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NaCl strongly modifies the physicochemical properties of aluminum hydroxide vaccine adjuvants

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ABSTRACT

The immunostimulation capacity of most vaccines is enhanced through antigen adsorption on aluminum hydroxide (AH) adjuvants. Varying the adsorption conditions, i.e. pH and ionic strength (I), changes the antigen adsorbed amount and therefore the ability of the vaccine to stimulate the immune system. Vaccine formulations are thus resulting from an empirical screening of the adsorption conditions. This work aims at studying the physicochemical effects of adjusting the ionic strength of commercial AH adjuvant particles suspensions with sodium chloride (NaCl). X-ray photoelectron spectroscopy data show that AH particles surface chemical composition is neither altered by I adjustment with NaCl nor by deposition on gold surfaces. The latter result provides the opportunity to use AH-coated gold surfaces as a platform for advanced surface analysis of adjuvant particles, *e.g.* by atomic force microscopy (AFM). The morphology of adjuvant particles recovered from native and NaCl-treated AH suspensions, as studied by scanning electron microscopy and AFM, reveals that AH particles aggregation state is significantly altered by NaCl addition. This is further confirmed by nitrogen adsorption experiments: I adjustment to 150 mM with NaCl strongly promotes AH particles aggregation leading to a strong decrease of the developed specific surface area. This work thus evidences the effect of NaCl on AH adjuvant structure, which may lead to alteration of formulated vaccines and to misinterpretation of data related to antigen adsorption on adjuvant particles.

1. Introduction

Vaccination plays an important role in preventing many infectious diseases. Vaccines prepared from attenuated or killed pathogens are directly effective and elicit a sufficient immune response. Nowadays, vaccines produced from antigenic subunits of pathogens, such as proteins or polysaccharides, or from pathogens toxoids, such as tetanus or diphtheria toxoids (Singh et al., 2006), are better tolerated by the body but also less immunogenic. They therefore fail to trigger both sufficient immune response and memory effect (Eppstein et al., 1989; Mbow et al., 2010), especially in children (Sivakumar et al., 2011). In 1926, Glenny et al. observed that diphtheria toxoid precipitated in presence of potassium alum ($KAl(SO_4)_2$) gave a higher immune response than the soluble toxoid after injection to guinea pigs (HogenEsch, 2002). The first adjuvant effect was just discovered.

Since then, many types of vaccine adjuvants have been studied, from bacterial extracts to emulsions passing by specialty polymers (Eppstein et al., 1989; Mbow et al., 2010; Sivakumar et al., 2011). These adjuvants have been used in vaccines with the objective to elicit a sufficient immune response and to limit secondary effects (Aguilar and Rodríguez, 2007; O'Hagan et al., 2001). Nowadays, the more widespread adjuvants used in human vaccines are aluminum-based suspensions. Commercially-used aluminum-containing adjuvants in vaccine formulation are prepared by precipitation of aluminum salts in controlled alkaline conditions (Lindblad, 2004).

Precipitation performed in presence of phosphate ions results in an amorphous gel of a non-stoichiometric compound having an $Al(PO_4)_x(OH)_y$ formula and commonly known as aluminum phosphate (AP) adjuvant. AP adjuvant is composed of platelet particles with a diameter of 50 nm forming irregular aggregates of 1–20 μm. The production process as well as different physicochemical characteristics of AP adjuvants have been well described in the literature (Burrell et al., 2000a, 2000b). Commercial AP adjuvants are composed of particles suspended in a NaCl solution.

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When the precipitation process is performed without added ions, an amorphous gel of aluminum hydroxide ($Al(OH)_3$) is obtained. Commercial aluminum hydroxide (AH) adjuvant is actually poorly crystalline boehmite. It is composed of a mixture of amorphous $Al(OH)_3$ and hydrated crystalline aluminum oxyhydroxide $AlOOH{\cdot}nH_2O$ (Hem and White, 1995; Shirodkar et al., 1990). This organized fraction is obtained by a hydrothermal treatment causing the dehydration of the primary $Al(OH)_3$ gel according to the following reaction:

$$Al(OH)_{3\ amorphous} \rightarrow AlOOH_{crystalline} + H_2O$$

Mineralogists have shown that AlOOH formation by dehydration of $Al(OH)_3$ is facilitated by high temperature and high sodium chloride concentration (3 M) (Tettenhorst and Hofmann, 1980; Yau et al., 2006). Adjuvant hydroxyl groups are coordinated with aluminum atoms. The surface charge of AH particles is pH-dependent, with a point of zero charge (PZC) of 11.4 (Rinella et al., 1998). Shirodkar et al. observed by transmission electron microscopy that AH adjuvants are composed of primary needle-like particles presenting the characteristic fibrous morphology of boehmite (Hem and White, 1995; Shirodkar et al., 1990), with average dimensions of $4.5 \times 2.2 \times 10\,nm^3$, and forming aggregates of 1–20 μm when dispersed in solution (Hem and HogenEsch, 2007). These aggregates are in dynamic equilibrium in suspension (aggregating and disaggregating continuously) and are known to be the functional units of adjuvants, i.e. the effective structure for antigen adsorption and immune system stimulation (Morefield et al., 2004). AH adjuvant specific surface area was determined by gravimetric/FTIR-based methods and X-ray diffraction (XRD) to be around $500\,m^2/g$ (Johnston et al., 2002). Stability studies of AH adjuvants over time or exposure to autoclaving showed that XRD spectra become sharper after treatment. It means that more order was developed in AH particles over time or after heat treatment as it enhances dehydration of amorphous $Al(OH)_3$ and formation of AlOOH. By acquiring more order, AH adjuvant showed a decrease in both specific surface area and adsorption capacity of antigens (Burrell et al., 1999, 2000c).

The World Health Organization (WHO) recommends for tetanus and diphtheria toxoids that 80% of antigen is adsorbed on adjuvant particles (Clausi et al., 2008). The antigenic subunits have indeed to be adsorbed on particles of an adjuvant suspension to produce an effective vaccine. It was shown that antigens adsorbed on aluminum-based adjuvants lead to a higher immune response compared to soluble antigens. Although mechanisms are still not completely understood, the higher immunostimulation of adsorbed antigens was explained by a prolonged retention time of aluminum-based adjuvants and adsorbed antigens at the injection site. This retention time, combined with a higher recruitment of immune system cells due to inflammatory response induced by adjuvants, is believed to have a positive effect on vaccine efficiency (Lindblad, 2004). Furthermore, Jones et al. showed that conformational changes of proteins upon adsorption on aluminum salts adjuvants lead to a higher immunogenicity of the vaccine. Antigen adsorption is thus considered as an important step of vaccine formulation (Jones et al., 2005).

To improve vaccine formulation, adsorption isotherms of model proteins were constructed experimentally to determine adjuvant adsorption capacity as well as involved mechanisms. Hem et al. showed that bovine serum albumin (BSA) and lysozyme respectively adsorbed on AH and AP suspensions at physiological pH (7.4). By rising the ionic strength (I) with NaCl, it was shown by Hem and coworkers that the adsorption capacity of BSA and lysozyme decreased on AH and AP, respectively. This was considered as a proof that electrostatic interactions were one of the major adsorption mechanisms of protein antigens on aluminum-based adjuvants (Al-Shakhshir et al., 1995; Hem and White, 1995). pH and I are thus tuned in the course of vaccine formulation to improve antigen adsorption.

The second principal driving force for antigen adsorption on adjuvant particles is believed to be a ligand exchange between antigen phosphate groups and adjuvant hydroxyl groups (Iyer et al., 2003; Morefield et al., 2005). This mechanism was deduced from the tunable adsorption of ovalbumin (OVA) on aluminum-based adjuvants. Highly phosphorylated OVA adsorbed more strongly and in higher amounts than dephosphorylated OVA on both AH and AP. As both adjuvants expose hydroxyl groups at the surface of particles, ligand exchange between hydroxyl and phosphate groups was proposed as adsorption mechanism. It was shown that the interaction force between adjuvant and antigen adsorbed by ligand exchange is stronger than by electrostatic interactions.

Adsorption of model antigens was often studied at pH 7.4 to be close to the physiological conditions of vaccines for parenteral administration. Regarding the latter administration way, the vaccine also needs to be as close as possible to the isotonicity, which is around 0.15 M in NaCl.

NaCl is thus frequently used in vaccine formulation (i) to optimize antigen adsorption and (ii) to adjust the vaccine I before injection. It was however shown that high NaCl concentration (3 M) during AH adjuvant production facilitates crystalline aluminum oxyhydroxide formation (Yau et al., 2006). As the formation of crystalline areas of AH adjuvant modifies its structure, vaccine efficiency could be modified after I adjustment. The potential effects on AH adjuvant structure of moderate NaCl concentration used to improve antigen adsorption or to adjust vaccine ionic strength have not been addressed yet.

This work aims at using advanced surface characterization techniques, i.e. atomic force microscopy and X-ray photoelectron spectroscopy, to characterize the effect of adding NaCl to commercial aluminum hydroxide adjuvant suspensions thereby mimicking the influence of body isotonicity. Adjuvant suspensions were used as such or after exposure to 150 mM NaCl and analyzed as freeze-dried powders or after deposition on a gold substrate depending on the further applied characterization method. Nitrogen adsorption, followed by computation of surface specific area, and scanning electron microscopy were also used to characterize the samples. Physicochemical properties such as surface chemical composition, particle morphology or developed specific surface area could indeed be modified upon exposure to NaCl during vaccine production steps. AH structure modification may finally alter vaccine efficiency.

2. Materials and methods

2.1. Materials

Two commercial aluminum hydroxide adjuvants were chosen for this work. ALHYDROGEL® “85” 2%–Ph. Eur. (AH1) was kindly provided by BRENNTAG (Brenntag Biosector, Denmark). REHYDRAGEL® LV (AH2) was purchased from General Chemical (NJ, USA). Both adjuvants consist in a suspension of particles in water. When needed, ionic strength of adjuvant suspensions was adjusted to 150 mM with sodium chloride (NaCl, Merck, Germany).

2.2. Methods

2.2.1. Sample preparation

Both AH powders and surfaces elaborated by immobilizing particles from AH suspensions on a solid substrate were analyzed. AH powders were obtained from adjuvant suspensions by freeze