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Polarized immune responses modulated by layered double hydroxides nanoparticle conjugated with CpG

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ABSTRACT

Modulation of the immune response is an important step in the induction of protective humoral and cellular immunity against pathogens. In this study, we investigated the possibility of using a nanomaterial conjugated with the toll-like receptor (TLR) ligand CpG to modulate the immune response towards the preferred polarity. MgAl-layered double hydroxide (LDH) nanomaterial has a very similar chemical composition to Alum, an FDA approved adjuvant for human vaccination. We used a model antigen, ovalbumin (OVA) to demonstrate that MgAl-LDH had comparable adjuvant activity to Alum, but much weaker inflammation. Conjugation of TLR9 ligand CpG to LDH nanoparticles significantly enhanced the antibody response and promoted a switch from Th2 toward Th1 response, demonstrated by a change in the IgG2a:IgG1 ratio. Moreover, immunization of mice with CpG-OVA-conjugated LDH before challenge with OVA-expressing B16/F10 tumor cells retarded tumor growth. Together, these data indicate that LDH nanomaterial can be used as an immune adjuvant to promote Th1 or Th2 dominant immune responses suitable for vaccination purposes.

1. Introduction

Historical vaccines have comprised attenuated or killed pathogens which are potent immunogens, but due to concerns regarding the potential for pathogenicity, modern vaccine development is aimed at utilizing purified antigenic components [1,2]. Due to the low immunogenicity of many purified antigens [3], immune adjuvants are required to enhance and regulate the immune response. However, the inclusion of adjuvant compounds may also provide a mechanism by which to modulate the immune response or switch its polarity. Aluminium hydroxide phosphate (Alum) is approved by the FDA for use in humans because of its safety and efficacy [4]. However, Alum typically induces a classical antibody-mediated (Th2) response rather than cell-mediated (Th1) immunity, and therefore is not suitable for vaccination against diseases such as intracellular infections or cancers [5,6]. In most cases, multiple immunization boosts are required due to the short duration of immune responses induced by Alum. Furthermore, the adjuvant frequently produces a strong inflammatory reaction at the injection site [7].

Th1 and Th2 represent the two extremes ('poles') of the adaptive immune responses, and are characterized by different antibody isotypes and cytokine secretion profiles [8]. Th1 cells evoke cell-mediated immunity and phagocyte-dependent inflammation whereas Th2 cells induce strong antibody responses and eosinophil accumulation, but inhibit functions of phagocytic cells (phagocyte-independent inflammation) [9,10]. The Th1/Th2 paradigm can provide the basis for the development of vaccines or other strategies against infectious agents, allergic and autoimmune disorders [11]. Cell-mediated (Th1) responses, particularly involving the cytotoxic ($CD8^+$) T lymphocyte (CTL), are the prime mediators of anti-tumor immunity [12]. CTL can be activated by an antigen or peptide (CTL epitope) coupled to major histocompatibility complex (MHC) class I molecules on the surface of antigen presenting cells (APCs) such as dendritic cells (DCs). After priming, the CTL is able to recognize the specific epitope on the cancer cell surface, and ultimately kill the cancer cell [13]. Hence, the search for adjuvants that are able to direct the immune response towards the desired type (either Th2 or Th1), will aid development of new types of vaccines.

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MgAl-layered double hydroxide (LDH) nanomaterials have been extensively investigated as efficient delivery vehicles for genes and drugs [14,15], but may also have potential application as vaccine adjuvants. LDHs are hydrotalcite-like (anionic clay) materials with the general chemical formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}(A^{n-})_{x/n}\,mH_2O$, and are readily synthesized in the laboratory at low cost. The peculiar 'sandwich-like' structure of LDHs consists of positively charged hydroxide layers ($[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}$), exchangeable anions and water molecules ($(A^{n-})_{x/n}\,mH_2O$) located between the hydroxide layers. Their anion exchange property enables LDHs to carry anionic biomolecules and drugs such as oligonucleotides [16], DNAs [17], RNAs [15,18], anti-cancer drugs (e.g. methotrexate [19]) and anti-inflammatory drugs (e.g. diclofenac, gemfibrozil, ibuprofen, naproxen) [20]. Moreover, the inherent positive charge of the MgAl-LDH hydroxide layers facilitates the adsorption of proteins to their surface [21,22], and such a kind of adsorption (e.g. polyelectrolyte adsorption on positively charged surfaces) has been reviewed recently by Szilagyi et al. [23]. Recent studies have demonstrated the capacity of LDH nanoparticles to target specific cells [24] or subcellular compartments [25]. These properties, combined with their low toxicity and excellent biocompatibility [14,26], indicate that MgAl-LDH nanoparticles may provide a mechanism by which to deliver vaccine antigens and other ligands (e.g. for Toll-like receptors) to modulate the immune response towards either Th2 or Th1 type, depending on the required immune response.

Pathogen-associated molecular patterns (PAMP) are widely used as effective immune-modulators, interacting with APCs via the toll-like receptor (TLR) signalling pathway [27]. In particular, oligodeoxynucleotides containing immunostimulatory CpG motifs (CpG ODN) have been shown to interact with TLR-9 in the endosome [28] and promote Th1 responses against tumor antigens [29,30]. However, the efficacy of CpG by itself is very low due to ineffective delivery to the endosome and rapid degradation [31]. Thus, an ideal adjuvant should be able to carry and protect CpG against enzymatic degradation, while delivering both antigen and CpG to the same APC [32]. Adjuvants, such as Alum, biodegradable microparticles and nanoparticles, liposomes and inorganic materials (such as silicon oxide, iron oxide, calcium phosphate, gold, carbon materials) [33–35] can be used to (co)deliver antigen and CpG, and significantly increase immune responses over the longer term [36]. Since MgAl-LDH nanoparticles are capable of incorporating both antigen and co-stimulatory ligand (e.g. CpG), as schematically shown in Fig. 1, they are ideal vehicles to carry, protect and efficiently deliver antigen and CpG to APCs. Thus we hypothesized that MgAl-LDH is an efficient nanoadjuvant. Just very recently, Williams et al. examined immunity promoted by various LDH nanomaterials and found that the adjuvant activity is determined purely by LDH chemistry [37].

The objectives of this study were to: (1) examine the loading capacity of LDH nanoparticle for a model antigen, ovalbumin (OVA); (2) compare the adjuvant activity of LDH and Alum; (3) determine the immune-stimulatory potency of LDH co-delivering OVA and CpG after *in vivo* subcutaneous immunization, and test anti-tumor efficacy in an animal model.

2. Materials and methods

2.1. Chemicals and reagents

Sample preparation was performed under sterile conditions. Sodium hydroxide pellets, magnesium chloride hexahydrate ($MgCl_2\,6H_2O$, 99.0–101.0%), aluminum chloride hexahydrated ($AlCl_3\,6H_2O$, 95–101%), albumin from chicken egg white (Ovalbumin, OVA, Grade V), CpG ODN 1826 (Class B CpG oligonucleotide – Murine TLR9 ligand) were purchased from Ajax Finechem, Chem-Supply, Scharlau and Sigma–Aldrich Pty Ltd, Invivogen, respectively.

2.2. LDH preparation

Mg_2Al–Cl-LDH nanoparticles were prepared by rapid precipitation and subsequent hydrothermal treatment. In brief, 10 mL of mixed salt solution containing $MgCl_2$ (0.70 M) with $AlCl_3$ (0.30 M) was added into 40 mL of NaOH solution (0.45 M) within 5 s with vigorous stirring. After 10 min stirring, LDH slurry was collected and washed twice with deionized water by centrifugation (SIGMA ®4–16 K Centrifuge). The slurry was dispersed in 40 mL of deionized water and transferred into a stainless steel autoclave with a Teflon lining (Parr Acid Digestion Vessels) for hydrothermal treatment at 100 °C for 16 h, giving rise to a well dispersed suspension after cooling down to room temperature. The final LDH mass concentration in suspension was around 10.0 mg/ml, with the yield of ~60%.

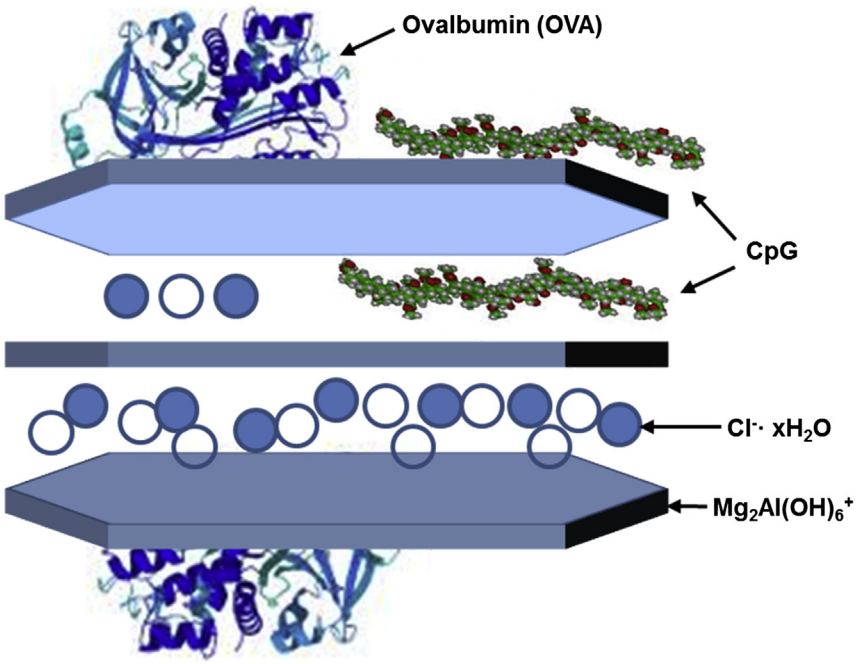

Fig. 1. Schematic representation of the Mg_2Al-LDH-based adjuvant-antigen hybrid system with OVA surface-adsorbed and CpG surface-adsorbed or intercalated.

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