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Aluminum hydroxide colloid vaccine encapsulated in yeast shells with enhanced humoral and cellular immune responses

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ABSTRACT

Aluminum salt (Alum) is one of the most important immune adjuvants approved for use in humans, however it is not suitable for vaccination against various chronic infectious diseases and cancers for not being able to induce cell-mediated (Th1) immunity. Here, we encapsulated an Alum colloid inside β-glucan particles (GPs), which are a type of natural particles derived from the yeast glucan shells, to prepare hybrid GP-Alum (GP-Al) adjuvant particles with a very uniform size of 2–4 μm. These hybrid particles can be used to load antigen proteins through a simple mixing procedure, and can be highly specifically targeted to antigen-presenting cells (APCs) and strongly activate dendritic cells (DCs) maturation and cytokine secretion. In an animal model, they elicit a strong Th1-biased immune response and extremely high antibody titer, and cause marked prophylactic and therapeutic effects against tumors. As Alum has been proven to be a safe adjuvant to induce strong humoral responses and β-glucans are safe for human use, this very uniform hybrid Alum particulate system could have important application as a vaccine carrier to stimulate humoral and cellular immune responses at the same time.

1. Introduction

Aluminum salt (known as Alum), with more than 80 years of use in humans recorded, is one of the most important immune adjuvants approved by the FDA for use in humans because of its safety and efficacy [1,2]. It has been used in more than 80% of the vaccines that have ever entered markets, such as the inactivated poliovirus, hepatitis A, streptococcus pneumonia, diphtheria pertussis tetanus, diphtheria-tetanus, hepatitis B virus (HBV), and human papilloma virus (HPV) vaccines [3,4]. The aluminum-salt-containing vaccines are formulated by adsorption of antigens onto highly charged aluminum hydroxide or aluminum phosphate gels, which are then phagocytosed by antigen-presenting cells (APCs), such as dendritic cells (DCs), macrophages and B cells [5]. To date, it is the most successful and important adjuvant for human use.

However, Alum typically induces a classical antibody-mediated (Th2) response rather than cell-mediated (Th1) immunity [6] and is therefore not suitable for vaccination against various chronic infectious diseases and cancers [7,8]. Furthermore, until now, there have also been no many other useful Th1-stimulating adjuvants for human use, and the lack of platforms that can elicit strong Th1-biased cell-mediated responses could be one the most key challenges restricting the development of related vaccines. The activation of Th1-biased cell-mediated responses, particularly involving cytotoxic T lymphocytes (CTLs), is necessary for therapeutic vaccines against chronic infections as well as cancer, where CTLs are the prime mediators eliminating infected or malignant cells [9,10]. Recently, many studies have demonstrated the preclinical or clinical usefulness of therapeutic vaccines against

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cancers, especially those based on neoantigens [11,12]. New vaccine delivery systems with strong humoral and Th1-biased cellular immune responses-stimulating potentials are very important for the improvement of their clinical potential. To expand the utilization of Alum adjuvant, attention should be paid to improving its adjuvant potency and Th1 stimulation capability.

The combination of Alum with Th1-biased immunostimulants could be an important strategy to efficiently induce Th1-biased cellular and humoral innate and adaptive immunity [13,14]. Among them, TLR agonists, such as unmethylated CpG oligodeoxynucleotides (CpG ODNs) [15], Poly (I:C) [16] and Lipid A (MPL) [17], are known, and some formulations containing them are under investigation for vaccines against certain types of cancers, as well as for infectious diseases [18–20]. For example, the Alum adjuvant combined with MPL, known as AS04, can induce a stronger antibody and cell-mediated response to HBsAg [21] and recombinant human papilloma virus antigens [22,23] than Alum alone. However, there are some drawbacks to the use of these immunostimulants when combined with Alum. The addition of these immunostimulants to Alum does not generally elicit a Th1-biased response. For example, many studies have shown that AS04 has a strong effect on $CD4^+$ T cell amplification, while there are no many reports on its effectiveness in the differentiation of $CD8^+$ T cells; the AS04-adjuvanted vaccine against herpes simplex virus was found to be ineffective [24]. Furthermore, after systemic administration, CpG ODN and Poly (I:C) are quickly eliminated by nuclease-mediated degradation under biological conditions and they are also poorly taken up by APCs when they are simply mixed together with Alum. Therefore, new systems with sufficiently high stability under biological conditions and high APC-specific delivery capability are required for inducing efficient Th1 stimulation using the Alum adjuvant.

Nano/micro-technology is another widely adopted strategy for the optimization of Alum adjuvant [25–28]. The activity of aluminum salt-based adjuvants can be significantly improved by rationally synthesizing nano/microparticulates, which can protect antigen from enzymatic degradation, promote a depot effect with gradual release of the antigen and improve the uptake and antigen cross-presentation by APCs [29,30]. For example, Wang and colleagues fabricated phospholipid bilayer-coated aluminum nanoparticles (PLANs) and found that they induced more robust antigen-specific humoral and cellular immune responses but less local inflammation than the widely used naked Alum-antigen [31]. Moreover, particle carriers can deliver both antigen and stimulants into the same lymph node-resident DCs [32,33], which were found to be very important for inducing a strong immune response and avoiding the risk of inducing specific tolerance if appropriate DC activation is absent after the systemic delivery of antigens [34,35]. However, Alum particles synthesized according to the usual methods do not always have an ideal size for APC uptake, and batch-to-batch variation of the particles' physical and chemical properties occurs. Moreover, preparing very uniform Alum particles with antigen and proper Th1-stimulant loading together may further increase the challenge. Generally, only particles with a sufficiently large size (several hundred nanometers to micrometers) can be specifically taken up by APCs, and small particles of tens to hundreds of nanometers are easily taken up by many other non-phagocytic cells [36,37], which could lead to immunogenic anergy [38]. Once the particle size is not sufficiently uniform, it is difficult to exclude the non-specific uptake of the particle vaccine by non-APCs. Manufacturing Alum particles with an ideal and uniform size would be very challenging, and such a new particle formulation would be helpful to improve the potency of Alum if it also provides high APC specificity and a strong Th1-biased stimulation at the same time.

β-Glucan particles (GPs) are a type of natural particles derived from the yeast glucan shells, with a very uniform particle size of 2–4 μm [39]. They are FDA approved as generally recognized as safe (GRAS) [40]. Due to their particle size and because they present a pathogen-associated molecular pattern (PAMP) recognized by dectin-1 and complement receptor 3 (CR3), GPs can be very specifically and efficiently taken up by phagocytic cells, including DCs and macrophages [39,41–43]. Moreover, GPs are a very strong Th1-biased immunostimulant, as the β-glucans on its surface can act as a PAMP and deliver “danger” signals to DCs, resulting in strong DC activation and high expression of the co-stimulatory molecules CD40, CD80 and CD86 [44,45]. Here, we want to encapsulate the aluminum hydroxide colloid inside GPs to prepare a hybrid Alum adjuvant with a very uniform particle size of 2–4 μm. In these hybrid adjuvant particles, the Alum was encapsulated first and then could absorb and fix the antigens inside the GPs easily, while the GPs could deliver the whole particles to APCs specifically and induce strong Th1-biased cell stimulation at the same time. As Alum has been proven to be a safe adjuvant inducing strong humoral responses, and β-glucans are safe for human utilization with high APC targeting and induce strong Th1-biased cellular response, the combination of Alum adjuvant with β-glucan particles may be an ideal vaccine delivery system.

The main aim of this study was to test the properties of this novel hybrid particulate system and whether they could result in strong humoral and cellular immune responses at the same time. The Alum was first encapsulated inside GPs to form uniform GP-Alum (GP-Al) particles, which are sufficiently stable for autoclave sterilization. Only a simple mixing of the antigen with the premade GP-Al particles is required before the immunization. This simple procedure avoids manipulation of the antigen with any complex chemical or physical operations, thus avoiding the introduction of new contamination and reducing the risk of denaturation and degradation of the antigens. We report here the preparation and characterization of this system, and the evaluation of the immune functions in initiating both humoral and cellular immune responses. This new Alum-based targeted delivery system may thus greatly expand the utilization potential of the Alum adjuvant.

2. Materials and methods

2.1. Materials

Ovalbumin EndoFit™ was obtained from InvivoGen (San Diego, CA, USA), and Alum adjuvant was obtained from Sigma (St. Louis, MO, USA). The micro bicinchoninic acid protein assay (Micro BCA) kit was obtained from Beyotime (Shanghai, China). DAPI (4′,6-diamidino-2-phenylindole) was purchased from Roche (Mannheim, Germany), and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Solarbio (Beijing, China). Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640, and fetal bovine serum (FBS) were ordered from HyClone (South Logan, UT, USA). Recombinant mouse GM-CSF and IL-4 (carrier free), APC anti-mouse CD11C antibody, PE anti-mouse CD40 antibody, APC anti-mouse CD80 antibody, APC anti-mouse CD86 antibody, PE anti-mouse MHC I antibody, PE anti-mouse MHC II antibody, TNF-α ELISA kit (Cat # 430904), IL-1β ELISA kit (Cat # 432604), IL-6 ELISA kit (Cat # 431304), IL-12 ELISA kit (Cat # 433604), IL-4 ELISA kit (Cat # 431104) and IFN-γ ELISA kit (Cat # 430804) were obtained from BioLegend (San Diego, CA, USA). PE anti-mouse CD8 antibody and APC-labeled anti-mouse IFN-γ antibody were purchased from Sungene Biotech Co., Ltd. (Tianjin, China). All other chemical