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Intranasally administered Endocine™ formulated 2009 pandemic influenza H1N1 vaccine induces broad specific antibody responses and confers protection in ferrets

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ABSTRACT

Influenza is a contagious respiratory disease caused by an influenza virus. Due to continuous antigenic drift of seasonal influenza viruses, influenza vaccines need to be adjusted before every influenza season. This allows annual vaccination with multivalent seasonal influenza vaccines, recommended especially for high-risk groups. There is a need for a seasonal influenza vaccine that induces broader and longer lasting protection upon easy administration. Endocine™ is a lipid-based mucosal adjuvant composed of endogenous lipids found ubiquitously in the human body. Intranasal administration of influenza antigens mixed with this adjuvant has been shown to induce local and systemic immunity as well as protective efficacy against homologous influenza virus challenge in mice. Here we used ferrets, an established animal model for human influenza virus infections, to further investigate the potential of Endocine™ as an adjuvant. Intranasal administration of inactivated pandemic H1N1/California/2009 split antigen or whole virus antigen mixed with Endocine™ induced high levels of serum hemagglutination inhibition (HI) and virus neutralization (VN) antibody titers that were also cross reactive against distant swine viruses of the same subtype. HI and VN antibody titers were already demonstrated after a single nasal immunization. Upon intratracheal challenge with a homologous challenge virus (influenza virus H1N1/The Netherlands/602/2009) immunized ferrets were fully protected from virus replication in the lungs and largely protected against body weight loss, virus replication in the upper respiratory tract and pathological changes in the respiratory tract. Endocine™ formulated vaccines containing split antigen induced higher HI and VN antibody responses and better protection from body weight loss and virus shedding in the upper respiratory tract than the Endocine™ formulated vaccine containing whole virus antigen.

1. Introduction

Influenza A viruses cause annual seasonal epidemics, sporadic avian influenza virus infections and influenza pandemics such as the H1N1 pandemic virus of 2009–2010 [1]. Seasonal influenza A virus infections cause substantial mortality and morbidity, particularly in high risk groups, such as children younger than age 5, elderly, people with certain chronic medical conditions and immune-compromised individuals [2]. Active immunization is the most cost effective way of limiting influenza related morbidity and mortality. Current split-virion or subunit seasonal influenza vaccines, of which hemagglutinin (HA) is considered the major immunogenic component, are effective against circulating homologous virus strains [3]. Antigenic drift caused by mutations in the HA, necessitates regular updates of the vaccine composition. Furthermore, more pathogenic viruses such as the newly emerged pandemic H1N1 virus of 2009 (pH1N1/09) for which among others,

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relatively young people were at an increased risk, highlight the need for improved influenza vaccines that induce better, more cross-protective, and longer lasting immunity than the current seasonal vaccines do.

Vaccines administered parenterally induce effective systemic immune responses, but only limited local immunity in the respiratory tract. Locally produced specific antibodies, in particular secretory IgA (S-IgA) can provide immunity via their unique capability to neutralize a pathogen before it even passes the mucosal barrier [4,5]. Moreover S-IgA antibodies have been demonstrated to contribute to the establishment of increased cross-protection from influenza [6]. Nasal administration of vaccine has the potential of establishing mucosal immune responses at the first site of natural infection [7]. In addition, nasal administration using a needle free delivery system is non-invasive, simply accessible and painless. The currently licensed nasally administered influenza vaccines are live attenuated influenza vaccines (LAIV). The LAIV vaccine manufactured by Medimmune, sold under the trade name FluMist in the US and Fluenz in Europe, has proven to be effective against seasonal infection and to provide better cross-protection against drifted influenza virus strains than the non-live seasonal vaccines [8–10]. However, the use of LAIV is currently restricted to the age group of 2 to 59 years, thus excluding children below age 2 as well as the elderly, both populations classified as major high risk groups by the WHO [2]. Therefore, nasal administration of an inactivated influenza vaccine that would be safe and protective through systemic and mucosal immunity, would be an attractive alternative to currently used influenza vaccines.

Appropriate adjuvants or carrier systems have shown to be indispensable to ensure effective stimulation of the mucosal immune system when non-replicating split or subunit antigens were used [11]. A mucosal adjuvant would ideally increase the uptake of the antigen through the mucus and mucous membrane and reduce the required antigen dose while eliciting mucosal as well as systemic immunity. Moreover, the adjuvant should ideally not cause adverse side effects. Concerns about the safety of mucosal adjuvants are real, since the reporting of an increased incidence of Bell's palsy syndrome seen after using an intranasally administered inactivated influenza vaccine, adjuvanted with an apparently insufficiently detoxified mutant of the *E. coli* heat labile enterotoxin [12,13]. Nevertheless, research on the design and development of effective and safe intranasal adjuvants is ongoing and several mucosal adjuvants which support influenza immunity are currently under investigation [14–18].

Endocine™, an adjuvant system based on endogenous lipids, was tested in three clinical phase I studies. A nasal diphtheria vaccine formulated with Endocine™ (1 or 4%) was evaluated in a phase I study in 2002, and was found to be safe and tolerable. Subjects receiving the diphtheria vaccine with 4% Endocine™ had a higher increase in neutralization titers compared to subjects receiving unadjuvanted vaccine (unpublished data). An inactivated whole virus influenza vaccine and an HIV vaccine, and was shown to be safe and tolerable in all studies [19,20]. Pre-clinical studies with split virion influenza vaccines showed that Endocine™, (previously known as L3B), significantly increases both local and systemic immune responses after intranasal immunization [21]. Addition of the adjuvant to a subunit influenza antigen given intranasally to mice conferred protection (measured by detection of viral RNA) against homologous virus challenge [22].

To further investigate the potential of Endocine™ to adjuvant inactivated nasal influenza vaccines we used the ferret as a model for influenza. Ferrets are considered to be the most suitable animal model for the different forms of human influenza and are naturally susceptible to infection with all wildtype human influenza A viruses causing clinical changes in ferrets similar to those observed in humans. Also the pathogenesis and antibody responses observed in ferrets are quite similar to those in humans [23,24]. Furthermore ferrets share similarities in lung physiology and airway morphology with humans [25,26] and the pattern of influenza virus attachment and replication in the ferret respiratory tract is largely similar to that in humans [27].

In the current study the efficacy of nasal Endocine™ adjuvanted split virion and whole virus pH1N1/09 candidate vaccines was evaluated using the homologous wildtype H1N1 A/The Netherlands/602/2009 (wt-pH1N1) virus as a challenge. Humoral, hemagglutination inhibiting (HI) and virus neutralizing (VN) antibody responses against homologous and three distant swine H1N1 viruses were evaluated. Efficacy was measured by evaluating clinical, virological and pathology parameters. In addition computed tomography (CT) imaging was performed as a newly developed read out parameter of efficacy by quantifying alterations in aerated lung volumes (ALV) [28,29].

2. Materials and methods

2.1. Vaccines

Vaccine nasal drops: Endocine™ 20 mg/ml formulated inactivated H1N1/California/2009 split virion antigen at 5, 15 and 30 μg HA/0.2 ml and whole virus antigen at 15 μg HA/0.2 ml were provided by Eurocine Vaccines AB (Stockholm, Sweden). Parenteral vaccine: Fluarix®, season 2010/2011, also containing inactivated H1N1/California/2009 (GlaxoSmithKline).

2.2. Ferrets

Healthy female ferrets (*Mustela putorius furo*: outbred), approximately 12 months of age, with body weights of 760-1210 g and seronegative for antibodies against circulating influenza viruses B, A/H1N1, A/H3N2 and A/pH1N1 as demonstrated by hemagglutination inhibition (HI) assays were used. Animals were housed in standard cages, in groups of maximal 8 animals during the pre-immunization phase and in study groups of 6 animals during the immunization phase. The study groups were transferred to negatively pressurized glovebox isolator cages on the day of challenge. During the whole study animals were provided with commercial food pellets and water *ad libitum*. The experimental protocol was approved before start of the experiments by an independent institutional animal ethics committee according to the Dutch law.

2.3. Immunization

Five groups of six ferrets received three intranasal immunizations (droplets: 100 μl in each nostril, using a pipet with filtertip) under anesthesia with ketamine and domitor at days 0, 21 and 42. Groups 3, 4 and 5 were intranasally immunized with 200 μl Endocine™ formulated H1N1/California/2009 split antigen containing 5, 15 and 30 μg HA, respectively. Group 6 was intranasally immunized with 200 μl Endocine™ formulated H1N1/California/2009 whole virus antigen containing 15 μg HA. Control group 1 received 200 μl of saline intranasally. One group of six ferrets (group 2) received two subcutaneous immunizations (days 21 and 42 using 25Gx5/8" needles) with 0.5 ml Fluarix®, season 2010/2011, a non-adjuvanted trivalent influenza vaccine (TIV) that also contains the pH1N1 (15 μg HA) component. Blood samples for serum preparation were collected prior immunization on days 0, 21 and 42 and before challenge on study days 64 and 70.