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Alum boosts TH2-type antibody responses to whole-inactivated virus influenza vaccine in mice but does not confer superior protection

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Summary Clinical trials with pandemic influenza vaccine candidates have focused on aluminium hydroxide as an adjuvant to boost humoral immune responses. In this study we investigated the effect of aluminium hydroxide on the magnitude and type of immune response induced by whole-inactivated virus (WIV) vaccine. Balb/c mice were immunized once with a range of antigen doses (0.04–5 μg) of WIV produced from A/PR/8 virus, either alone or in combination with aluminium hydroxide. The hemagglutination inhibition (HI) titers of mice receiving WIV + aluminium hydroxide were 4–16-fold higher than HI titers in mice receiving the same dose of WIV alone, indicating the boosting effect of aluminium hydroxide. WIV induced a TH1 skewed humoral and cellular immune response, characterized by strong influenza-specific IgG2a responses and a high number of IFNγ-secreting T cells. In contrast, immunization with WIV adsorbed to aluminium hydroxide resulted in skewing of this response to a TH2 phenotype (high IgG1 levels and a low number of IFNγ-producing T cells).

To assess the effect of the observed immune response skewing on viral clearance from the lungs mice immunized once with 1 μg WIV without or with aluminium hydroxide were challenged with A/PR/8 virus 4 weeks later. The immunized mice showed a significant decrease in viral lung titers compared to control mice receiving buffer. However, despite higher antibody titers, mice immunized with WIV adsorbed to aluminium hydroxide suffered from more severe weight loss and had significantly higher virus loads in their lung tissue than mice receiving WIV alone. Major difference between these groups of mice was the type of immune response induced, TH2 instead of TH1, indicating that a TH1 response plays a major role in viral clearance.

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Introduction

Influenza virus continues to be a major health burden. According to the World Health Organization (WHO) the estimated number of worldwide excess deaths due to influenza is 0.25—0.5 million each year [1]. In addition to the seasonal epidemic influenza burden there is the risk of a pandemic caused by an influenza virus to which the majority of the world population is immunologically naïve. Morbidity and mortality of a pandemic influenza strain will likely be much higher than that of epidemic influenza [2]. Currently, H5N1, H7N2, H7N3, H7N7 and H9N2 have crossed the species barrier from birds to man to cause human infections on multiple occasions ([3,4], reviewed in Ref. [5]). H5N1 is considered a likely candidate for the next pandemic, having been confirmed to infect 357 people resulting in 225 deaths (WHO, Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) 1 February 2008).

The best protection against influenza virus infection remains effective vaccination [6]. Inactivated vaccines against influenza virus include whole-inactivated virus (WIV) vaccines, split virus vaccines, and subunit vaccines [7]. WIV vaccine is prepared by inactivation of influenza virus with β-propiolactone or formaldehyde, resulting in presence of all viral proteins in their native organization without viral replication. Split influenza virus vaccine consists of chemically disrupted inactivated influenza virus. Subunit vaccines are prepared by purification of the HA and NA from inactivated and detergent-solubilized influenza virus. All of these vaccine formulations have been reported to be in clinical trials as pandemic vaccine candidates [8].

A major objective in the context of the development of pandemic vaccines is to ensure sufficient supply of vaccines despite limited production capacities for vaccine virus. Dose-sparing strategies are being developed to solve this problem. One of these dose-sparing strategies is the use of WIV instead of split virus or subunit since WIV induces higher antibody responses, especially at low antigen doses and in an immunologically naïve population [9,10]. Another dose-sparing strategy to increase the efficacy of pandemic influenza vaccines is addition of adjuvants to the vaccines to boost immune responses.

Adjuvants used most frequently for human vaccination are aluminium compounds, including aluminium hydroxide and aluminium phosphate ([11,12], reviewed in Ref. [13]). Prior to injection the antigen is adsorbed onto a preformed aluminium gel (Alhydrogel® or Adju-phos®). Aluminium adjuvants generally induce a TH2-type of immune response and result in a stronger and more rapid induction of antibody titers. This makes them potentially suitable for the pandemic situation where likely only one vaccination can be achieved before the first wave of the pandemic [14]. Accordingly, in the majority of clinical trials performed to date with pandemic influenza vaccine candidates' aluminium hydroxide or aluminium phosphates were used as the adjuvant ([15—19], for an overview of all clinical pandemic influenza vaccine trials see [8]). Outcomes of these clinical trials are reported predominantly as hemagglutination inhibition titers and seroconversion rates. So far, six clinical studies have been reported which compare the response to non-adjuvanted and alum-adjuvanted pandemic influenza vaccines, respectively [8]. Three of these studies did not find a benefit or even a negative effect of alum adjuvants (8: Baxter study A/Vietnam/1194/2004 WIV, Sanofi Pasteur study A/Vietnam/1203/2004 split vaccine in adults (Ref. [17]), Novartis study A/Vietnam/1203/2004 subunit vaccine). Two studies found inconsistent results ([8]: GSK study H9N2 WIV, Sanofi Pasteur study A/Vietnam/1203/2004 split vaccine in the elderly). One study reported improvement of seroprotection, seroconversion and rise of GMT by alum (8: GSK study A/Vietnam/1194/2004 WIV).

The mode of action of aluminium compounds is not completely clear yet, they may form a depot for the antigen, either at the site of injection or in the draining lymph nodes. The depot enables slow release from the site of injection and/or longer contact with cells of the immune system [13,20]. Another mode of action could be the conversion of soluble antigen to particulate antigen facilitating phagocytosis by antigen-presenting cells [21]. Furthermore, aluminium compounds induce priming of B cells and accumulation of IL-4 producing Gr1 + myeloid cells facilitating B cell responses [22].

In this study we aim to determine if aluminium hydroxide is indeed a suitable adjuvant for influenza vaccines, using two vaccine formulations, WIV vaccine and subunit vaccine, in a mouse model system. H5N1 vaccines are known to be poorly immunogenic, inducing low antibody titers in both experimental animals and man [23—25]. To mimic this, H1N1 A/Puerto Rico/8/34 (PR8) virus was used as a model influenza virus. PR8 has a low immunogenicity compared to the commonly used H3N2 strains (L. Bungener, unpublished observation). Moreover, PR8 causes symptomatic infection in mice and can therefore be used in a challenge model to test the protective efficacy of the vaccines. We determined the effect of addition of aluminium hydroxide to PR8 influenza vaccines on antibody titers, phenotype of immune response and protective capacity of the vaccines upon challenge. Aluminium hydroxide boosted the antibody response but altered the phenotype of the immune response. Moreover, despite 4—16 times higher hemagglutination inhibition titers in mice receiving aluminium hydroxide-adjuvanted WIV clearance of influenza virus from the lungs was decreased.

Materials and methods

Virus, subunit and virosomes

PR8 influenza virus (H1N1 subtype) grown on Madin—Darby canine kidney cells (MDCK) was kindly provided by Solvay Pharmaceuticals (Weesp, The Netherlands). The virus was purified, inactivated by treatment with formaldehyde and used as whole-inactivated virus (WIV). For inactivation, virus was incubated twice with a freshly prepared 4% formaldehyde/10% sucrose solution in buffer containing 5 mM Hepes, 150 mM NaCl and 0.1 mM EDTA (HNE buffer) at a final concentration of 0.02% formaldehyde. Inactivation was carried out for 24 h at 4 °C under continuous stirring, maintaining a neutral pH during the entire incubation. After inactivation, the virus was dialyzed against HNE buffer. Virus inactivation was confirmed by standard titration of the virus preparation on MDCK cells.

Subunit material was prepared by solubilizing inactivated virus for 3 h under continuous rotation in 0.3 mg/ml