated in mice in order to characterize the antiviral immune response elicited and its capacity to protect against challenge with an RSV-Luciferase recombinant virus. Viaskin®-N vaccination elicited a strong antigen-specific cellular response in mice and piglets. In mice, Viaskin®-N promotes a Th17/Th1 oriented immune response and conferred protection against virus replication in lungs.

2. Material and methods

2.1. Plasmid construction, protein expression and purification of N-nanorings

Expression and purification of recombinant nucleoprotein (N) was performed as previously described [9]. Briefly, the purification of the recombinant N protein is permitted by its specific interaction with the C-terminal region of RSV P protein (PCT) (residues 161–241) fused to GST. *E. coli* BL21 (DE3) bacteria were co-transformed with pGEX-PCT and pET-N and then grown at 37 °C for 8 h in 1 L of Luria-Bertani (LB) medium containing 100 μg/mL of ampicillin and 50 μg/mL of kanamycin. The same volume of LB medium was then added, and protein expression was induced by adding 80 μg/mL isopropyl-β-D-thiogalactopyranoside (IPTG) to the medium. Bacteria were incubated for a further 15 h at 28 °C and harvested by centrifugation. Protein complexes were purified from the bacterial pellets on glutathione-sepharose 4B beads (GE Healthcare, Uppsala, Sweden) as previously described [28]. To isolate N-nanorings, beads containing bound complexes were incubated with biotinylated thrombin (Merck Millipore, Darmstadt, Germany) for 16 h at 20 °C. Thrombin was then removed using the cleavage capture kit according to the manufacturer's instructions (Merck Millipore, Darmstadt, Germany). Purified N-nanorings were then concentrated on Vivaspin® centrifugal concentrator (Sartorius, Goettingen, Germany) and sterilely filtered. Of note, a small amount of PCT was co-purified with chimeric N-nanorings. However, we previously demonstrated that PCT is poorly immunogenic and induced a low anti-P antibody response by comparison with the entire P protein [29]. N-nanorings fused to Green Fluorescent Protein (N-GFP) was constructed as previously described [11]. Briefly, the EGFP coding sequence was cloned onto pET-N plasmid. The resulting plasmid was designated as pET-N-GFP and encode for a chimeric N protein fused to EGFP at its C-terminus. The expression and the purification protocols of N-GFP were identical to those of N protein alone.

2.2. Production of N-nanorings-loaded Viaskin® patches (Viaskin®-N)

N- and N-GFP- nanorings were purified as described above. Then, a homogeneous mix between N- or N-GFP nanorings and CpG was made in PBS to a respective concentration of 1 mg/mL. 50 μL (mice immunization) or 100 μL (piglets immunization) were then homogeneously deposited on Viaskin® patches and dried 1 h at 30 °C in a ventilated oven to produce Viaskin®-N and Viaskin®-N-GFP, respectively. The stability of N-nanorings after their deposition on Viaskin® patches was then controlled by native gel electrophoresis, electronic microscopy and DLS after recovery from the matrix of the patch (Fig. S1). Results showed that N-nanorings integrity was preserved upon deposition and drying on Viaskin® patches.

2.3. Mice: immunization, challenge protocols and sample collection

Female BALB/c mice aged 6 weeks were purchased from Janvier's breeding center (Le Genest, St Isle, France) and housed under Bio-Safety Level (BSL)-2 conditions in the animal facility (IERP, INRA, Jouy-en-Josas, France). All experiments were carried out in INRA (Rodent Experimental Infectiology Platform, Jouy-en-Josas, France) and approved by the ethics committee COMETHEA (Ethical Committee for Animal Experimentation, INRA and AgroParisTech; authorization number 12-126). All efforts were made to optimize animal welfare (environmental enrichment) and avoid suffering.

For Viaskin® patches immunization, mice were anesthetized with a solution of ketamine and xylazine (50 and 10 mg/kg respectively) and hairs were removed from the back of each mouse using an electric clipper and then a depilatory cream (Veet®, Reckitt Benckiser, Slough, Berkshire, United Kingdoms). N-rings loaded Viaskin® (Viaskin®-N) was applied on the depilated back the day after and secure using a bandage (Urgoderm® band-aid, Urgo laboratories, Chenôve, France).

For intra-nasal immunizations, mice were anesthetized with a solution of ketamine and xylazine (50 and 10 mg/kg respectively) and vaccinated twice at 2 weeks interval by intra-nasal instillation of 50 μL of 0.9% endotoxin-free NaCl, containing 10 μg of N-nanorings, associated to 10 μg of CpG ODN (1826) (T*C*C*A*T*G*A*C*G*T*T*C*C*T*G*A*C*G*T*T, Sigma-Aldrich, Saint Louis, Missouri).