Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Characterization of a novel oil-in-water emulsion adjuvant for swine influenza virus and *Mycoplasma hyopneumoniae* vaccines

A. Galliher-Beckley[a], L.K. Pappan[a], Rachel Madera[a], Y. Burakova[a,c], A. Waters[a], M. Nickles[a], X. Li[a], J. Nietfeld[b], J.R. Schlup[c], Q. Zhong[d], S. McVey[e], S.S. Dritz[b], J. Shi[a,*]

[a] *Department of Anatomy and Physiology, Pretoria, GA, USA*
[b] *Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Manhattan, NY, USA*
[c] *Department of Chemical Engineering, College of Engineering, Kansas State University, Manhattan, NY 66506, USA*
[d] *Department of Food Science and Technology, University of Tennessee, Knoxville, TN 37996, USA*
[e] *United States Department of Agriculture, Agricultural Research Service, Arthropod Borne Animal Disease Research Unit, Manhattan, NY KS 66502, USA*

ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form 14 April 2015
Accepted 16 April 2015
Available online xxx

Keywords:
Adjuvant
Oil-in-water emulsion
Swine influenza virus
Mycoplasma
Vaccine

ABSTRACT

Vaccines consisting of subunit or inactivated bacteria/virus and potent adjuvants are widely used to control and prevent infectious diseases. Because inactivated and subunit antigens are often less antigenic than live microbes, a growing need exists for the development of new and improved vaccine adjuvants that can elicit rapid and long-lasting immunity. Here we describe the development and characterization of a novel oil-in-water emulsion, OW-14. OW-14 contains low-cost plant-based emulsifiers and was added to antigen at a ratio of 1:3 with simple hand mixing. OW-14 was stable for prolonged periods of time at temperatures ranging from 4 to 40 °C and could be sterilized by autoclaving. Our results showed that OW-14 adjuvanted inactivated swine influenza viruses (SIV; H3N2 and H1N1) and *Mycoplasma hyopneumoniae* (*M. hyo*) vaccines could be safely administered to piglets in two doses, three weeks apart. Injection sites were monitored and no adverse reactions were observed. Vaccinated pigs developed high and prolonged antibody titers to both SIV and *M. hyo*. Interestingly, antibody titers were either comparable or greater than those produced by commercially available FluSure (SIV) or RespiSure (*M. hyo*) vaccines. We also found that OW-14 can induce high antibody responses in pigs that were vaccinated with a decreased antigen dose. This study provides direct evidence that we have developed an easy-to-use and low-cost emulsion that can act as a powerful adjuvant in two common types of swine vaccines.

1. Introduction

Although attenuated live organisms are frequently used as vaccines to control infectious diseases, these modified live vaccines (MLV) may pose potential safety risks when administered to immune-compromised animals or the virus/bacteria is capable of reverting to a virulent form. Another disadvantage of using modified live vaccines in disease management is that it is difficult to differentiate infected from vaccinated animals. Inactivated virus/bacteria, purified proteins, and synthetic peptides are considered to be much safer compared to live pathogens, but they are often less immunogenic [1,2]. Consequently, these vaccines rely on adjuvants to stimulate the innate immune response which in turn facilitates a strong adaptive response [3]. The incorporation of an adjuvant into a vaccine can achieve qualitative and quantitative alteration of the immune protection and provide functionally appropriate types of immune responses. Adjuvants also act to reduce the antigen dose required to generate a protective response and extend the duration of effective immunity [4]. New vaccine candidates have been developed recently against not only infectious agents but also allergic and autoimmune diseases, cancer, and infertility [5–10]. All these applications require adjuvants with desirable functions and performance in order to successfully achieve vaccination-induced immune protection and therapeutic effects.

Adjuvants can be broadly divided into two categories, the first being antigen vehicles, such as emulsions and liposomes, which act to present vaccine antigens to the immune system in a more efficient way and prolong the release of antigens to increase the specific immune responses [11]. The second category of adjuvants

* Corresponding author. Tel.: +1 785 532 4506; fax: +1 785 532 4557.
E-mail address: jshi@vet.k-state.edu (J. Shi).

http://dx.doi.org/10.1016/j.vaccine.2015.04.065
0264-410X/

are immuno-stimulants, such as Toll-like receptor (TLR) agonists, aluminum hydroxide, saponins and cytokines [12,13]. One major limiting factor of adjuvants is that many of them have unacceptable side effects and lack of biocompatibility. The most commonly used adjuvant in the U.S. is aluminum hydroxide (ALOH). ALOH is considered safe and is currently a component of several veterinary and human vaccines [14]. However, recent reports suggest that ALOH adjuvants lack efficacy for several pathogens [15]. Conventional oil-in-water emulsions use various chemical emulsifiers (i.e. Tween 80 and Span 80), but the safety of these chemicals when injected intramuscularly remains controversial [16]. Here, we use food-grade plant-derived surfactants commonly used in human food processing as emulsifiers to stabilize a novel oil-in-water emulsion, referred to as OW-14. OW-14 uses inexpensive, readily available materials, is stable at temperatures up to 40 °C, and can be autoclaved for sterilization. Furthermore, OW-14, when mixed with whole inactivated swine influenza and mycoplasma antigens can elicit higher and prolonged antibody responses than commercial vaccines for the same pathogens. Here, we provide evidence that OW-14 emulsion is a low-cost, easy-to-use alternative adjuvant for use in swine vaccines.

2. Materials and methods

2.1. Materials

Whole killed swine influenza virus (H3N2, 1.6 mg/ml) and whole killed *Mycoplasma hyopneumoniae* (*M. hyo*) bacterin (20 mg/ml) were purchased from Newport Labs (Worthington, MN). Commercial SIV antigen from FluSure® (H1N1 and H3N2) without adjuvant, FluSure® vaccine, and RespiSure® (*M. hyo*) vaccines were purchased from Zoetis (Florham Park, NJ). Ticamulsion A-2010 was purchased from Tic Gums (White Marsh, MD). Penreco Drakeol 5 oil was purchased from Penreco (Karns City, PA).

2.2. Emulsion and vaccine formulation

To make the OW-14 emulsion adjuvant, Ticamulsion A-2010, a Gum arabic emulsifier was dissolved in deionized water with a 7.5% (W/V) final concentration. Mineral oil (Penreco Drakeol 5) was added to the water phase (15%, w/v) and mixed on a Silverson Lab mixer (L5M-A, Silverson, East Longmeadow, MA) for 15 min at 10,000 rpm. The emulsion was then passed five times through a Microfluidizer (M-110P, Microfluidics, Newton, MA) at ~10,000 psi. Emulsions were sterilized by autoclaving for 20 min at 117 °C and stored at 4 °C, room temperature, or 40 °C. Vaccines were prepared by simple hand mixing OW-14 with antigens to produce a final 5% w/v of mineral oil and 2.5% w/v of the Ticamulsion A-2010. The viscosity of OW-14 was measured using a Barnant falling ball viscometer (Fisher Sci., Waltham, MA).

2.3. Transmission election microscopy

OW-14 emulsion was visualized used a Transmission Election Microscope housed within the Department of Biology at Kansas State University. Undiluted OW-14 was placed on a 200 mesh formvar-carbon filter (Electron Microscopy Sciences, Hatfield, PA) for 5 min at room temperature. Samples were then counterstained with uranyl acetate for 5 min at room temperature. Grids were air-dried and imaged on FEI CM100 Transmission Electron Microscope (TEM) equipped with an AMT digital image capturing system using a magnification of 130,000 and an accelerating voltage of 100 kV.

2.4. Particle size and zeta potential characterization

Adjuvants were diluted in deionized water before being analyzed on a Malvern Zetasizer Nano ZS 90 instrument (Malvern Instruments, Westborough, MA). The particle size distribution was measured by dynamic light scattering (DLS), where the distribution of diffusion coefficients was determined through the measurement and correlation of the statistical fluctuations in the light scattered from a system of particle diffusion under the influence of Brownian motion. Adjuvants were then assayed for zeta potential using automatic software determination. Measurements were taken in triplicate to assess repeatability of the results and highlight any sample changes such as agglomeration or sedimentation during measurements.

2.5. Swine vaccination experiments

All animal experiments were approved by the Institutional Animal Care and Use Committee at Kansas State University. Conventional large White-Duroc crossbred weaned specific-pathogen free piglets (3 weeks of age) were used in all studies. The adjuvant/vaccine efficacy evaluation and adjuvant thermal stability/efficacy studies were conducted at the Swine Research Unit, Kansas State University. The antigen sparing experiment was conducted at Large Animal Research Center (LARC) facility, Kansas State University. All piglets were confirmed sera-negative for antibodies to swine influenza virus (by hemagglutination inhibition test) and *M. hyo* on day 0. For each experiment, pigs were immunized intramuscularly using a 20 gauge needle on day post-vaccination (DPV) 0 and 21 with experimental or commercial vaccines. During the adjuvant/vaccine efficacy evaluation experiment, blood samples were collected on day 0, 3 weeks, 5 weeks, 2 months, 4 months, and 5 months post initial vaccination. During adjuvant thermal stability/efficacy studies, blood samples were collected on DPV 0, 21, and 35. During the antigen sparing experiment blood samples were collected at DPV 0 and 42. Serum was separated from clotted blood and preserved at −20 °C. Vaccine injection sites were examined by trained veterinary pathologists on the day of slaughter (5 months post initial vaccination) for any pathological changes to the muscle or surrounding tissues. Pigs vaccinated with experimental adjuvant/vaccines were slaughtered/euthanized and disposed at the end of the experiments.

2.6. Antibody response

Serum was sent to Iowa State University Veterinary Diagnostic Laboratory (Ames, IA) for the evaluation of *M. hyo* antibody production using IDEXX *M. hyo.* Ab Test (IDEXX Laboratories, Westbrook, ME) according to their standard protocols.

2.7. Hemagglutination inhibition titer

The hemagglutination inhibition (HAI) test is the most accurate serologic method of determining a swine herd's immune status [17]. Serum was heat inactivated for 30 min at 56 °C then diluted 10 fold and incubated with chicken red blood cells (RBC's) overnight at 4 °C. Samples were serially diluted (1:2), mixed with 4 HA units (HAU) of whole killed H3N2 swine influenza virus, and incubated for 30 min at room temperature. Chicken RBC's (0.5%) were added to the samples and incubated 3 h at room temperature. The HAI titer was defined as the highest serum dilution that completely inhibited hemagglutination.

Please cite this article in press as: Galliher-Beckley A, et al. Characterization of a novel oil-in-water emulsion adjuvant for swine influenza virus and *Mycoplasma hyopneumoniae* vaccines. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.04.065