INSTITUT PASTEUR

Microbes and Infection 12 (2010) 11–18

www.elsevier.com/locate/micinf

Original article

Protection against nontypeable *Haemophilus influenzae* challenges by mucosal vaccination with a detoxified lipooligosaccharide conjugate in two chinchilla models

Wenzhou Hong[1], Daxin Peng[2], Maritza Rivera, Xin-Xing Gu*

Vaccine Research Section, National Institute on Deafness and Other Communication Disorders, Rockville, Maryland 20850, USA

Received 12 June 2009; accepted 11 September 2009
Available online 24 September 2009

Abstract

Otitis media (OM) can occur following outset of upper respiratory tract infections. Inhibition of bacterial colonization in nasopharynx (NP) by mucosal vaccination may prevent OM by reducing bacterial invasion of the middle ears (MEs). In this study, 80 chinchillas were intranasally (i.n.) immunized with a detoxified lipooligosaccharide (dLOS)-tetanus toxoid conjugate vaccine of nontypeable *Haemophilus influenzae* (NTHi) mixed with cholera toxin (CT) or CT alone. All vaccinated animals responded with elevated levels of mucosal and serum anti-LOS antibodies. Two weeks after the last immunization, 40 chinchillas were challenged i.n. with NTHi to evaluate NP colonization and ME infection while the rest of the animals were challenged transbullarly (T.B.) to examine the development of OM. Compared to the control group, the vaccination inhibited not only bacterial colonization in NP and transmission to MEs in the i.n. challenge group but also bacterial colonization in NP and transmission to unchallenged ears in the T.B. challenge group. Though no difference was found in the challenged ears of either group right after the T.B. challenge, an early clearance of NTHi from NP and unchallenged ears as well as less severity of OM in the unchallenged ears were observed in vaccinated animals. Current results along with our previous data indicate that mucosal vaccination is capable of inhibiting NTHi NP colonization and preventing OM occurrence in chinchillas; the i.n. challenge model is preferable for testing the mucosal vaccines while the T.B. challenge model is superior for testing the systemic vaccines.
Published by Elsevier Masson SAS.

Keywords: Mucosal immunization; Nontypeable *Haemophilus influenzae*; Chinchilla models; Lipooligosaccharide conjugate vaccine

1. Introduction

Otitis media (OM) is most common disease in young children [1]. Although the major bacterial pathogens, *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis*, have not changed over the past decades, it has been found that there is a shift in the distribution of these organisms. Because of immunization with pneumococcal polysaccharide and conjugate vaccines, the proportion of OM caused by the vaccine covered pneumococcal strains has been decreasing while NTHi and *M. catarrhalis* are becoming more frequent [2,3]. Though there are no licensed NTHi and *M. catarrhalis* vaccines available, a recent report showed that a pneumococcal polysaccharide vaccine conjugated to NTHi protein D prevented both pneumococcal and NTHi OMs with an overall 33.6% reduction [4]. Currently, antibiotics are still the most common approach for treatment of the disease, and such treatment can encourage the emergence of new drug-resistant bacterial strains [5]. There is a need to develop effective vaccines as well as immunization strategies against OM caused by all of these bacterial species,

* Corresponding author. 5 Research Court, Room 2A31, Rockville, MD 20850, USA. Tel.: +1 301 402 2456; fax: +1 301 435 4040.
E-mail address: guxx@nidcd.nih.gov (X.-X. Gu).
[1] Department of Otolaryngology and Communication Sciences, Medical College of Wisconsin, 9200 W. Wisconsin Avenue, Milwaukee, WI 53226, USA.
[2] School of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu 225009, China.

1286-4579/$ - see front matter Published by Elsevier Masson SAS.
doi:10.1016/j.micinf.2009.09.006

Table 1
Chinchilla antibody responses to NTHi 9274 LOS elicited by intranasal immunization with dLOS-TT conjugate vaccine.

Immunogen[a]	Isotype	GM antibody ELISA units (GM ± SD range)[b]	
		Nasal lavage	Serum
dLOS-TT + CT	IgA	143 (17∼1066)[c]	6 (1∼32)[d]
	IgG	134 (23∼790)[c]	9 (3∼26)[c]
CT	IgA	1 (0∼2)	0 (0∼1)
	IgG	1 (0∼2)	1

[a] Chinchillas from each group were given an intranasal immunization on days 0, 7, 14, 21 and 28 with dLOS-TT + CT, or CT alone. Samples were collected one week after the last immunization. dLOS: detoxified lipooligosaccharide. TT: tetanus toxoid. CT: cholera toxin.
[b] LOS antibody levels were measured by ELISA using strain 9274 LOS as a coating antigen, and expressed as the reciprocal of geometric mean (GM) and GM ± one standard deviation (SD) range within brackets.
[c] $p < 0.001$, compared to CT group.
[d] $p < 0.01$, compared to CT group.

especially for NTHi that causes a high percentage of chronic or recurrent OM in children [6].

Several NTHi vaccine candidates are under development including outer membrane proteins and lipooligosaccharide (LOS)-based conjugate vaccines [7–10]. Most of these vaccine candidates were administered by parental route and induced systemic antibodies while some of them showed protection against bacterial challenge in animal models. Since NTHi is a mucosal pathogen that needs to colonize in the nasopharynx prior to the onset of AOM, inhibition or reduction of pathogenic microorganisms colonizing in upper respiratory tract by mucosal immunity is believed to be an alternative. It may be even more effective in overcoming or preventing the occurrence of OM [11,12]. Several *in vitro* studies indicated that adherence of NTHi to human nasopharyngeal epithelial cells was inhibited by mucosal NTHi-specific S-IgA [13,14]. In murine models, intranasal immunization with outer membrane proteins or LOS based conjugates of NTHi can induce protective immunity including antibody production in the nasopharynx or middle ears (MEs) and enhancement of bacterial clearance from the upper respiratory tract [15–18]. However, there is a lack of information as to whether the mucosal immunization would generate immunity against NTHi NP colonization and subsequently prevent OM in a chinchilla model of OM. Previous studies have documented that the chinchilla model is a state-of-the-art model which mimics OM in humans and has been used for evaluating the efficacy of vaccine candidates by systemic vaccination against pneumococcal or NTHi-caused OM [19–22]. In the present study, we investigated the roles of mucosal immunization with a detoxified LOS-tetanus toxoid (dLOS-TT) conjugate [23] in the inhibition of bacterial colonization in the nasopharynx as well as for infection in MEs in chinchillas. Two challenge models, intranasal and transbullar infections, were used for evaluation and comparison of the protective properties elicited by the mucosal vaccination.

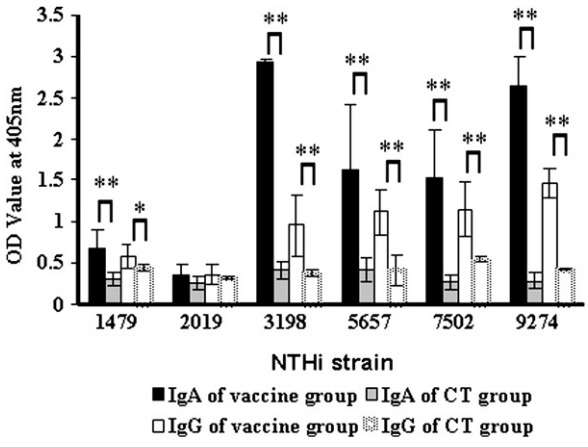

Fig. 1. Binding activities of mucosal antibodies elicited by dLOS-TT plus CT or CT alone to NTHi strains in the whole cell ELISA. Mucosal IgA and IgG (nasal lavages) bound to homologous strain 9274 and heterologous strains 1479, 3198, 5657 and 7502 but not to 2019. *$p < 0.05$; **$p < 0.01$.

2. Materials and methods

2.1. Conjugate vaccine

NTHi strain 9274, a clinical isolate from ME fluids of a patient with OM, was provided by Dr. M.A. Apicella, University of Iowa. The bacterial growth, purification of LOS from strain 9274, detoxification of the LOS, conjugation of dLOS to tetanus toxin (TT), and characterization of dLOS-TT were described previously in Ref. [24]. The composition of dLOS-TT was 638 ug/ml of dLOS and 901 μg/ml of TT with a molar ratio of dLOS to TT of 35: 1.

2.2. Immunization

80 Outbred, healthy chinchillas (weighing between 250 and 350 g) with no evidence of ear infection by otoscopic examination were purchased from Moulton Chinchilla Ranch, Rochester, Minn., and housed in individual cages. The animals underwent procedures in accordance with the National Institutes of Health guidelines under Animal Study Proposal 1074-2. Animals were randomly assigned to two groups. A vaccine group was immunized intranasally (i.n.) with 50 μl of phosphate-buffer saline (PBS) containing 25 μg of dLOS-TT (dLOS content) plus 2 μg of cholera toxin (CT) as an adjuvant (List Biological Laboratories, Campbell). A control group was immunized with 50 μl of PBS containing 2 μg of CT. The immunization was performed 4 times at one-week intervals. Each dose was inoculated into the nostrils (25 μl each side) under anesthesia with an intramuscular injection of a mixture of ketamine (1.0 mg/kg of body weight) and Xylazine (0.5 mg/kg of body weight) in PBS.

2.3. Nasopharyngeal (NP) lavage and blood collection

NP lavage samples were collected before the immunization, 1 week post-immunization, and days 3, 7, 14, 21 and 28 post-challenge as described previously in Refs. [25,26]. Briefly, under anesthesia, the chinchillas were laid on their sides while