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Procedia in Vaccinology 9 (2015) 66 – 79

Procedia in Vaccinology

www.elsevier.com/locate/procedia

8th Vaccine & ISV Congress, Philadelphia, USA, 2014

Construction and synergistic effect of recombinant yeast co-expressing pig IL-2/4/6 on immunity of piglets to PRRS vaccination

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Abstract

In order to develop cost-effective immunomodulator, the recombinant Pichia pastoris were firstly constructed to co-express porcine IL-2/4/6 genes, and then fermented to feed 45-days Tibetan piglets at different doses to evaluate its effects on immunity of piglets to PRRS vaccination, which simultaneously received intramuscular injection of inactivated PRRS vaccine. The results were found that the leukocytes, IgG and specific antibody to PRRSV, Th and Tc cells increased significantly in the blood of treated piglets in comparison with those of the control ($P<0.05$); the mRNA expression of TLRs (TLR-2, 3, 4, 7, 9), IFN-γ, IL-2, IL-4, IL-6, IL-7, IL-12 and IL-15 genes were elevated significantly in the immune cells from the blood of treated piglets ($P<0.05$). Moreover, the growth of the treated piglets also markedly improved whose average net weight gain was significantly higher than the control on 58 days post inoculation ($P<0.05$). These results suggest that the recombinant yeast can effectively enhance the systematic innate and adaptive immunity of piglets as well as promote the growth of piglet, which could be further developed as cost-effective promising immunomodulator to improve the control of pig PRRS disease.

Selection and peer-review under responsibility of the 8th Vaccine Conference Organizing Committee.

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Selection and peer-review under responsibility of the 8th Vaccine Conference Organizing Committee.
doi:10.1016/j.provac.2015.05.011

Keywords: piglet; pig interleukin genes; recombinant *Pichia pastoris;* immunity; growth.

1. Introduction

Nowadays pigs in intensive feeding farms are susceptible to various pathogens which could not be controlled only through vaccination. Viral and bacterial infectious diseases not only bring huge economical loss to pig industry but also result in severe public health problems in China. Although vaccines are very important for prevention of pig infectious diseases, immune failures of animal frequently happen worldwide due to inadequate immune responses or immunosuppression of animals caused by many reasons[1, 2]. Therefore, development of effective immunomodulator to enhance overall immunity of animals is one of practicable and economical choices to prevent animal diseases.

Cytokines have potent effects on the immune system, regulation haematopoietic cells differentiation, proliferation and/or activation. Since the early 1990s, many groups have examined to use cytokines as vaccine adjuvant [3], and have evidenced that most cytokines have the ability to modify and re-direct the immune response[4]. IL-2, IL-4 and IL-6 are very important cytokines that play key roles in regulation of innate and adaptive immunity, produced by various cells in animal. IL-2 is involved in T-cell proliferation and the induction of T regulatory responses, it not only can enhance cellular immune response, but also stimulate proliferation of activated B lymphocytes and induce immunoglobulin secretion[5]; IL-4 can influence humoral and cellular immune responses, such as the production, class switching and secretion of immunoglobulin[6]; IL-6 also has many biological activities that to promote interleukin gene expression, B cell differentiation, T cell activation, and play a important role in acute phase reaction[7, 8].

Our previous studies had demonstrated that pig IL-2 or IL-6 with CpG immunostimulatory sequences shuffled gene [9,10], or the fusion pig IL-4/6 gene could effectively enhance the resistance of animals against pathogen infection[11, 12]. The oral administration can elicit both local and systemic immune responses[13]. Yeast expression systems have a number of properties such as high expression levels, adjuvant properties, post-translational modifications, and "generally recognized as safe" status, these properties make them have potential advantages as live delivery systems for oral inoculation[14, 15]. In addition, yeast can be internalized by DCs and macrophages and promote presentation of antigens[16]. Moreover, yeast is a good probiotic that can resist the local stresses in gastrointestine, showing high survival rate in stomach and small intestine, and can carry out the bioconservation reaction throughout the digestive tract[17,18,19]. Therefore, we suppose that recombinant yeast could be utilized to express cytokine genes to effectively promote animal immunity via oral inoculation.

For sake of developing novel cost-effective immunomodulator for pig, and based on our previous reports, the present experiment was conducted to construct recombinant yeast to co-express pig IL-2 gene and fused IL-4/6 gene by 2A self-cleavage technique use[20], and to evaluate its effect on the immune response and growth of piglets at different dose.

2. Materials and methods

2.1 The recombinant *Pichia pastoris* and its construction

The cDNA of fused pig IL-4/6 gene and IL-2 gene were cloned respectively from recombinant VR1020 plasmids containing fused pig IL-4/6 gene or pig IL-2 gene, named VRIL4/6 and VRIL2 preserved in our lab.

The IL-4/6 gene was firstly cloned into pGAPZαA (Invitrogen) under the control of GAP promoter with an N-terminal α-factor of *Saccharomyces cerevisiae* as secretion signal. Then, IL-2 gene was linked by 2A-α fragment that contained gene of FMDV 2A peptide and α-factor gene to guarantee the secreted expression of IL-2. Meanwhile, the termination codon of fused pig IL-4/6 gene was removed. Afterwards, the recombinant plasmid (pGAPZαAJMPIL4/6-PIL2) was transformed into *Escherichia coli (E. coli)* DH5α and positive transformants were screened out on low salt Lurea Bertani (LLB) plates containing 25μg/ml zeocin, and further identified by direct PCR and sequencing.