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Cloning of interleukin-15 gene of Tibetan pig and adjuvant effect of its recombinant plasmids packed with PEG and PEI modified chitosan nanoparticles on immunity of mice to FMD vaccination

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Abstract

IL-15 cDNA of Tibetan pig was firstly cloned from its activated lymphocytes, and then was sub-cloned into VR1020 to construct recombinant VRTIL-15 plasmid to study the *in vitro* and *in vivo* biological effects on animal. The VRTIL-15 was entrapped with chitosan modified with PEG-PEI (CS-PEG-PEI) to transfect HEK293 cells for the preliminary study of its expression in eukaryotic cells. The total RNA of HEK293 cell was isolated in 48h, and the successful expression of IL-15 was detected by RT-PCR and the supernatant of HEK293 cells was found to stimulate significant proliferation of lymphoblasts of pig. Subsequently, VRTIL-15 packed with CS-PEG-PEI was utilized to intramuscularly inoculate Kunming female mice at the age of 21 days. Their bloods were collected before and after inoculation on 1, 2, 3, 4 and 5 weeks to detect the changes of innate and adaptive immunity of animals. The results were found that Th and Tc, specific antibody to FMD, IgG, IgG1, IgG2a content markedly increased in the blood of treated mice compared with the control group ($P<0.05$). The mRNA expression of TLR1, TLR4 TLR6, TLR9, TGF- β , IL-2, IL-4, IL-6 and IL-23 were significantly higher in the treated group than those of the control ($P<0.05$). These results indicate that the VRTIL-15 wrapped with CS-PEG-PEI can significantly improve the innate, humoral and cellular adaptive immunity of animal, which could inspire the development of effective immune adjuvant to improve the comprehensive immune protection of animals against FMD.

Keywords: Chitosan and its derivatives, nanoparticle package and delivery, gene expression, piginterleukin-15, mice, immunity

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1. Introduction

The Tibetan pig is the unique indigenous pig breed in China, and has evolved for nearly nine thousands of years through natural selection in the Qinghai-Tibetan Plateau with an average altitude of more than 4200 m above sea level. It become adapted to marginal feeding and harsh environment on the plateau and is well known for strong resistance against various diseases and hypoxia environment[1]. But up to now little is known about the molecular mechanisms for these unique performances, especially for its special immunity against diseases.

The interleukin-15 (IL-15) is an important cytokine in immune regulation of animal immune system, and has a key role in the proliferation, survival and activation of CD8+ T, natural killer (NK) and other immune cells[2,3,7]. Although its function is similar to IL-2, IL-15 utilizes different mechanisms of signaling with IL-15 receptors to affect multitudinous target cells. Nowadays there is no report about IL-15 of Tibetan pig and far less than its role in the immune responses to viral vaccination of animal. To further explore and clarify the immunogenetic characteristics of Tibetan pig, here we conducted the present experiment to clone IL-15 gene of Tibetan pig and analyze its potential as an immunoadjuvant to boost the immune responses to Foot and Mouth Disease(FMD) vaccine which is still in urgent need of safe and effective adjuvant.

As a non-toxic, biocompatible and biodegradable polysaccharide, chitosan (CS) has attracted great attention in recent years, and a number of applications in drug delivery have been found due to its favorable biological properties[4,5,16,18]. In order to improve the efficiency of *in vivo* gene transfection in naimal, different chitosan derivatives were prepared and employed to pack the recombinant expression plasmid for IL-15 gene in animal vaccination experiment.

2. Materials and methods

2.1. TPIL-15 gene cloning and sequencing

Total RNA was extracted from the collected leukocytes of a Tibetan pig which was stimulated by LPS for 12 hours. RT-PCR is used for TPIL-15 gene cloning into pMD®19-Tvector (Takara), and then transformed into DH5α competent cells. Primers were designed by Primer 5.0 with *BamHI* and *BglII* sites according to conserved ORF sequence of IL-15 gene of Duroc pig, Landrace pig and other mammalians from NCBI/GenBank. PCR primers for the IL-15 sequences are: TPIL-15-F: 5'ATGTGTTTGAGAAGTACTTG3', TPIL-15-R:5'GTTCATCAACCCTTCTTGA3'. Plasmids were assessed for conformity by PCR and digested by *BamHI* and *BglII*.Subsequently, the pMD®19-T/IL-15plasmids were sent toBGI biological company for sequencing. TPIL-15sequence was aligned with domestic pigs and other mammalians by NCBI/Blast. Plasmid VR1020 is a eukaryotic expression vector (Vical Company of America). We cloned the TPIL-15 into VR1020 to construct VTPIL-15 to get secreted protein for *in vitro* and *in vivo* bioactivity test using the same fragment and methods as above mentioned.

2.2. Large-scale preparation of recombinant VTPIL-15, chitosan nanoparticles and characterization

A monoclonal cell line of recombinant DH5a *E. coli*, containing VTPIL-15or VR1020 plasmid, was inoculated in LB broth with kanamycin (100 mg/ml) at 37℃， 200rpm overnight. Plasmid was extracted following the spermine precipitation method described as Jason then the plasmid was resuspended in sterile water and stored at -20℃ until use.

The VTPIL-15 plasmids were entrapped with chitosan (CS, provided by Chengdu Organic Chemistry Institute of Chinese Academy of Science, China, MW: 50 kD and its deacetyl degree is over 95 percent) and its derivatives (CS-PEG–PEI and CS-PEG-LAC, prepared in our lab as reported previously[19,20]) by ionotropic gelation method[15, 16]. The zeta potential and average diameter of the nanoparticles were characterized by Zeta-sizer 3000 HS/IHPL (Malvern Instruments Ltd., Malvern, UK).

2.3. Gene transfection and bioactivity analysis

HEK293 cells were seeded in 12-well plates (1.0×10^5 cells/well) respectively. The cells were incubated in Dulbecco's modified Eagle medium (DMEM, Invitrogen Corporation.). The nanoparticles enwrapped with CS, CS-PEG–PEI and CS-PEG-LAC containing 3 μg of DNA were added into the wells, respectively, then they were incubated at 37℃in a 5% carbon dioxide humidified atmosphere.

The bioactivity of the TPIL-15 protein was measured by its ability to provoke the proliferation and cell viability of pig lymphoblast stimulated with Concanavalin A (Con A) through CCK8 colorimetry. Lymphocyte Separation Medium (LSM, ficoll 400) was used to separate pig peripheral blood mononuclear immune cells for *in vitro* bioactivity test.