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SCIENCE DIRECT
Vaccine 24 (2006) 2017–2026
Vaccine
www.elsevier.com/locate/vaccine

Enhanced immunogenicity of microencapsulated multiepitope DNA vaccine encoding T and B cell epitopes of foot-and-mouth disease virus in mice

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Received 8 October 2005; received in revised form 10 November 2005; accepted 15 November 2005
Available online 1 December 2005

Abstract

The role of poly(D,L-lactic-co-glycolic acid, PLGA) microparticles on enhancing immune responses of multiepitope DNA vaccines was investigated in vitro and in vivo. pcDNA-SG encoding T and B cell epitopes of foot-and-mouth disease virus (FMDV) was encapsulated into PLGA microparticles. PLGA microparticles could protect themselves from nuclease degradation in vitro. PLGA–pcDNA-SG microparticles could be uptaken by cells and expressed His-tagged SG immunogen in vitro and in vivo. A prolonged expression and presentation of SG immunogen were observed by confocal laser scanning microscopy in the lymphocytes from the mice incubated with PLGA–pcDNA-SG microparticles, compared with the mice immunized with naked pcDNA-SG. PLGA–pcDNA-SG microparticles displayed a significant stronger immunogenicity than naked DNA vaccines with a higher titer of virus-specific antibody, elevated IFN-γ production and enhanced lymphocyte proliferation. PLGA–DNA microparticle could elicit augmented humoral and cellular responses with reduced amounts and times of immunization.

Keywords: Microparticle; DNA vaccine; FMDV; PLGA

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals and has a great potential for causing severe economic losses [1]. Short-term control of the disease is achieved by vaccination with a chemically inactivated whole virus vaccine. Although conventional inactivated vaccines have thus far succeeded in generating neutralizing antibody, protection via cellular immunity is still lacking [2]. Moreover, it is not completely safe, due to the fact the inactivation processes are technically challenging and can sometimes yield incompletely inactivated virus. Therefore, more safer and effective vaccines should be developed [3].

DNA vaccination is potentially capable of generating humoral immunity as well as cellular immunity [4]. DNA vaccines for prevention of FMD are studied by three strategies [5–12], in which the multiepitope DNA vaccine is focused on for its several potential advantages in recent years. The advantages include the increased "safety" of this immunization strategy that mimics antigen processing and presentation during natural infections, without actually causing disease, and the "flexibility" in epitope selection, which allows induction and optimization of the desired type of immunity [13]. Five sites containing B cell epitopes were defined on the four structural proteins of FMDV (1–4) through monoclonal antibody escape mutant studies [14,15]. In our previous research, four B cell epitopes and a single T cell epitope (VP1) were tandem arranged and inserted into an eukaryotic expression vector, producing a multiple-epitope DNA vaccine [16]. Both virus-specific humoral and cellular immune responses could be observed

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0264-410X/$ – see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2005.11.042

in mice immunized with the multiple-epitope DNA vaccine.

Large dosage of DNA vaccine is required to induce immune responses in large animals and human. It is a major obstacle for the broad application of the multiepitope DNA vaccine. Thus reduction of the dosage and enhancement of immunogenicity of plasmid DNA required may represent the key factors for successful DNA vaccine [17]. In this study, we addressed this question by using a novel DNA delivery system, based on PLGA microparticles. Poly(D,L-lactic-co-glycolic acid, PLGA) is a biodegradable polymer that has been approved of human use for many years [18]. PLGA microparticles encapsulating or absorbing plasmid DNA have many potentials such as protecting against biological degradation of plasmid DNA by nucleases and directing target delivery to macrophages and dendritic cells (DCs), resulting in an augmented immune responses [19]. However, there is still no report concerned with FMDV.

Here, the multiepitope DNA vaccine was encapsulated into PLGA microparticles forming a novel vaccine formulation against FMDV. The potential of PLGA–DNA microparticles to induce augmented immune responses with reduced amounts and times of vaccination was explored. Moreover, the mechanisms of immunopotentiation effects were also investigated in this article.

2. Materials and methods

2.1. Plasmid construction

B cell antigenic epitopes of FMDV were selected from five sites, as following: residues 70–78 and 131–134 of VP2, residues 56–58 on VP3, residues 41–60 of VP1, residues 135–167 of VP1 and residues 141–160 and 200–213 of VP1. These B cell epitopes and a single T cell epitope (residues 21–40 of VP1) were tandem arranged. Linkers, comprised of glycine and serine, were included to avoid the interference resulting from the direct ligation between epitopes or subsequent generation of new epitopes. Gene encoding these multiple epitopes and linkers was synthesized according to the corresponding sequence (subtype O1K, accession no. X00871, in GENEBANK)[16]. Multiepitope gene, named SG gene, was cloned into plasmid vector of pcDNA3.1/V5-His (Novagen). The fusion expression product of pcDNA-SG is SG immunogen with His-tag.

2.2. Preparation of plasmid-loaded microparticles

Multiepitope DNA vaccine, pcDNA-SG, was microencapsulated in PLGA as described previously [20]. Briefly, solutions of two PLGA polymers, RG502 and RG503 (Boehringer Ingelheim), were mixed 50:50 in ethyl acetate (4 ml) and emulsified with 0.4 ml pcDNA-SG (10 mg ml^{-1}) in STE buffer (100 mM NaCl, 10 mM Tris/HCl, 1 mM EDTA, pH 8.0). This emulsion was added immediately to an 8% aqueous polyvinyl alcohol solution (66 ml) preheated to 30 °C and emulsified, resulting in the formation of a water-in-oil-in-water emulsion. Microparticles were harvested by centrifugation, washed several times to remove the polyvinyl alcohol and residual solvent and finally lyophilized. A control formulation with the empty vector pcDNA3.1 encapsulated into PLGA microparticles was similarly manufactured [20]. For the phagocytosis experiment, PLGA–pcDNA-SG microparticles containing encapsulated rhodamine B (red fluorescence dye) were prepared individually with a target load of 0.25% (w/w) rhodamine in PLGA by the same process [20].

2.3. Phagocytosis of PLGA–pcDNA-SG microparticles in vitro

A 12-well plate with glass slides (14 mm × 14 mm) was seeded with suspensions of the mouse monocyte macrophage cell line RAW264.7 in DMEM containing 10% FCS and 1.5 mM L-glutamine, 100 units ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin to give approximately 80% confluence after overnight incubation. Fluorescence-labeled PLGA–DNA microparticles were suspended and added to monolayers at concentration of 20 μg/well without microparticles in control wells. On different incubation time points, slides were washed to remove excess microparticles and the cells were fixed. Phagocytosis was observed directly and photographed using a digital camera linked to a fluorescence microscope (Olympus, Japan).

2.4. Expression of SG immunogen in RAW 264.7 cells

Expression of the plasmid-encoding SG immunogen in vitro directly from phagocytosed microparticles was investigated on monolayers of RAW 264.7 cells. Cells were cultured and seeded as described previously [16]. Briefly, RAW 264.7 cells with 80% confluence were incubated with PLGA–pcDNA-SG microparticles or PLGA-encapsulated empty vector pcDNA3.1 (negative control) at concentration of 20 μg/well. After 3 h at 37 °C, cell sheets were washed three times with PBS to remove excess microparticles and fresh growth medium was added. Following incubation for 72 h, cell monolayers were washed in PBS and fixed with 1% paraformaldehyde for 10 min at room temperature. Cells were permeabilized by treatment with permeabilization buffer (PBS, 0.25% Triton X-100, 0.5% DMSO) for 10 min. After washing three times, nonspecific reactive sites were blocked by incubation with 2% BSA in PBS for 2 h and cells were then incubated overnight at 4 °C with 1:500 dilution of mouse monoclonal antibody anti-His·tag (Novagen). Bound antibody was detected after a further incubation for 1 h at room temperature with 1:1000 dilution of Alexa 555-conjugated goat anti-mouse $IgF(ab')_2$ (Molecular Probes, Eugene, USA). Washed cells then were incubated with Hoechst 33258 for 10 min and viewed by fluorescence microscopy (Olympus, Japan).